ORIGINAL ARTICLE

# Sputum Inflammatory Cells and their Impact on Asthma Control in Adults: A Prospective Study

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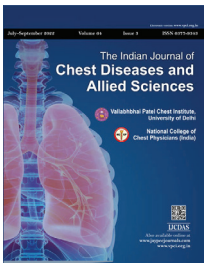


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## Abstract

**Background:** Asthma is broadly categorized as eosinophilic or noneosinophilic. Noneosinophilic asthma (NEA) can be paucigranulocytic asthma (PGA), mixed granulocytic asthma (MGA), or neutrophilic asthma (NeuA). A relationship between the cytological type of inflammation and response to treatment with inhaled corticosteroids (ICS) in asthma has been of great interest. The objective of the current study was to predict the control of asthma according to sputum inflammatory cells.

**Materials and methods:** A total of 58 patients were evaluated. Sputum was induced and sent for cytological examination. Patients were prescribed controller and reliever medications as per the GINA guidelines. Accordingly, subjects were divided into eosinophilic, neutrophilic, mixed granulocytic, and paucigranulocytic asthma. The response to treatment was classified as poorly controlled based on ACT score.

**Results:** Out of 58 patients, eosinophilic asthma (EA) was 24% and noneosinophilic 76% (NeuA 17%, MGA 23%, and PGA 36%). After treatment, 14 (24.13%) patients were found poorly controlled. Poor control was in 5.17% among EA and 18.97% in NEA phenotypes. Poor control was significantly higher in females, NeuA, and MGA. Peripheral eosinophilia affects control of asthma adversely.

**Conclusion:** Pretreatment sputum analysis can predict the asthma control and steroid responsiveness. Mixed granulocytic asthma and NeuA are difficult to control, and PGA is the best responder.

**Keywords:** Asthma, Eosinophilic, Mixed granulocytes, Noneosinophilic, Phenotypes, Paucigraulocytes.

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## Abbreviations used in this Article

NEA = Noneosinophilic asthma; PGA = Paucigranulocytic asthma; MGA = Mixed granulocytic asthma; NeuA = Neutrophilic asthma; ICS = Inhaled corticosteroids; EA = Eosinophilic asthma; DTT = Dithiothreitol; ACT = Asthma control test.

## Introduction

Asthma is a disease of wide heterogeneity and variability. Although the majority of asthmatics can be controlled on standard therapy, a significant number of patients remain uncontrolled. Severe asthmatics are often referred to as corticosteroid-dependent, refractory, or corticosteroid-insensitive asthmatics. Induced sputum can be used to monitor the presence and severity of airway inflammation in asthma.[1] Previous papers established a relationship between cytological type of inflammation and the response to treatment with ICS in asthma. Sputum eosinophil levels of greater than 2–3% have been used to define EA.[2] Noneosinophilic asthma with defining values of sputum neutrophilia ranging from as low as 40% to as high as >76% can be further differentiated into paucigranulocytic, mixed granulocytic, or neutrophilic asthma.[3,4] While in eosinophilic asthma, the pathobiologic pathway is well-understood to be a Th2-mediated, neutrophilic asthma, usually a non-type-2 inflammatory process, involves type-1 and/or type-17 inflammations and is resistant to corticosteroids.[4,5]

Technical requirements for sputum processing and cell counting may limit the feasibility of using sputum eosinophil counts in all clinical centers. Raised absolute eosinophil counts are reported as a predictor of response to treatment, but blood eosinophil counts do not necessarily reflect sputum eosinophil counts also. Studies of the anti-IL-5 mepolizumab required only peripheral blood eosinophilia greater than or equal to 150 cells/µl at screening or 300 cells/µL in the preceding year.[6,7] Almost all patients with more than 400 cells/µl can be expected to have significant sputum eosinophils.[8] Paucigranulocytic asthma is an asthma phenotype with no evidence of elevated numbers of eosinophils or neutrophils in sputum or blood, is known as a "benign" asthma phenotype with better lung function and less frequency of severe refractory asthma,[9] whereas the mixed cellular phenotype was reported to be the most severe phenotype among the four groups.[10] Occasionally, NeuA together with PGA is referred to as "noneosinophilic asthma".[3]

The identification of the type of airway inflammation for making the optimal treatment decision can be very helpful. The

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current study is done at a tertiary care center in New Delhi for the inflammatory cell characteristics and control of asthma as per asthma control test (ACT) score.

## Materials and Methods

This was a prospective observational study, conducted at the Allergy and Immunotherapy Clinic of National Institute of TB and Respiratory Diseases, New Delhi, for about 22 months. Ethical clearance was taken from the Institutional Ethical Committee. All asthmatic patients between 18 and 65 years of age referred to our center were evaluated using a detailed questionnaire, including history and physical examination. Asthmatics excluded were: pregnant and lactating women, smokers, patients with generalized skin disease, cardiac, neuromuscular diseases, COPD, and any other respiratory diseases like acute and chronic infections, bronchiectasis, etc. A total of 74 patients were enrolled after inclusion and exclusion criteria.

They further underwent a complete hemogram, sputum for AFB to rule out TB, and spirometry on the first visit. For sputum cytology, sputum induction was performed by using hypertonic saline (NaCl 3%) aerosolized by nebulizer for three periods of 5 minutes. The patient was made to cough sputum into a plastic container. Fresh sputum sample was homogenized with dithiothreitol (DTT). The sample was agitated and after keeping at room temperature for 30–60 minutes, was centrifuged, slide prepared, and then stained by hematoxylin and eosin and Giemsa stains. The slide was examined by microscope, and results were given as predominantly eosinophilic (sputum eosinophils >2%), neutrophilic (sputum neutrophils >40%), paucigranulocytic (sputum eosinophils <2% and neutrophils <40%), and mixed granulocyte (sputum eosinophils >2% and neutrophils >40%). Patients were prescribed controller and reliever as per the GINA guidelines stepwise management up to Steps 4 and 5, i.e,. high dose of ICS/LABA medications for 4–6 weeks. Accordingly, subjects were divided into controlled and poorly controlled categories based on the ACT score. Asthma control test score >19 was taken as controlled and <19 as poorly controlled.

### Statistical Analysis

Data were entered in Microsoft Excel and analyzed using SPSS 22. For descriptive statistics, quantitative variables were described by mean and standard deviation. Qualitative variables were described by percentage distribution. For inferential statistics between groups, comparison of qualitative variables was analyzed by Chi-square test and quantitative variables were compared by student *t*-test and proportion *z*-test. *P*-value of less than 0.05 was considered as level of significance.

## Results

Out of a total 74 asthma patients, 2 were found to have ABPA, 8 were found noncompliant to the treatment, and 6 were not using proper technique and hence, were excluded from the study. A total of 58 patients were analyzed, which includes 34 males and 24 females. Males had significantly better asthma control than females (66% vs 34%). The mean duration of illness in both the groups is statistically insignificant. The mean age at the time of presentation was 31 years in poorly controlled asthma as compared with 35 years in the controlled group, which is statistically significant (Table 1).

In 58 patients, 14 (24%) were EA and 44 (76%) were NEA. Among the NEA, 10 (17%), 13 (23%), and 21 (36%) were NeuA, MGA, and PGA, respectively. Hence, PGA patients are maximum followed by EA (Table 2).

**Table 1:** Demographic profile of asthmatics

| *Profiles* | *Controlled* | *Poorly controlled* | *p-value* |
|---|---|---|---|
| Male | 29 (65.9%) | 5 (35.7%) | |
| Female | 15 (34.1%) | 9 (64.3%) | 0.046 |
| Total | 44 (100%) | 14 (100%) | |
| Age at presentation mean (years) | 35.4 | 30.54 | 0.04515 |
| Age at onset of symptoms Mean (years) | 26.07 | 25.5 | 0.44 |
| Duration of illness Mean (years) | 7.51 | 5.22 | 0.09 |

**Table 2:** Sputum inflammatory cells profile of study patients

| *Sputum cytology* | *n (%)* |
|---|---|
| A. Eosinophilic | 14 (24%) |
| B. Noneosinophilic | 44 (76%) |
| *1. Neutrophilic* | *10 (17%)* |
| *2. Mixed granular* | *13 (23%)* |
| *3. Paucigranular* | *21 (36%)* |
| Total | 58 (100%) |

After ICS/LABA treatment as per GINA guidelines, out of 58 patients, 14 (24.13%) patients remained poorly controlled. The individual phenotypes showed great variation. While poor control among EA was in 3 (5.17%), it was 11 (18.97%) in NEA phenotypes (Table 3). Overall, among 58 asthmatics enrolled, poor control was seen in 3 (5.17%) EA, 3 (5.17%) NeuA, 6 (10.34%) MGA, and 2 (3.45%) PGA patients implying that PGA phenotypes were associated with the best control of asthma and MGA with worst control (Table 3).

Peripheral eosinophilia (>500 cells/μL) was present in 10 (17.24%) of poorly controlled asthmatic groups as compared to 4 (6.9%) in patients with eosinophils <500 cells/μL (Table 4). Hence, peripheral eosinophilia has been found as a strong risk factor for poor control of asthma in our study.

## Discussion

In our study, 76% of patients irrespective of type of inflammation were well controlled, and 24% remained poorly controlled even after full compliance to treatment. In one study, control of asthma was 58–76% in different groups of Asian patients in the initial phase of treatment for 12 weeks though it increased on the continuation of treatment for a longer time.[11] Another study reported asthma control to be 24.8% after 1 month of ICS/LABA, and this proportion gradually increased to 67.7% at 6th-month follow-up.[12]

The proportion of EA and NEA is approximately equal in different studies,[13–16] but sputum eosinophilia (>2% eosinophils) in only 36% of subjects with asthma not on ICS and in 17% of ICS-treated subjects with asthma was reported.[17] In our study, also 24% of patients were eosinophilic and 76% were noneosinophilic. In fact, many patients at the time of reaching the tertiary center

**Table 3:** Asthma control in different phenotypes

| *Phenotype* *n = 58* | *Eosinophilic (EA) = 14 (24.13%)* | *Noneosinophilic (NEA) = 44 (75.86%)* | | | |
|---|---|---|---|---|---|
| | | *NeuA* | *MGA* | *PGA* | *Total* |
| Controlled *n* = 44 (75.86%) | 11 (18.9%) | 7 (12%) | 7 (12%) | 19 (32.76%) | 33 (56.9%) |
| Poorly controlled, *n* = 14 (24.14%) | **3 (5.17%)** | 3 (5.17%) | 6 (10.34%) | 2 (3.45%) | **11 (18.97%)** |

**Table 4:** Peripheral eosinophilia and asthma control

| *Peripheral eosinophils* | *Controlled = 44* | *Poorly controlled = 14* | *p-value* |
|---|---|---|---|
| <500 cells/µl *n* = 14 (24.14%) | 10 (17.24%) | 4 (6.9%) | |
| >500 cells/µl *n* = 44 (75.86%) | 34 (58.62%) | 10 (17.24%) | 0.000 |
| Total = 58 | 44 (75.86%) | 14 (24.13%) | |

like ours had already received the ICS irregularly. The role of ICS in affecting the cellular pattern is controversial. Several studies have shown that ICS therapy decreases sputum eosinophils,[16,18] but earlier authors did not find any statistically significant difference in the sputum cellularity in spite of treatment with ICS.[19] They reported that 42% of patients were eosinophilic, 16% neutrophilic, 4% mixed granulocytic, and 38% paucigranulocytic. In our study, 24%, 17%, 23%, and 36% were eosinophilic, neutrophilic, mixed, and paucigranular, respectively. The difference could be because of the criteria adopted (eosinophil >3% and neutrophil >76%) in the above study and also due to the use of inhaled/oral steroids irregularly by our patients.

In our study, the poor control of NEA (≈19%) is almost 4 times more as compared to EA (5.17%). Significantly reduced response was reported to inhaled mometasone in patients with non-eosinophilic asthma compared with eosinophilic asthma,[20] consistent with the findings of previous uncontrolled studies. However, an equal response was found to ICS in both EA and NEA.[21]

In this study, PGA and EA are better controlled than mixed and neutrophilic cellular inflammation which is similar to the findings of earlier studies.[19] Whether PGA is associated with asthma severity is still unclear. Paucigranulocytic asthma most likely represents a group of patients with a good response to treatment rather than a real asthma phenotype.[9] However, uncontrolled paucigranulocytic patients despite optimal treatment represent an asthmatic population that requires further study. Paucigranulocytic asthma patients expressed lower levels of inflammatory markers in exhaled air and sputum, and severe refractory asthma occurred less frequently in PGA than in EA and MGA.[9]

Blood eosinophils do not always correlate well in approximately 40% of patients,[22] but they can serve as a useful marker to assess patient's phenotype as they are significantly more likely to have pretreatment elevated eosinophil counts with moderate-to-severe asthma than those with mild disease.[23] In our study, the control of asthma was poor in patients with peripheral eosinophils >500 cells/µl as compared to <500 cells/µl (17.24% vs 6.9%). In a cohort of 54 patients, no correlation was found between sputum and blood eosinophil counts.[24] But, a group of asthmatic subjects with very few sputum eosinophils and a poorer response to corticosteroids was identified.[15]

## Conclusion

Though the sputum inflammatory cell analysis is a cumbersome procedure, it can help in determining the treatment response to ICS. Four phenotypes viz., eosinophilic, neutrophilic, mixed, and paucigranulocytic asthma are identified on the basis of predominance of eosinophils or neutrophils. As such, NEA is the worst controlled but PGA shows a very good control. Mixed granulocytic asthma and NeuA are difficult to control as compared to EA. In our study, peripheral eosinophilia is a marker of poor asthma control.

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