

Nasal smear eosinophils and allergic rhinitis

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Abstract

Our aim was to find out the association between nasal smear eosinophil count and allergic rhinitis (AR) and to determine a cutoff value that is significant for a diagnosis of AR. We also wanted to determine whether this count is related to the predominant symptoms, duration, or type and severity of AR, or to the presence of coexisting asthma. We selected 100 patients with a clinical diagnosis of allergic rhinitis across all age groups and an equal number of age- and sex-matched controls for the study. Their nasal smear eosinophil counts were recorded in terms of the number of eosinophils per high-power field (HPF). All patients were then clinically assessed for asthma and underwent spirometry. The data were recorded and appropriate statistical analysis done. The difference in the mean eosinophil counts of patients with AR and controls was found to be statistically significant ($p = 0.000$). A nasal smear eosinophil count of >0.3 per HPF had a 100% specificity and a 100% positive predictive value for AR. Asthma was associated with allergic rhinitis in 40% of patients; an association was not found between nasal smear eosinophil count and the symptoms, duration, type, and severity of allergic rhinitis or coexistent asthma. We conclude that an eosinophil count of >0.3 per HPF in nasal smears is a highly specific criterion for the diagnosis of AR. However, nasal smear eosinophil counts are poor indicators of the degree, duration, or type of upper or associated lower airway inflammation due to allergy.

Introduction

Nasal smear eosinophilia is accepted as a useful finding in the diagnosis of allergic rhinitis (AR). Although

not pathognomonic, it is considered an additional confirmation of nasal allergy. However, a nasal smear eosinophil count is hardly ever performed in the diagnosis of AR because its validity and reliability are not clear. Therefore, it is pertinent to determine the utility of doing the eosinophil count of nasal smears in patients with AR.

Studies clearly document the coexistence of AR and asthma. Approximately 19 to 38% of patients with AR have concomitant asthma, and 30 to 80% of patients with asthma have AR.¹ The concept of “one airway, one disease” was highlighted in the Allergic Rhinitis and Its Impact on Asthma (ARIA) guidelines in 2001.² This concept had arisen because of the well-established link between the upper and lower airways. This relationship may represent a causal association or, more simply, may result from a common mucosal susceptibility. Therefore, it will be of interest to see the relationship between the nasal eosinophil count and the coexistence of asthma in patients with AR.

The objectives of the present study were: (1) to determine the association between nasal smear eosinophil count and AR, (2) to determine a cutoff value of the nasal smear eosinophil count that is significant for patients with AR, (3) to determine whether the eosinophil count in nasal secretions is related to the predominant symptoms, duration of symptoms, or the type and severity of AR, and (4) to determine whether the eosinophil count in nasal secretions is related to the presence of coexisting asthma in AR.

Patients and methods

One hundred patients with a clinical diagnosis of AR, confirmed by two qualified otorhinolaryngologists, attending the Otorhinolaryngology Outpatient Clinic at a tertiary referral health centre in West Bengal, India, were selected for the study. Patients' ages were across all age groups. AR was diagnosed clinically based on the presence of at least two of the four nasal symptoms of running, sneezing, itching, and stuffiness, along with a relevant history of triggering factors and the presence of pale and grayish nasal mucosa visible on anterior rhinoscopy.

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The patients were classified into *intermittent* and *persistent* groups and further subclassified into *mild* and *moderate-to-severe* groups. The details of the study and the involved procedures were explained to the patients, and written informed consent was obtained.

An equal number of age- and sex-matched subjects without any active nasal or pulmonary disease formed the control group.

The exclusion criteria were coexistent respiratory tract pathology other than AR or asthma, medical therapy for rhinitis or asthma, and the use of antihistamines, β_2 agonists, or systemic, intranasal, or inhaled corticosteroid therapy during the preceding month.

Nasal smears were collected from all participants by scraping the mucous membrane of the medial surface of both inferior turbinates with a sterile cotton swab soaked in saline. The secretions were then spread thinly onto two glass slides (one for each side) and air dried. The slides were numbered and sent to the pathologist for examination.

The smears were stained with May-Grünwald-Giemsa stain, and the eosinophil count was recorded in terms of the number of eosinophils per high power field (HPF). As the cells were unevenly distributed in smears, the eosinophils were counted in at least 50 HPFs, and the average count was recorded. This was done for smears from both the left and right nostrils; finally, an average of the two counts was calculated.

To prevent subjective judgment by the pathologist examining the smears, all the smears from patients and controls were coded before they were sent to the pathologist. All the smears were examined by the same observer.

The patients and controls were then assessed by the pulmonologist for asthma. They were clinically assessed and underwent spirometry using a Helios 401 spirometer (Recorders and Medicare Systems Pvt., Ltd.; Gurgaon,

India), which meets all the specifications of American Thoracic Society standardization.

Bronchodilator reversibility was assessed 15 minutes after administration of 400 μ g of inhaled salbutamol (albuterol). The spirometric values of forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC), and FEV₁/FVC were recorded before and after bronchodilator administration. An FEV₁/FVC ratio <0.7 was considered an airflow limitation or obstructive lung defect. The degree of reversibility in FEV₁ indicating a diagnosis of asthma is accepted as a 12% increase in the FEV₁ value and a 200-ml absolute increase in FEV₁ after bronchodilator administration [*Global Initiative for Asthma* (GINA) updated guidelines, 2012].³

The data were recorded in a predesigned, semistructured case record proforma, and appropriate statistical analysis was done using the Statistical Package for the Social Sciences, v. 17. The study was performed with the approval of the Institutional Ethics Committee.

Results

Of the 100 patients diagnosed with AR, there were 47 males and 53 females. The mean age of the study population was 30.21 ± 12.864 years (range: 8 to 64). The age and sex distribution of the patients and controls are described in table 1.

Predominant symptoms of AR were sneezing in 87 patients and rhinorrhea in 83 patients. Nasal congestion, or stuffiness, was the least common symptom, present in only 56 patients.

The mean eosinophil count was 5.23 ± 9.076 per HPF in patients with AR while in the control group it was 0.07 ± 0.231 per HPF. The difference was statistically significant ($p = 0.000$, with a 95% confidence interval [CI] of -0.963 to -3.360). The p values for different levels of eosinophil counts per HPF are provided in table 2.

Table 1. Age and sex distribution of patients and controls

Age group (yr)	Allergic rhinitis present			Allergic rhinitis absent			Total
	Male	Female	Total	Male	Female	Total	
0-14	5	4	9	5	4	9	18
15-24	13	15	28	13	15	28	56
25-34	13	17	30	13	17	30	60
35-44	9	9	18	9	9	18	36
45-54	3	6	9	3	6	9	18
55-64	4	2	6	4	2	6	12
Total	47	53	100	47	53	100	200

Table 2. Nasal smear eosinophil counts in patients and controls with their respective sensitivities and specificities

Eosinophil count	AR	non-AR	Total	p Value	Sensitivity (%)	Specificity (%)
≤0.1 per HPF	17	72	89	0	83	72
>0.1 per HPF	83	28	111			
≤0.2 per HPF	17	72	89	0	83	72
>0.2 per HPF	83	28	111			
≤0.3 per HPF	38	100	138	0	62	100
>0.3 per HPF	62	0	62			
≤0.5 per HPF	38	100	138	0	62	100
>0.5 per HPF	62	0	62			

Key: AR = allergic rhinitis; HPF = high-power field.

It should be noted that the differences in eosinophil counts are significant for all the three values of 0.1, 0.2 and 0.3 eosinophils per HPF ($p < 0.05$). However, there was no further change in sensitivity or specificity values above the value of 0.3 eosinophils per HPF. Therefore, that is the cutoff we used for identifying an elevated eosinophil count in nasal smears in our study. Thus, a nasal smear eosinophil count of >0.3 per HPF can be considered diagnostic for AR with 100% specificity and a 100% positive predictive value. The sensitivity with this criterion is 62%. The positive likelihood ratio reaches infinity, and the negative likelihood ratio is 0.38 with this eosinophil count value.

No association was found between an elevated nasal smear eosinophil count (>0.3 per HPF) or the mean eosinophil count and the symptoms of AR (i.e., sneezing, rhinorrhea, itching, and stuffiness). We also found no association between the duration of disease and the eosinophil count and failed to find a relationship between the eosinophil count and the type of AR (intermittent or persistent) or its severity (mild, moderate, severe). The results of the statistical analysis in this regard are detailed in table 3.

Asthma was associated with AR in 40% of the patients in our study. In 35% (14 of 40) of the asthma patients, obstructive lung defect was identified on spirometry; 71.4% (10 of 14) of the patients with obstructive lung defect showed significant reversibility of FEV1 to bronchodilator administration. However, no statisti-

cally significant relationship was found between the coexistence of asthma in patients with AR and the nasal smear eosinophil count, either using the >0.3 eosinophil count per HPF cutoff ($p = 0.933$) or using the patient's mean nasal smear eosinophil count ($p = 0.958$, 95% CI -3.677 to 3.877), even in the presence of obstructive lung defect ($p = 0.081$, 95% CI -0.565 to 9.162).

Discussion

AR is a localized immediate or type I hypersensitivity reaction of the nasal mucosa in response to stimulation by inhaled allergens. Such a reaction has two well-defined phases—an immediate, or initial, response and a late-phase reaction.⁴

The initial response is characterized by vasodilatation, vascular leakage, and glandular secretion and starts within 5 to 30 minutes of exposure to an allergen. It subsides by 60 minutes. Immunoglobulin E (IgE) antibodies and mast cells play a pivotal role in this phase.⁵

The late-phase reaction sets in 2 to 24 hrs after initial exposure and may last for several days. Infiltration of tissue by various inflammatory cells (including eosinophils and neutrophils) is the characteristic feature of this phase. Interleukin 5 (IL-5) secreted by T-helper type 2 cells is the most important chemokine, responsible for eosinophil recruitment. IL-5 also activates eosinophils that are important effectors of type I hypersensitivity reaction.^{6,7} The eosinophils liberate proteolytic enzymes and proteins such as major basic protein and eosinophil

Table 3. Nasal smear eosinophil counts vs. various parameters of allergic rhinitis (N = 100)

	Eosinophil ≤0.3 per HPF	Eosinophil >0.3 per HPF	Total	<i>p</i> Value
Symptom				
Rhinorrhea	33	50	83	0.423
Itchiness	26	48	74	0.319
Sneezing	34	53	87	0.788
Stuffiness	20	36	56	0.595
Type and severity				
Intermittent				
<i>Mild</i>	6	10	16	0.763
<i>Moderate-severe</i>	2	1	3	
Persistent				
<i>Mild</i>	20	37	57	0.576
<i>Moderate-severe</i>	10	14	24	
Duration of symptoms (mo)				
>3	36	59	95	1
>6	34	57	91	0.954
>12	34	51	85	0.327
>18	33	47	80	0.181
>24	25	32	57	0.165
Coexistent asthma present	15	25	40	0.933

cationic protein, which are toxic to epithelial cells. The activated eosinophils and other recruited cells sustain the inflammatory response without additional exposure to the allergen. The eosinophilic infiltration in the target tissue appears by 24 hrs of exposure to an allergen. Thus, all our patients were examined well after the eosinophilic response had fully developed.

Studies have used various methods to measure the nasal eosinophilic response. Some have reported the number of eosinophils seen per HPF,⁸ while others have measured it as the eosinophil count per 100 leukocytes.^{9,10} Still others have used cytopsin for preparation of nasal smears and reported the eosinophil infiltration in terms of the percentage of inflammatory cells in the smears.¹¹

In our study, we used the absolute eosinophil count per HPF to determine a cutoff value of the eosinophil count, to differentiate AR smears from non-AR smears. An important drawback of estimating the absolute eosinophil count in a smear is the uneven distribution of the nasal secretion specimen. To minimize that problem, we scrutinized at least 50 HPFs for every smear and then calculated the average eosinophil count from them.

AR was diagnosed clinically based on the presence of at least two of the four nasal symptoms of running, sneezing, itching, and stuffiness, along with a history of triggering factors and the presence of pale and grayish nasal mucosa visible on anterior rhinoscopy. The diagnosed patients were then classified according to the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines,¹² into *intermittent* and *persistent* groups and further subclassified into *mild* and *moderate-to-severe* groups. Skin prick tests were not done, as they have a positive predictive value of <50%. Our data showed a fairly uniform distribution of the different types and severity of AR across all age groups and both sexes.

The increase of eosinophils in nasal secretions in AR was first reported in 1927.¹³ Our study reaffirms the statistically significant difference of nasal smear eosinophil counts in AR and non-AR cases. The maximum eosinophil count per HPF seen in the control group was 0.3. Although we were getting *p* values of <0.05 for eosinophil counts of 0.1, 0.2 and 0.3 per HPF, we opted for 0.3 as the cutoff value for determining a high nasal

eosinophil count in AR as there was no further change in sensitivity or specificity values above this level.

This cutoff value gave us a specificity of 100% for diagnosing AR with a nasal smear eosinophil count; however, the sensitivity was only 62% with this value. Using a lower value of the eosinophil count as a cutoff value would increase the sensitivity but lower the specificity (table 2). Other studies also have found that using the eosinophil count as a diagnostic criterion for AR is more specific than sensitive.⁸

Opinion is divided on the utility of nasal smear eosinophil counts in the management of AR because its validity and reliability are unclear. It is therefore not a procedure done routinely on patients with AR. Numerous studies have attempted to clear the air on this matter. Klaew-songkram et al found a positive correlation between the nasal eosinophil count and the symptom of nasal stuffiness ($p=0.037$), although not with total nasal symptom scores.¹⁴

Ciprandi et al reported that the number of eosinophils in nasal scrapings highly correlated with nasal flow and spirometric values.¹⁵ In another study, the same author found a close relationship between the nasal eosinophil number and the percentage of predicted FEV1 in perennial AR patients with mild asthma.¹⁶

Lambrou et al, in their study of 34 patients and 10 controls, found that the inflammatory infiltration, characterized by the presence of eosinophils and CD4+ T cells (mature T-helper cells), was similar in the nasal mucosa in noninfectious rhinitis (including AR) irrespective of the presence of asthma or the allergic status of the patient.¹⁷ Chen et al, however, in their study of 160 patients with perennial AR, reported the nasal eosinophil count and serum allergen-specific IgE to be independent predictors of the severity of AR.¹⁸

One study of 37 pediatric patients found a converse correlation between nasal peak inspiratory flow rates and nasal eosinophil counts and a direct relation to nasal airflow resistance in patients who had AR with asthma (not in patients with AR only).¹⁰ However, the same study found no correlation between nasal symptoms and eosinophil counts. A 2012 study of 37 pediatric patients with AR by Yukselen et al found a significantly higher nasal eosinophil count in patients with AR and coexisting asthma compared to patients with AR alone.¹¹

Canbaz et al, in a study of 37 patients with persistent AR, found a lower nasal airflow and a higher nasal eosinophil count in those with coexistent bronchial hyper-responsiveness.¹⁹ According to the article, a cutoff point of 68% nasal eosinophils yielded a sensitivity of 100% (63.1 to 100) and a specificity of 58.6% (38.9 to 76.5) to determine the presence of bronchial hyper-responsiveness. The positive likelihood ratio for the value of eosinophils above the cutoff value was 2.42 (1.8 to 3.3).¹⁹ Sood, in a study of 40 patients with AR, found no association between

nasal eosinophil counts and the type of AR, type of nasal discharge, or color of nasal mucosa.¹⁰

In our study, we failed to find any statistical link between any of the four symptoms of AR and nasal eosinophil counts in either patients with AR only or those with coexistent asthma. This also included those asthma patients who had obstructive lung defect and significant bronchodilator reversibility. Our study also found no relation between the eosinophil count and either the type (intermittent or persistent) or severity (mild or moderate-to-severe) of the rhinitis. Nor did we find any relationship between the nasal eosinophil response and the duration of the disease.

Our study negates any relationship between nasal inflammation and upper or lower airway functions. What we did find was that the eosinophil count in patients with AR was highly specific, with a very high positive likelihood ratio value but low sensitivity and a low negative likelihood ratio value. This implies that this criterion is not good for ruling out the disease but is very strong for confirming it.

The diagnosis of asthma was done clinically by the pulmonologist, based on the presence of characteristic symptoms such as episodic breathlessness, wheezing, cough, and chest tightness.²⁰ Measurement of lung function and the demonstration of reversibility of lung function abnormalities greatly enhance the diagnostic confidence for a diagnosis of asthma.

The association of AR with asthma is well known and documented. Studies have found 10 to 40% of patients with AR to also have asthma.²¹ Conversely, there is a coexistence of AR in as many as 78% of patients with asthma,²² which contrasts with the prevalence in the general population of 20%. In our study asthma was associated with AR in 40% of patients; 35% of the asthma patients had obstructive lung defect on spirometry, indicated by an FEV1/FVC ratio of <0.7 . Furthermore, 71.4% of these patients showed significant reversibility of FEV1 on bronchodilator administration indicated by a 12% increase in the value of FEV1 and a 200-ml absolute increase in FEV1 after bronchodilator administration.

Conclusion

Our study proves that an eosinophil count of >0.3 per HPF in nasal smears is a highly specific criterion for the diagnosis of AR with a 100% positive predictive value. Its sensitivity is somewhat lower at 62%. There is no association between any of the four symptoms, duration, or the type or severity of AR with nasal smear eosinophil counts. There is also no statistical relationship between nasal eosinophil counts and the coexistence of asthma. Therefore, we can say that nasal smear eosinophil counts are poor indicators of the degree, duration, or type of upper or associated lower airway inflammation due to allergy.

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