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# Nasal Cellularity in 183 Unselected Schoolchildren Aged 9 to 11 Years

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**ABSTRACT.** *Objective.* Although rhinitis is extremely frequent in children, methods for assessing the severity of nasal inflammation produce results with wide variability and hence weak clinical significance. We designed this epidemiologic investigation to define the clinical usefulness of assessing nasal cellularity in children.

*Methods.* We studied 183 of 203 eligible unselected schoolchildren who were aged 9 to 11 years and whose parents gave informed consent and completed a questionnaire on the history of atopic and respiratory symptoms. In all children, nasal swabs were obtained from both nostrils and eluted in saline and slides were prepared from cytospin preparations for staining and white cell counts. Children also underwent determination of nasal volume, skin prick tests with 7 common local allergens, flow volume curves, and nitric oxide measurement in expired air. Blood samples were drawn for the measurement of total immunoglobulin E, eosinophil percentage, and detection of *Chlamydia pneumoniae* antibodies. *C pneumoniae* DNA was also sought in eluates from nasal swabs. The percentage, standard deviations, and percentiles of the various nasal white cell populations were determined.

*Results.* No correlation of the percentage of these cells was found with the history of allergies or respiratory disease or with functional or laboratory finding. Repeat nasal swabs obtained 1 month after the initial examination in 31 children (20 with neutrophils higher and 11 lower than 14%) in 77.4% of the cases confirmed the previous (high or normal) result. Twelve of the 16 eligible children with persistently high nasal neutrophil counts completed a 15-day cycle of intranasal flunisolide therapy (200 µg twice a day). Therapy significantly reduced nasal neutrophil percentage and increased nasal volume.

*Conclusions.* Increased nasal neutrophils, although related neither to the clinical history nor to laboratory variables, are a common important finding in children. A 15-day cycle of intranasal flunisolide is sufficient to restore normal nasal neutrophilia. *Pediatrics* 2002;110:1137–1142; *nasal cellularity, neutrophils, rhinitis, allergy, schoolchildren.*

ABBREVIATIONS. NO, nitric oxide; PCR, polymerase chain reaction; eNO, exhaled nitric oxide; ppb, part per billion; Ig, immunoglobulin; CI, confidence interval.

Rhinitis is an extremely common clinical condition in children and is fundamentally supported by an inflammatory state.<sup>1</sup> The nasal manifestations vary from a severe inflammatory state to extremely mild or transiently asymptomatic processes.

Over the years, numerous techniques have been developed for objectively assessing inflammatory problems in the nasal cavity.<sup>2,3</sup> Despite technological advances, no method for studying the nose and the severity of nasal inflammation in children has been fully standardized and widely accepted.<sup>4</sup> A review of the relevant literature suggests that most existing assessment techniques have 2 basic defects: wide variability and, hence, weak clinical significance of the reported values.

The most direct method for studying the severity of inflammation in the nasal mucosa is the assessment of nasal cellularity, for example, by nasal washing, nasal scraping, and nasal biopsy.<sup>2,5,6</sup> Among these methods, nasal scraping is the most simple and noninvasive procedure suitable for studying a large population such as unselected schoolchildren. Few studies have validated measurements of nasal cellularity, and most results refer to preselected population. Because few epidemiologic studies have enrolled unselected children, sound data on the range of normal values and on the repeatability of results are lacking.<sup>7</sup> Hence, this technique still is not in widespread clinical use.

We designed this study to provide normal values (including mean, standard deviations, and percentile distribution) of nasal cellularity in an unselected population of schoolchildren who were 9 to 11 years of age and to seek an association between nasal cellularity and gender; questionnaire-derived clinical history of respiratory disease and atopy; various respiratory variables and laboratory findings of atopic and infective markers, including expired nitric oxide (NO) concentration, a recognized marker of allergic inflammation<sup>8</sup>; and antigen and antibodies for chlamydia, a microorganism known to be associated with asthma.<sup>9</sup> We also assessed the reproducibility of nasal cell counts and investigated whether local corticosteroid therapy would restore altered nasal cellularity to normal.

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**TABLE 1.** Cell Populations by Demographic Variables

	Total (100%)*	Boys (44.8%)	Girls (55.2%)	<10 Years (36.1%)	≥10 Years (63.9%)
Neutrophils	15.0 ± 17.4	15.6 ± 18.6	14.5 ± 16.5	17.0 ± 18.1	13.8 ± 17.0
Lymphocytes	1.5 ± 3.8	1.2 ± 3.1	1.7 ± 4.3	1.0 ± 3.3	1.7 ± 4.1
Squamous epithelial cells	23.2 ± 20.6	24.4 ± 21.6	22.1 ± 19.7	19.1 ± 16.3	25.5 ± 22.4
Ciliated epithelial cells	60.5 ± 22.2	59.0 ± 22.5	61.7 ± 22.0	63.1 ± 20.5	59.0 ± 23.1

Values are mean ± standard deviation.

\* Percentage of the 183 studied children considered in the column.

## METHODS

### Study Population

The study and the procedure had the approval of the ethics committee of the Pediatric Clinic at the University of Rome, La Sapienza. We studied an unselected population of children who attended an elementary school in Ronciglione (Viterbo; age range: 9–11 years). The population was recruited by sending a letter to parents of all children in classes IV and V. There were 203 eligible children. The parents of 200 gave informed consent for their children to participate. Before the study, 200 parents completed a questionnaire seeking information on the history of atopic and respiratory symptoms; information on questions and on derived definitions of atopic and respiratory disease have been detailed elsewhere.<sup>8</sup> Mainly, they reproduce the definitions internationally agreed on in the International Study of Asthma and Allergies in Childhood.<sup>10</sup>

Only 183 of the children who were enrolled completed the programmed procedures. The 17 children who were excluded (because of absences or refusal to undergo important procedures) did not differ as a group for history or available variables from the cases that were finally included in the study. All children then underwent a preliminary clinical examination, and those who had acute respiratory symptoms were asked to return and entered the study when their symptoms had cleared. All procedures were accomplished within 45 days.

### Nasal Swabs

Nasal cytology was studied in eluates from nasal swabs obtained in the middle third of the inferior turbinate using sterile swabs mounted on aluminum sticks. During the examination, both nostrils were scraped with the same swab, which was subsequently eluted in 3 mL of saline solution and transported within 6 hours to the laboratory at the Pediatric Clinic "Policlinico Umberto I," Rome. Slides were prepared from cytospin preparations and stained with May-Grünwald-Giemsa for identification of squamous epithelial cells, ciliated epithelial cells, lymphocytes, eosinophils, and neutrophils on the basis of cell shape, size, and nuclear configuration. White cells were counted with an optic microscope (Leitz Aristoplan, Wetzlar, Germany), and the percentage of each white cell population was determined.<sup>11</sup> Because most samples contained no eosinophils and only a few had 1% or fewer (2% in 2 samples), in all samples the percentage of eosinophils was assumed to be 0.

### Nasal Cell Repeatability

Nasal cell percentages above the 66th percentile were defined as elevated. To assess the repeatability of nasal cellularity 1 month after the first measurement, we asked 20 children with elevated neutrophil percentages (>14%) in nasal swabs eluated and 11 with neutrophil counts within the normal limits to repeat the nasal swab; all of them agreed to undergo this procedure.

### Effects of Local Steroid Therapy

To assess the effects of local corticosteroid therapy in restoring altered nasal cellularity, we asked 16 children in whom we had found increased nasal neutrophils on two occasions to undergo a 15-day trial of nasal flunisolide inhalation.

### DNA Polymerase Chain Reaction for *Chlamydia pneumoniae*

A total 100 µL of nasal aspirates was proteinase K digested (50 µg/ml) for 1 hour at 55°C, phenol extracted, ethanol precipi-

tated, then resuspended in Tris-ethylenediaminetetraacetic acid. Samples were assayed in a nested DNA polymerase chain reaction (PCR) using specific primers selected from major outer membrane protein.<sup>12</sup> The outer (first round) primers used were APNOU (5'-AATCTCTGTAAACAAACCC-3') and APNOL (5'-ATTAA-GAAGCTCTGAGCATA-3'), and the inner (second round) primers were APN1 (5'-AGCCTAACATGTACTACTCTGAT-3') and APN2 (5'-TGCCAACAGACGCTGCGT-3') generating a 487-bp product detected by gel electrophoresis.

PCR reaction buffers, AmpliTaq Gold DNA polymerase, and oligonucleotide primers were provided by Perkin Elmer Applied Biosystems (Foster City, CA). The final PCR mixture contained 10 × PCR buffer II, 2 mmol/L MgCl<sub>2</sub>, 1 µM of each primer, 0.8 mmol/L dNTPs, 1 U of AmpliTaq Gold, and 2 µL of template. After preincubation at 94°C for 5 minutes, the reaction cycle consisted of denaturation at 94°C for 75 seconds and annealing at 52°C for 75 seconds for the first round or 63°C for 75 seconds for the second round, and the extension at 72°C for 60 seconds, with 30 cycles for the first round and 35 cycles for the second round. The sensitivity by second-round gel was 4.5 fg of *C pneumoniae* DNA. A short (550 bp) cloned fragment of *C pneumoniae* genome DNA and 2 clinical samples known to be positive for *C pneumoniae* from a previous study<sup>13</sup> were used for positive controls. Negative controls were noninfected phosphate-buffered saline. All positive samples were reconfirmed by repeat analysis, and all DNA extractions, primary PCR amplifications, and secondary PCR amplifications were undertaken in distinct areas, using dedicated pipettes and filtered tips.

### Flow Volume Curves

A Cosmed Altair 1000 pneumotachograph (Cosmed Srl, Rome, Italy) was used for spirometry. Forced expiratory volume in 1 second was selected from 3 repeatable maneuvers of forced vital capacity, and results were expressed as a percentage of the predicted values.

### Exhaled NO Measurements

Exhaled NO (eNO) was measured at the mouth as previously described<sup>14</sup> with a chemiluminescence NO analyzer (Sievers NOA 280, Boulder, CO; response-time: 0.02 seconds; sensitivity: <1 part per billion [ppb], range of measures: <1–500 000 ppb, repeatability: ±1 ppb; sampling flow: 200 mL/s). The 0 signal was calibrated with an air filter (Sievers ACT 01400), and the measurement scale was calibrated with a gas containing 10 parts per million NO in nitrogen (SIAD Srl, Bergamo, Italy). Exhaled gas was collected into 1-L Mylar bags. Children exhaled through a calibrated needle restrictor (internal diameter: 1.55 mm) keeping a constant pressure of 10 cm H<sub>2</sub>O. All samples were transported to the laboratory and analyzed with a maximum delay of 2 hours after collection; in each child, eNO was calculated as the mean from 2 samples.

### Nasal Volume

The equipment used for nasal volume measurements (Acoustic Rhinometer EccoVision, SensorMedics, Yorba Linda, CA) consisted of a pulse generator, a sound tube with a microphone mounted in the wall and a nosepiece attached, a microphone amplifier, and a computer for data analysis. A wave tube was subsequently introduced into each nostril at an angle of 45° and fitted tightly with a conical nosepiece. An audible sound was propagated into nasal cavities. Local changes in acoustic impedance were expressed as local changes in the cross-sectional area. Two consecutive measurements were obtained in each nasal cavity at the end of an oral blowing within approximately 1 second, and the maximum value was used for calculation.

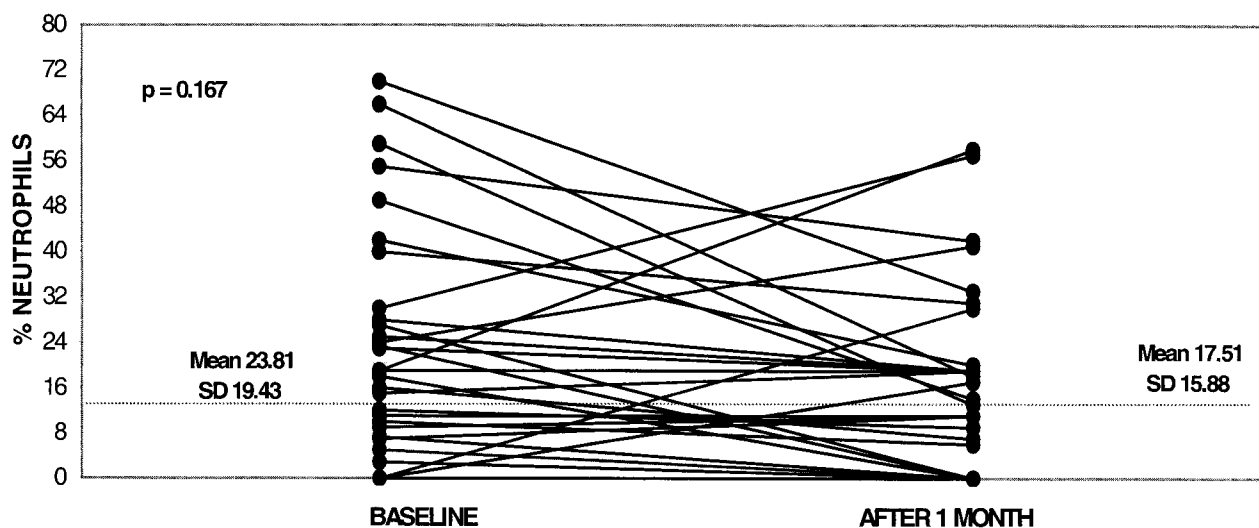


Fig 1. Repeatability of percentage nasal neutrophil counts in 2 measurements obtained 1 month apart in 31 children. Dotted line represents the 66th percentile (14%); Cohen's  $\kappa$ : 0.535; 95% CI: 0.233–0.837;  $P = .002$ .

### Blood Samples

Blood cytometric evaluation included a differential white cell count and eosinophil percentages. Serum samples were stored in aliquots at  $-70^{\circ}\text{C}$  and subsequently tested for total immunoglobulin (Ig) E by the immuno-CAP method (Pharmacia, Uppsala, Sweden).

Microimmunofluorescence testing for IgG, IgA, and IgM antibodies to purified *C pneumoniae* elementary bodies (strain 2023, ATCC VR 1356) was done with a Chlamydia MIF Kit (Diamedix, Miami, FL). The prevalence of *C pneumoniae*-specific IgG, IgA, and IgM antibodies was based on the presence of IgA at the titers of 1:16 and IgG at the titers of 1:64.

### Skin Prick Test

Allergen sensitization was measured by means of skin prick tests in the volar aspect of the forearm by means of a previously described technique.<sup>15</sup> The battery of allergens comprised *Dermatophagoides pteronyssinus*, cat hair, *Aspergillus fumigatus*, *Alternaria tenuis*, mixed grass, mixed tree pollen, *Parietaria officinalis*, egg, and cow milk (Soluprick; ALK-Abellø, Horsholm, Denmark). As positive and negative controls, we used histamine dihydrochloride (10 mg/mL) and diluent (50% glycerol and 50% physiologic saline). After 10 minutes, the wheal size was recorded in millimeters as the long axis and its perpendicular; the mean of these 2 measurements was calculated. A wheal  $\geq 3$  mm in size was considered a positive skin reaction.

### Statistical Analysis

Contingency tables ( $\chi^2$  test) were used for comparison of proportions. Cohen's  $\kappa$  with 95% confidence interval (CI) was calculated for measuring repeatability of nasal neutrophils. Reproducibility of 2 nasal volume measurements (intrasession) was determined by the "coefficient of repeatability," ie, 2 standard deviations, which is the measure interval including 95% of the differences between couples of measures.<sup>16</sup> The Student  $t$  test for independent samples was used to compare the changes in neutrophil counts and nasal volumes after the therapeutic cycle. All data were computed with the software program SPSS (SPSS, Inc, Chicago, IL).  $P < .05$  was considered statistically significant.

## RESULTS

### Epidemiologic Results

The mean value of nasal volume was  $4.16 \pm 0.94$   $\text{cm}^3$ ; the mean difference between duplicate intrasession measurements was  $0.006 \pm 0.369$   $\text{cm}^3$ , with a coefficient of repeatability of  $0.738$   $\text{cm}^3$ .

Readings of the cytospin preparations from nasal swab eluates in 183 children disclosed the percentages of cell populations reported in Table 1. No significant difference in percentages for any white

TABLE 2. Cell Populations by Clinical History

	Normal Subjects* (37.1%)	Chronic Rhinitis† (15.1%)	Chronic Cough‡ (14.5%)	Previous Asthma Diagnosis (15.1%)	Previous Eczema Diagnosis (15.1%)
Neutrophils	$12.9 \pm 13.3$ (80th percentile: 22%) (66th percentile: 14%)	$12.4 \pm 16.2$	$12.2 \pm 14.5$	$16.1 \pm 15.5$	$19.5 \pm 22.8$
Lymphocytes	$1.9 \pm 4.2$ (80th percentile: 2%) (66th percentile: 0%)	$1.2 \pm 2.3$	$0.46 \pm 1.2$	$1.9 \pm 3.5$	$2.24 \pm 5.31$
Squamous epithelial cells	$24.2 \pm 18.7$ (80th percentile: 39%) (66th percentile: 24%)	$23.4 \pm 25.5$	$20.0 \pm 13.3$	$16.2 \pm 18.9$	$25.5 \pm 28.4$
Ciliated epithelial cells	$61.0 \pm 20.2$ (80th percentile: 77%) (66th percentile: 67%)	$62.9 \pm 25.1$	$67.3 \pm 15.9$	$66.5 \pm 22.4$	$52.6 \pm 25.3$

Values are mean  $\pm$  standard deviation.

\* No chronic rhinitis, no chronic cough, no asthma, no eczema, no positive prick tests.

† Blocked or runny nose on most days of the previous 12 months.

‡ Cough on most days of the previous 12 months.

TABLE 3. Cell Populations by Laboratory Findings

	At Least 1 Positive Prick Test (39.7%)	Blood Eosinophils >3% (34.4%)	Total IgE >200 (20.7%)	eNO >17 ppb (24.7%)	FEV <sub>1</sub> <95% (18.6%)	Nasal Volume <3.4 cm <sup>3</sup> (18.6%)	Nasal Carriers of <i>C pneumoniae</i> Antigen (10.4%)	<i>C pneumoniae</i> Antibodies (IgG or IgA or IgM) (39.3%)
Neutrophils	13.8 ± 17.1	13.9 ± 17.7	17.7 ± 20.5	15.6 ± 17.3	15.3 ± 15.2	14.7 ± 13.6	13.7 ± 17.0	16.5 ± 18.2
Lymphocytes	1.8 ± 4.0	1.3 ± 2.9	1.6 ± 3.8	1.8 ± 4.0	1.1 ± 2.3	0.8 ± 2.2	1.5 ± 2.9	1.2 ± 3.3
Squamous epithelial cells	24.1 ± 23.2	22.9 ± 23.8	20.4 ± 20.0	17.0 ± 18.2	24.1 ± 18.9	19.8 ± 14.0	25.2 ± 18.9	22.1 ± 19.0
Ciliated epithelial cells	60.2 ± 25.1	61.8 ± 24.6	60.4 ± 23.4	65.6 ± 22.6	54.3 ± 20.2	65.2 ± 17.5	59.5 ± 19.2	60.3 ± 21.3

FEV<sub>1</sub> indicates forced expiratory volume in 1 second. Values are mean ± standard deviation.

cell population was found according to demographic subdivisions (boy versus girl, age 9 vs 10–11 years; Table 1). The repeat examination after 1 month in 31 children, 11 with normal neutrophil percentages (<14%) and 20 with elevated values (≥14%), overall disclosed no significant variations in the percentages of any white cell populations (Fig 1). In particular, in the group of 20 children who originally had high neutrophil percentages, 15 (75%) still had high values, whereas in the group of 11 children who originally had lower values, 9 (81.8%) had percentages that remained low. Overall, the examination yielded a reproducible result for neutrophil percentages in 24 of 31 children (77.4%; Cohen's  $\kappa$ : 0.535; 95% CI: 0.233–0.837;  $P = .002$ ). Percentages of nasal cell populations were not significantly different according to the questionnaire-derived clinical history (Table 2). Similarly, children with laboratory findings outside the normal range (including elevated atopic variables, reduced nasal volumes, or abnormal respiratory function variables, evidence of current or previous chlamydia nasal infection) and those with laboratory findings within the normal range had similar cell populations (Table 3).

#### Effect of Nasal Nebulization With Corticosteroids

The parents of 16 children in whom the second measurement of nasal cytology had demonstrated elevated neutrophilia were contacted by a physician of our team and their children were invited to complete a 15-day course of flunisolide nasal nebulization, in agreement with the family physician. There were 2 refusals. Of the remaining 14 children, 2 failed to comply fully with the instructions for the programmed course of therapy (200  $\mu$ g twice daily for 15 days) and were excluded from further analysis. Studies of nasal cytology and measurements of nasal volume before and after therapy showed that both variables improved significantly after flunisolide (Figs 2 and 3).

#### DISCUSSION

We determined nasal cytology in a sample of unselected children who were aged 9 to 11 years. Neither any cell population nor the neutrophils—considered the expression of nasal inflammation<sup>17,18</sup>—correlated with age, gender, history of nasal and lower respiratory tract symptoms, or atopic history. Several laboratory findings, including atopic and respiratory variables, also showed no correlation with nasal cytology. Nevertheless, when we repeated nasal cytology measurement 4 weeks later, the finding remained remarkably stable: approximately 80% of the children with increased or normal nasal neutrophils showed a persistent finding at the second measurement. This result induced us to suggest that the small group of the children who had persistent nasal neutrophilia should undergo a 15-day therapeutic trial with corticosteroids. Flunisolide therapy substantially reduced nasal neutrophils and increased nasal volume.

Our results therefore suggest that nasal neutrophilia, of intensity higher than 14% (66th percentile in our population), although unrelated to demo-

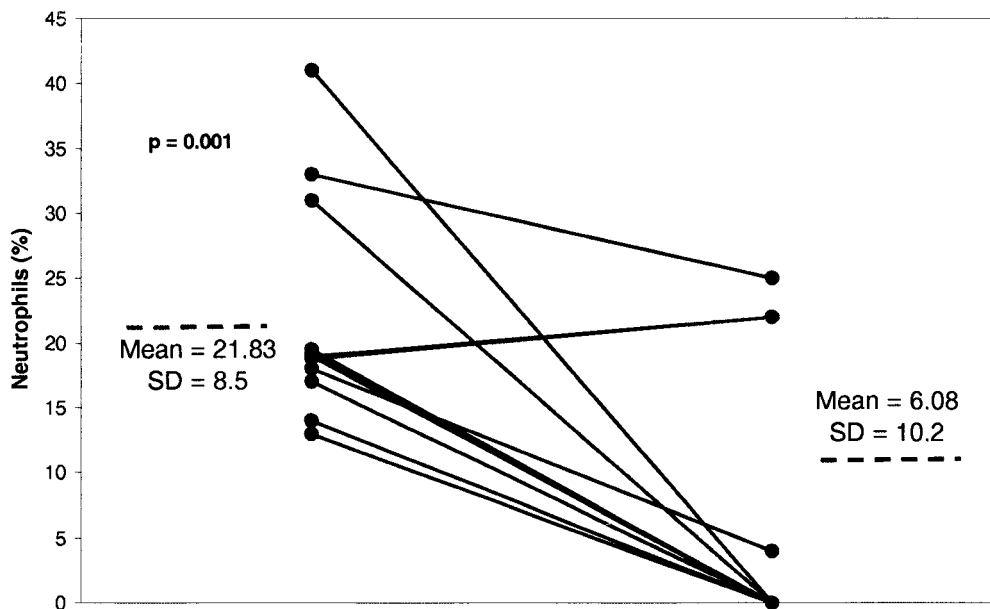


Fig 2. Neutrophil percentages in the 12 children who had >14% neutrophils in nasal white cell counts on 2 occasions and who underwent local nasal therapy with flunisolide.

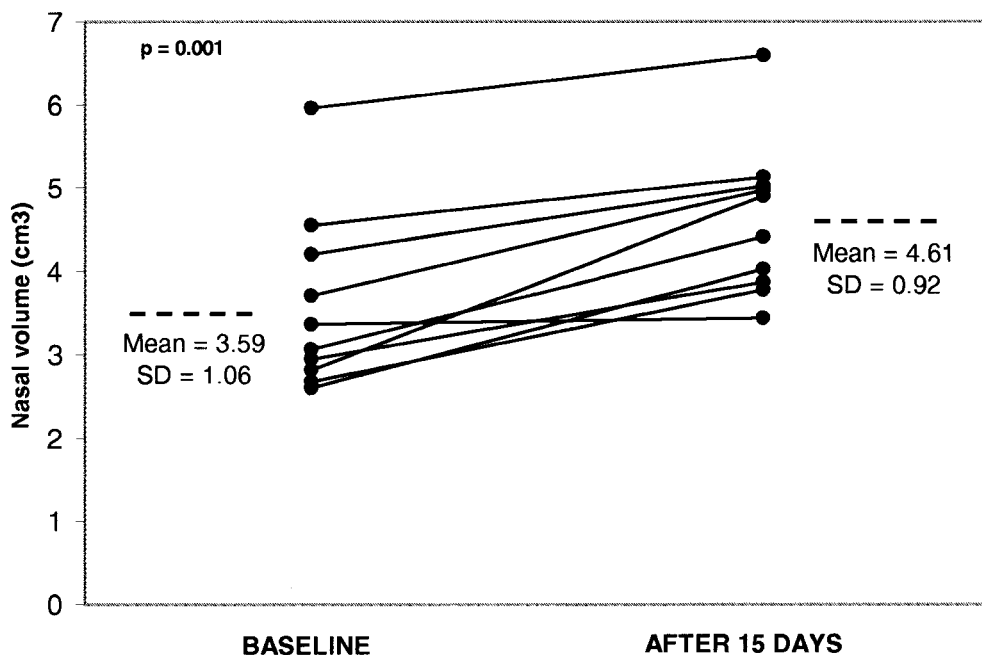


Fig 3. Nasal volumes in the 12 children who had >14% neutrophils in nasal white cell counts on 2 occasions and who underwent local nasal therapy with flunisolide.

graphic, respiratory, or atopic indices, tends to persist if untreated and is significantly reduced by corticosteroid treatment. Nasal neutrophilia (at a rate found in 33% of an unselected child population) represents a persistent inflammatory state that can be relieved only by a therapeutic intervention. However, because corticosteroid therapy decreased the neutrophil count and in parallel increased the volume of the nasal cavities, the inflammation presumably has cellular as well as clinical importance.

In evaluating the importance of this local inflammation, it is relevant to remember that we conducted our initial examination only in children without

acute symptoms and in a period of the year (November–January) when in our geographic setting seasonal allergic stimulation from pollen is unlikely and that from *D pteromyssimus* is not at its maximum. Nasal neutrophils (together with eosinophils and basophils) are known to increase in subjects with pollen sensitization after specific nasal challenge both in immediate and in late-phase reactions.<sup>18–20</sup> In nonallergic subjects, a challenge with irrelevant allergens or with pure diluent causes a significant increase in neutrophils but leaves eosinophils and basophils unchanged.<sup>2</sup>

Even if the role of neutrophils in nasal eluates

remains unclear, these cells seem to play a nonspecific role and to represent a reaction of the nasal mucosa after any kind of stimulus.<sup>2</sup> That our study population included a subgroup of children in whom neutrophils were persistently high suggests that either these children live in environments abnormally rich in irritative stimuli or that they have unusual nasal reactivity to normal environment stimulation (or a variable combination of these 2 components).

Numerous observations show that neutrophil activity characterizes several forms of acute or chronic nasal inflammation.<sup>21,22</sup> Neutrophil activity is influenced by several mediators that enhance their migration, adhesion, and degranulation.<sup>17</sup> Neutrophils can also be activated by their exposure to substances (eg, platelet adherence factor, leukotrienes, and cytokines) that enhance the effects of other agonists (priming effect).<sup>23,24</sup> The number of neutrophils in the nasal fluid of schoolchildren with allergic rhinitis correlate to the levels of interleukin-8.<sup>25</sup> The most intriguing finding of our investigation is that this neutrophilic nasal inflammation, present in a large proportion of our unselected population, is in no way predicted by or associated with the variables normally used for the clinical assessment of children.

Atopy is considered one of the most important cause of nasal symptoms in childhood: the incidence of allergic rhinitis has substantially increased in the past 30 years. In our population, however, we found no relationship of nasal cytology with the results of prick tests or total serum IgE or blood eosinophils or with a history of chronic rhinitis, asthma, eczema, or chronic cough.

Respiratory function tests such as spirometry and eNO concentrations also failed to predict neutrophilic nasal inflammation. This finding suggests that nasal inflammation could be a strictly local phenomenon and that the concept of "one airway, one disease" supported by the frequent association at the epidemiologic level between asthmatic and rhinitic symptoms has some exceptions at least at the cellular level.<sup>26,27</sup> The evidence of a nasal infection with *C pneumoniae*, a microorganism strongly associated with respiratory illness,<sup>9,28</sup> also did not predict nasal neutrophilia.

## CONCLUSION

The findings of this study suggest that no other investigation can substitute a study of nasal cellularity. Nonetheless, the practical meaning of altered nasal cellularity and the threshold of severity for therapeutic intervention needs to be clarified better by means of additional studies, including measurements of nasal cytokines in association with nasal neutrophils. However, this simple, noninvasive examination can already help to guide the clinician in assessing patient compliance and the effectiveness of nasal corticosteroid inhalation therapy.

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