

Repeatability and validity of cell and fluid-phase measurements in nasal fluid: a comparison of two methods of nasal lavage

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Summary

Background There is little information on the repeatability of cell counts and fluid-phase measurements in nasal fluid obtained by different methods of nasal lavage.

Objective To compare the repeatability and validity of total and differential cell counts and eosinophil cationic protein (ECP) in nasal secretions obtained by two methods of nasal lavage.

Patients and methods Twelve healthy subjects and twelve subjects with clinically stable allergic rhinitis were randomly assigned to two nasal lavages (separated by 48 h), by one of two methods in the first week and by the second method in the following week. One method was a modification of the method described by Greiff et al. and Grunberg and coworkers and the other was that described by Naclerio and coworkers.

Results Both methods of nasal lavage gave poorly repeatable eosinophil counts and ECP in normal subjects but better repeatability in subjects with rhinitis. The modified Greiff/Grunberg method gave higher and more repeatable total cell count and, in subjects with rhinitis, more reproducible ECP levels compared with the Naclerio method. Both methods were able to discriminate between healthy and rhinitic subjects: mean $\pm$ SD eosinophil percentage count and eosinophil cationic protein differences were $4.5 \pm 4\%$ ($P < 0.05$) and $24.5 \pm 46.9 \mu\text{g/L}$ ($P < 0.05$), respectively, with the modified method and $7.0 \pm 4\%$ ($P < 0.05$) and $26.9 \pm 68 \mu\text{g/L}$ ($P < 0.05$), respectively, with the Naclerio method.

Conclusion Both methods are valid and discriminate between normal and rhinitic subjects. In subjects with rhinitis, although the repeatability of eosinophil counts is similar by both methods, the modified Greiff/Grunberg method gives more reproducible ECP measurements, compared with the Naclerio method.

Keywords: nasal lavage, validity, reproducibility, cell counts, ECP

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Introduction

Allergic rhinitis is associated with nasal mucosal inflammation involving eosinophils, basophils, mast cells, lymphocytes and their mediators [1]. Various methods have been used to obtain nasal cytology specimens to study

inflammation in the nasal cavity [2]. The most commonly used techniques of nasal lavage are that described by Naclerio et al. [3] and by Greiff and colleagues [4], which was later modified by Grünberg and coworkers [5] by using a Foley's catheter to instill saline solution in the nostril.

Although the different methods of nasal lavage have been used to compare patients with rhinitis and normal subjects [6], and to assess the responses to various allergens and drugs [7–9], a direct comparison of their reproducibility in the

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Table 1. Subject characteristics

	Normal subjects	Rhinitis subjects
<i>n</i>	12	12
Males, <i>n</i>	4	5
Age, yr	43 (12)	43 (10)
Atopy, <i>n</i>	3	12
FEV ₁ , % predicted	101 (13)	98 (9)
FEV ₁ /VC, %	92 (11)	90 (14)

The numbers represent mean and standard deviation Atopy means one or more positive early responses in allergy skin-prick tests.

same group of subjects has not been made. This is important in comparing the results obtained by both methods. We compared the reproducibility and validity of the cell counts and fluid-phase measurements in nasal lavage samples obtained using the two methods. The reproducibility was examined by comparing measurements in two specimens of nasal lavage obtained on 2 separate days in a group of normal subjects and subjects with rhinitis. Since there is no gold standard for the diagnosis of allergic rhinitis, we checked the construct validity by comparing the eosinophil differential cell count in the lavage fluid in the two groups of subjects.

Methods

Subjects

Twelve healthy, mainly non-atopic, non-smoking subjects and twelve non-smoking, atopic patients with allergic rhinitis were recruited from the Firestone Regional Chest and Allergy Clinic (Table 1). Allergic rhinitis was defined according to the international consensus report [10] as symptoms of sneezing, itching, rhinorrhea or nasal congestion for more than 3 months, positive skin prick test to at least one of 14 prevalent allergens in our area and total serum IgE level higher than 50 IU/mL. Atopic subjects did not have variable exposure to the relevant allergens and had stable symptoms during the study period and the preceding 4 weeks. None of the subjects had used nasal corticosteroids or oral antihistamines for at least 4 weeks prior to the study. The study was approved by the hospital ethics committee and written informed consent was obtained from all subjects.

Study design

We performed a cross-sectional, randomized study with blind assessment of outcome measurements over 4 days.

Nasal lavages were performed after a standard anterior rhinoscopy and completing a symptom questionnaire. On day 1, subjects were randomly assigned to nasal lavage by one method and 2 days later the same method was repeated. In the next week, nasal lavage was performed by the other method on 2 separate days. The primary outcome measures were the total and differential cell count and fluid-phase levels of eosinophil cationic protein (ECP).

Procedures

Allergy skin tests were performed using the modified prick technique [11] with 14 common allergen extracts. Nasal lavage by the modified Greiff and Grünberg method was performed using a 14G all-silicone Foley catheter (C.R. Bard Inc., Covington, GA, USA). The tip of the catheter was cut just distal to the balloon while keeping the balloon intact and introduced about 1.5 cm into the frontal nasal chamber (not against nasal septum) with the subject holding it in place. With the subject in a writing position, the balloon was inflated and 10 mL of isotonic saline (0.9%) was instilled using a syringe which was left attached to the other end of the catheter which was trimmed to fit the syringe snugly. After 5 min the mixture of saline and mucus was recovered into the syringe. The balloon was then deflated and the procedure repeated on the other nostril.

Nasal lavage by the Naclerio method was performed with the subjects seated with their necks extended approximately 30° from the vertical. Five millilitres of isotonic saline (0.9%) at room temperature were instilled into one nostril with a syringe. The subjects were instructed not to breathe or swallow. After 10 s the subjects flexed their necks and expelled the sample of mucus and saline into a container. The procedure was then repeated in the other nostril. The volume recovered by both methods was noted. The patient's acceptance of the methods was checked using a Borg scale of discomfort (0: no trouble at all, 10: maximum discomfort similar to congestion from a 'cold').

The samples were processed within 2 h of collection using the same procedure employed for processing sputum [12], except that instead of using four times the volume of 0.1% dithiothreitol (Sputolysin, Calbiochem Corp., San Diego, CA, USA) to disperse the cells, one-tenth of the nasal lavage volume was used. The total cell count was obtained in a modified Neubauer haemocytometer. The cell viability was determined by the trypan blue exclusion method. Cytospins were prepared by centrifuging the fluid at 7 g for 6 min (Shandon III cytocentrifuge, Shandon Southern Instruments, Sewickley, PA, USA) and stained with modified Wright's stain. Four hundred non-squamous cells were counted and the results expressed as a percentage of the total count. The concentration of ECP (µg/L) in the

Table 2. Repeatability of cell counts and ECP in normal subjects ($n = 9$)

Measurement	Modified Greiff/Grunberg method			Naclerio method		
	Day 1	Day 2	ICC	Day 1	Day 2	ICC
Viability, %	69 (24)	73 (40)	0.8	36 (24)	80 (41)	0.2
TCC, $10^6/\text{mL}$	0.3 (0.6)	0.1 (0.2)*	0.7	0.01 (0.1)	0.01 (0.1)	0.1
N, %	52.8 (40.1)	35.9 (35.2)	0.8	12.8 (15.8)	20.3 (27.6)	0.1
Eo, %	0.1 (0.3)	0.4 (1.2)	0.2	0.6 (1.6)	0 (0)	0.2
M, %	3.4 (8.5)	1.8 (3.5)	0.8	3.1 (5.2)	3.8 (6.0)	0.9
Ep, %	32.5 (35.5)	50.9 (37.1)	0.9	61.3 (39.4)	53.7 (39.4)	0.7
ECP, $\mu\text{g/L}$	8.5 (7.1)	16.4 (35.5)	0.2	2.2 (2.8)	1.7 (1.6)	0.1

Numbers represent mean and standard deviation. TCC = total cell count, N = neutrophil, Eo = eosinophil, M = mononuclear cell (predominantly monocyte/histiocyte), Ep = epithelial cell, ECP = eosinophil cationic protein, ICC = intraclass correlation coefficient. * $P < 0.05$ (for the comparison of the mean values of one method with the other method).

supernatant was determined using a radioimmunoassay (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden) with a limit of detection $< 2 \mu\text{g/L}$.

nasal lavage methods was coded and included in the model as a dummy variable. Significance was accepted at the 95% level.

Analysis

Descriptive statistics was used to summarize demographic data. Grouped data were summarized using means and standard deviations. Repeatability was assessed by analysing the differences between two samples collected within 48 h and was expressed by the intraclass correlation coefficient (ICC). Validity was checked by demonstrating differences in eosinophil counts and ECP levels between healthy and rhinitis subjects using the analysis of variance for repeated measures method, with 'diagnosis' and 'lavage method' as the between-subject factors. The order of the

Results

Of the 96 samples from the 24 subjects, 10 samples (from three normal subjects and one rhinitis subject) were not non-processable (six obtained by the modified Greiff/Grunberg method and four obtained by the Naclerio method). The recovered volume — mean (SD) of the percentage instilled — was similar using both methods: 65(14) and 65(27), respectively.

A significantly greater number of cells (total cell count per unit ml of recovered fluid) was observed in the lavage fluid obtained by the modified method in normal and

Table 3. Repeatability of cell counts and ECP in subjects with rhinitis ($n = 11$)

Measurement	Modified Greiff/Grunberg method			Naclerio method		
	Day 1	Day 2	ICC	Day 1	Day 2	ICC
Viability, %	64 (9)	39 (20)	0.3	50 (14)	47 (36)	0.2
TCC, $10^6/\text{mL}$	0.7 (2.0)	0.3 (0.6)*	0.7	0.2 (0.7)	0.05 (0.07)	0.3
N, %	65.2 (33.7)	32.9 (34.2)	0.6	38.0 (41.8)	35.0 (39.0)	0.7
Eo, %	3.3 (8.9)	5.9 (11.7)	0.9	7.0 (20.0)	7.5 (20.7)	0.9
M, %	4.3 (6.0)	7.6 (14.7)	0.7	11.0 (13.1)	3.9 (6.0)	0.6
Ep, %	18.8 (22.8)	26.4 (31.0)	0.6	36.2 (35.9)	45.1 (42.1)	0.8
ECP, $\mu\text{g/L}$	54.1 (142.8)	25.9 (36.1)	0.6	48.1 (132.0)	9.5 (8.4)	0.2

Numbers represent mean and standard deviation. TCC = total cell count, N = neutrophil, Eo = eosinophil, M = mononuclear cell (predominantly monocyte/histiocyte), Ep = epithelial cell, ECP = eosinophil cationic protein, ICC = intraclass correlation coefficient. * $P < 0.05$ (for the comparison of the mean values of one method with the other method).

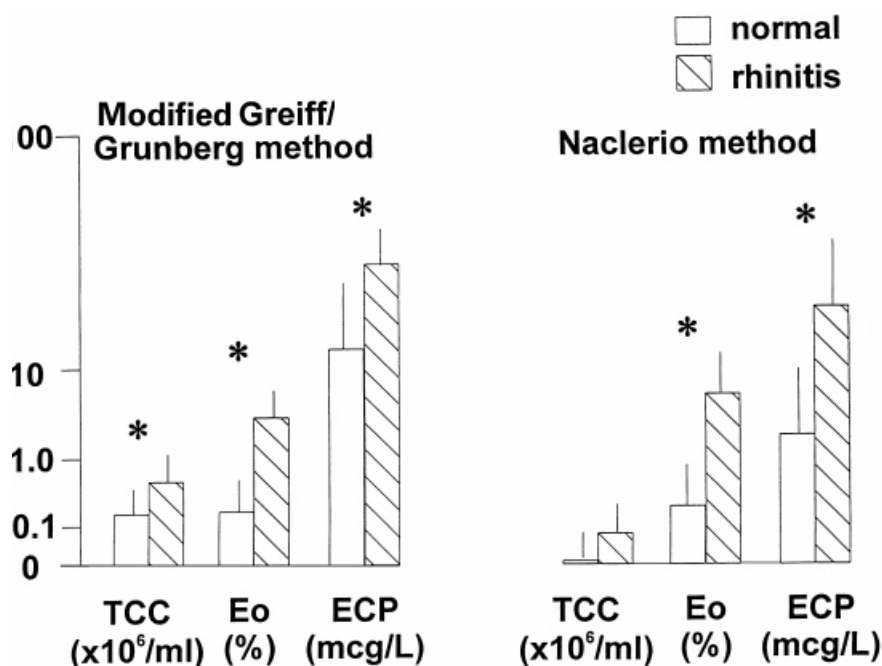


Fig. 1. Comparison of cell and fluid-phase measurements in nasal lavage fluid obtained by the modified Greiff/Grünberg method and the Naclerio method, in normal subjects (open bars) and rhinitis subjects (hatched bars). The subjects with rhinitis have significantly higher total cell count, eosinophil percentage count and eosinophil cationic protein compared with normal subjects. TCC = total cell count, Eo = eosinophil percentage count, ECP = eosinophil cationic protein.

rhinitic subjects (Tables 2 and 3). There were no significant differences in the mean values of differential cell counts and ECP levels in the fluid obtained by both methods (Tables 2 and 3). The repeatability of total cell count was higher using the modified method than the Naclerio method in both normal (ICC 0.7 vs. 0.1) and rhinitic subjects (ICC 0.7 vs. 0.3) (Tables 2 and 3). The repeatability for eosinophil differential count and ECP were poor in normal subjects by both methods; however, in subjects with rhinitis, the modified method gave a more reproducible ECP measurement (ICC 0.6 vs. 0.2).

Eosinophil differential cell count and ECP level were higher in subjects with rhinitis compared with the normal subjects in lavage fluids obtained by both methods (Fig. 1, Tables 2 and 3). The mean ($\pm$ SD) differences in eosinophil percentage count and eosinophil cationic protein level between normal and rhinitic subjects were $4.5 \pm 4\%$ ($P < 0.05$) and $24.5 \pm 46.9 \mu\text{g/L}$ ($P < 0.05$), respectively, with the modified method and $7.0 \pm 4\%$ ($P < 0.05$) and $26.9 \pm 68 \mu\text{g/L}$ ($P < 0.05$), respectively, with the Naclerio method ($P < 0.05$). The total cell count was also significantly higher in subjects with rhinitis compared with normal subjects when using the modified method.

The Borg score of discomfort from the procedure was comparable for both methods (median score 0.5 for the modified method and zero for the Naclerio method). Although both procedures caused hardly any discomfort, 13 of the 20 subjects preferred Naclerio's method to the modified method (four subjects did not indicate their preferences).

Discussion

The results of this study show that both methods of nasal lavage gave poorly reproducible eosinophil cell count and ECP in normal subjects, but the modified Greiff/Grünberg method gave more reproducible ECP measurement in subjects with rhinitis. The modified method also gave higher total inflammatory cell yield in both normal and rhinitic subjects. Both methods were able to discriminate between normal subjects and subjects with rhinitis.

Although biochemical, immunological and cellular measurements are conveniently made in blown nasal secretions and nasal scrapings, nasal lavage is usually necessary in order to obtain sufficient quantities of nasal secretion with minimal perturbation of the nasal mucosa. Nasal lavage can be performed in several different ways. One of the best known nasal lavage methods is the Naclerio method, which has good discriminative and evaluative properties. The procedure takes only 20 s. However, this technique requires good collaboration from the subject. Also, the distribution and concentration of the lavage solution in contact with the nasal mucosa is unknown and variable. The other method we used, which was a modification of the methods described by Greiff and Grünberg, requires little cooperation from the subject. The Foley's catheter that we used is readily available and is inexpensive. The technique is simple and can be easily performed with little training. However, it takes more time than the Naclerio method. Also, a greater volume of fluid is instilled into the nose.

This is the first direct comparison of the reproducibility

and validity of two methods of nasal lavage. It is important to compare studies employing the two methods and also to power studies using upper airway inflammatory markers as outcome measures. The modified method yielded a higher total number of inflammatory cells in both normal and rhinitic subjects. This may be a reflection of the longer time of contact of the lavage fluid with the nasal mucosa and the greater volume of lavage fluid instilled in the nose, although the volume of fluid recovered was similar by both methods. While both methods gave reproducible eosinophil counts in subjects with rhinitis, the low intraclass correlation coefficients of reproducibility in normal subjects may be because of the small range of the cell counts, rather than a reflection of the poor performance of the method. Both methods were valid and could detect higher eosinophil counts in subjects with rhinitis compared with normal subjects. The modified technique is unlikely to have affected the cell counts by a neuroreflex mechanism involving sensory neuropeptides because the sensation of discomfort was comparable for both the methods.

We measured ECP in the lavage fluid using a modification of the method used to process sputum. In order to correct for the increased volume of fluid compared with sputum, we used one-tenth the volume of dithiothreitol to disperse the cells. Despite the higher volume of fluid instilled by the modified method, the ECP measurement by this method was more reproducible (ICC 0.6) in subjects with rhinitis than when using the Naclerio method (ICC 0.2). The ECP levels in normal subjects are similar to those reported in other studies which compared different methods of obtaining nasal secretions [13]. Both methods are valid as demonstrated by the differences between normal subjects and those with atopic rhinitis.

In conclusion, cell counts in nasal lavage fluid obtained by the method described by Naclerio and colleagues and the modified method, which is a modification of that developed by Grieff et al. and Grunberg et al., are both valid and repeatable and equally well tolerated. The modified method yields higher number of cells and a more reproducible measurement of ECP in subjects with rhinitis, suggesting that the volume of fluid and time of contact of the fluid with the nasal mucosa are relevant in the interpretation of the results.

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