Validity and reproducibility of morphologic analysis of nasal secretions obtained using ultrasonic nebulization of hypertonic solution

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Background: Collection of nasal secretions is important for the evaluation of upper airways inflammation in many nasal disorders.

Objective: To study the validity and reproducibility of nasal secretion cellularity induced by nebulization of hypertonic solution in patients with allergic rhinitis (AR), patients with nonallergic rhinitis with eosinophilia syndrome (NARES), and control subjects.

Methods: Sixty-eight individuals (29 with AR [mean ± SD age, 33.3 ± 16.9 years], 23 with NARES [mean ± SD age, 46.4 ± 16.6 years], and 16 controls [mean ± SD age, 42.1 ± 15.1 years]) underwent ultrasonic nebulization of hypertonic (4.5%) saline solution on 2 different occasions to study the validity and reproducibility of total and differential cell counts of nasal secretions.

Results: The mean ± SD percentage of eosinophils was significantly higher in samples from patients with AR (20.8% ± 23.1%) and NARES (18.7% ± 22.8%) than in samples from controls (0.6% ± 0.6%; $P < .001$ for both). There was a significant correlation between 2 samples of nasal secretions obtained on 2 different occasions for percentages of macrophages, neutrophils, eosinophils, and epithelial cells.

Conclusions: The analysis of nasal secretions obtained using ultrasonic nebulization of hypertonic solution can distinguish patients with AR and NARES from controls. The reproducibility of this technique is good for macrophages, neutrophils, eosinophils, and epithelial cells. This method could be used to detect nasal airway inflammation in clinical settings.

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INTRODUCTION

Collection of nasal secretions is important for the evaluation of upper airways inflammation in many nasal disorders. Evidence has been provided that inflammatory cells are present not only in the airways of patients with asthma but also in the airways of patients with seasonal allergic rhinitis (AR).[1]

Physicians and researchers are often faced with the problem of collecting adequate samples of nasal secretions. Various techniques have been used to obtain specimens from nasal mucosa and to study inflammation in the nasal cavity.[2] The most commonly used techniques include nasal lavage, nasal biopsy, nasal brush, nasal scraping, nasal mucosa collection, application of a microsuction, and direct aspiration of secretion.[3–6] Each method has several disadvantages, including being invasive procedures and the unsuitability of some samples for analysis. Furthermore, direct comparison of the validity and reproducibility of these methods in the same group of patients has never been performed.

Recently, a new noninvasive method for obtaining nasal secretion samples based on nebulization of hypertonic solution was described.[7] Samples obtained using this method are suitable for morphologic analysis. To our knowledge, no studies have analyzed and validated the results of examination of nasal secretions obtained by means of nebulization of hypertonic solution from patients with AR, patients with nonallergic rhinitis with eosinophilia syndrome (NARES), and control subjects. Thus, the aim of the present study is to assess the validity (ability to distinguish disease vs normality) of the method based on nebulization of hypertonic solution for the analysis of nasal secretions obtained from patients with AR, patients with NARES, and controls by comparing total and differential cell counts. In addition, the reproducibility of the method was examined by comparing measurements in 2 samples of nasal secretions obtained on 2 different occasions in a group of patients with AR, patients with NARES, and controls.

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MATERIALS AND METHODS

Patients

Sixty-eight individuals were enrolled to study the validity of the nasal secretion method: 16 controls and 52 patients with either AR (n = 29) or NARES (n = 23) (Table 1). Twenty-nine of the 68 participants did not show up for the second induction of nasal secretion or did not produce adequate sample on either the first or the second occasion. Thus, the reproducibility of the method was studied using 39 individuals (17 with AR, 12 with NARES, and 10 controls). AR was diagnosed on the basis of a positive skin test reaction to 1 or more allergens, positive allergen specific IgE antibody test results, and a history of seasonal symptoms.[8] NARES was defined as a clinical syndrome comprising symptoms consistent with AR in which an absence of atopy has been demonstrated by allergen skin testing, and nasal cytologic analysis demonstrates eosinophils.[9]

Study Design

Each participant enrolled in the study underwent ultrasonic nebulization of hypertonic (4.5%) saline solution on 2 different occasions at the same hour of the day within 1 week to study the reproducibility of total and differential cell counts of nasal secretion. The validity of the method was evaluated by comparing differential cell counts of nasal secretions obtained from patients with AR, patients with NARES, and controls. The protocol was approved by the ethics committee of Fondazione Salvatore Maugeri, and all the participants provided written informed consent.

Ultrasonic Nebulization of Hypertonic Saline Solution

Ultrasonic nebulization of hypertonic (4.5%) saline solution was generated using an ultrasonic nebulizer (Orion 2; Nova, Heyer, Germany) at maximum output power (4.4 mL/min) for 5 minutes.[7] During nebulization the participants had their necks extended to facilitate penetration of the inhaled solution on the whole surface of the nasal mucosa, including the posterior nasal cavity. The mean size of particles inhaled through the nose was 2.5 to 5.0 μm. After 5 minutes of inhalation the participants were asked to press the left nostril and to blow the right nostril forcefully into a Petri dish. The blowing procedure was repeated for the left nostril.

Sample Processing

The secretions collected were processed.[7] In brief, the secretions were immediately weighted, and dithiothreitol 0.1% reagent (Sputolysin; Calbiochem Corp, San Diego, California) freshly prepared was added to the secretions. The volume of dithiothreitol was equal to 4 times the weight of the secretions portion. The sample was placed at 37°C for 20 minutes and was vortexed every 5 minutes to ensure cell dispersion. The sample was then filtered on sterile gauze, and a small volume (20 μL) was used to evaluate total cell counts using a standard hemocytometer. The sample was then divided into 2 aliquots: 1 was diluted to obtain a final cellular concentration of 2×10^5/mL; 100 μL of this sample was cytocentrifuged at 400 rpm for 5 minutes; the slides were stained with a Romanowski-based stain (Diff-Quik; Dade Behring AG, Düdingen, Switzerland), and a differential cell count was performed on 200 cells.

Statistical Analysis

Descriptive statistics were used to summarize clinical and demographic characteristics of the participants. The results are expressed as mean ± SD for age, lung function values, and cellular composition of nasal secretions. The comparison of differential cell counts between patients with rhinitis and controls was evaluated using *t* tests. The reproducibility of measurements was examined using the Pearson correlation coefficient. $P < .05$ was considered statistically significant.

RESULTS

Cellular Composition of Nasal Secretion Samples

The percentage of eosinophils was significantly higher in samples from patients with AR (20.8% ± 23.1%) and NARES (18.7% ± 22.8%) than in samples from controls (0.6% ± 0.6%; $P < .001$ for both) (Table 2). Samples of nasal secretions collected from patients with AR (1.2% ± 1.4%) and NARES (1.2 ± 1.0) were characterized by an increased percentage of lymphocytes compared with controls (0.0% ± 0.0%; $P < .001$ for both). The percentage of neutrophils increased significantly in samples from patients with NARES vs controls (78.0% ± 21.0% vs 65.4% ± 20.7%; $P = .04$), whereas this increase was not significant in patients with AR (70.8% ± 25.9% vs 65.4% ± 20.7%; $P = .22$). The percentage of epithelial cells (AR vs controls: 5.9% ± 12.5% vs 30.1% ± 19.6%; NARES vs controls: 2.0% ± 3.8% vs 30.1% ± 19.6%; $P < .001$ for both) and macrophages (AR vs controls: 1.2% ± 6.7% vs 3.9% ± 2.8%; $P = .03$; NARES vs controls: 0.0% ± 0.0% vs 3.9% ± 2.8%; $P < .001$) decreased significantly in samples of nasal secretions

Table 1. Demographic and Clinical Characteristics of the 68 Study Participants

Characteristic	AR group (n = 29)	NARES group (n = 23)	Control group (n = 16)	Total (N = 68)
Sex, M/F, No.	13/16	9/14	6/10	28/40
Age, mean ± SD, y	33.3 ± 16.9	46.4 ± 16.6	42.1 ± 15.1	39.8 ± 17.2
FEV_1, mean ± SD, % predicted	98.4 ± 16.6	93.9 ± 16.5	105.2 ± 18.1	98.7 ± 17.2
FVC, mean ± SD, %	102.2 ± 12.8	99.7 ± 28.5	109.6 ± 16.8	104.5 ± 14.8

Abbreviations: AR, allergic rhinitis; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; NARES, nonallergic rhinitis with eosinophilia syndrome.