

Letter to the Editor

Variability of sputum inflammatory cells in asthmatic patients receiving corticosteroid therapy: A prospective study using multiple samples*To the Editor:*

Sputum examination in patients with asthma is a noninvasive method for assessment of airway inflammation.¹ Typically, expectorated or induced sputum in patients with asthma is rich in eosinophils, but variable inflammatory cell profiles have been reported,² prompting a classification with 4 phenotypes: eosinophilic, neutrophilic, mixed, and paucigranulocytic. These profiles might change with time,³ but the extent to which inflammatory patterns are conserved has not been extensively explored.

The current study assesses the characteristics and stability of airway inflammation in subjects with moderate and severe asthma. Induced sputum was analyzed monthly for patients with severe disease and every 3 months for patients with moderate disease over 1 year. Respiratory physicians at 2 tertiary centers identified 37 patients who met the American Thoracic Society criteria for severe asthma.⁴ The subjects were participating in the Severe Asthma Program, which has been the subject of several previous publications.⁵ Physicians also recruited 24 age- and sex-matched patients with moderate asthma.

The subjects were taking 176 to 880 $\mu\text{g/d}$ fluticasone (or equivalent) with or without β -agonists, a leukotriene receptor antagonist, or theophylline; used 2 or fewer steroid bursts in the previous year and none in the previous 3 months with less than 30 days of oral steroids in the previous 12 months; had an FEV₁ of 70% or greater of predicted value and 90% or greater of personal best in previous 2 years; and had 1 or more unscheduled visits for asthma in the previous 12 months.

The study was approved by the research ethics committee of each participating center. All subjects provided written informed consent, and their clinical characteristics are found in Table I.

The study procedure was as previously described.⁵ Subjects with severe asthma underwent a 1-month run-in period to assess compliance. They were provided with metered-dose inhalers containing 250 μg of fluticasone and 25 μg of salmeterol per inhalation and took 2 inhalations twice daily. An electronic device (MDILog; Medtrac Technologies, Lakewood, Colo) was attached to monitor use. Only subjects who inhaled 4 or more inhalations per day on 70% or more of the days were included. The subjects completed the Asthma Control Questionnaire.⁶ Sputum was induced by using aerosolized 0.9% NaCl, followed by 3% to 5% NaCl through an ultrasonic nebulizer.¹ Subjects were asked to blow the nose, rinse the mouth, and swallow water to minimize contamination by postnasal drip and saliva. They then coughed into a sterile container, and expectorated sputum was processed within 2 hours. Cytospin preparations were prepared on poly-L-lysine-coated slides and fixed for 7 minutes with acetone/methanol (60:40) followed by air drying.⁵ Sputum induction was successful in approximately 74% of visits. A sputum eosinophil count of 3% or greater was established as "eosinophilic inflammation"; sputum neutrophil counts of 65% or greater were considered "neutrophilic inflammation."⁷

ANOVA and the *post hoc* Student *t* test with a Bonferroni correction were used. The Mann-Whitney *U* test was used for

TABLE I. Clinical characteristics

	Severe asthma* (n = 37)	Moderate asthma* (n = 24)	P value
Age (y)	47.6 $\pm$ 11.76	51.6 $\pm$ 10.2	NS
Sex (M/F)	21/16	14/10	NS
FEV ₁ (% predicted) [†]	66.6 $\pm$ 21.9	85.0 $\pm$ 12.2	.0003
Prebronchodilator FEV ₁ /FVC ratio	65.7 $\pm$ 11.9	73.1 $\pm$ 5.5	.0058
BMI (kg/m ²)	28.2 $\pm$ 6.1	27.2 $\pm$ 6.3	NS
Subjects with atopy [‡]	31 (84%)	21 (88%)	NS
Dose of inhaled corticosteroid ($\mu\text{g/d}$) [*]	1,275 $\pm$ 324	618 $\pm$ 325	<.0001
Asthma exacerbation per subject per visit in the previous year	0.1 $\pm$ 0.1	0.03 $\pm$ 0.07	.007
Exsmokers	14 (38%)	7 (29%)	NS
Symptom score	1.8 $\pm$ 1.0	1.0 $\pm$ 0.6	.001
Subjects taking oral steroids	25 (68%)	3 (13%)	<.0001
Subjects taking long-acting β -agonists [†]	36 (97%)	17 (71%)	.0036

Values are presented as means $\pm$ SDs or numbers.

BMI, Body mass index; F, female; FVC, forced vital capacity; M, male; NS, not significant.

*All subjects were taking inhaled corticosteroids.

[†]Means $\pm$ SDs based on observations for all visits.

[‡]Subjects underwent skin testing to 13 aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, horse, ragweed, grass mix, tree mix, weed mix, feather mix, and *Alternaria*, *Aspergillus*, and *Cladosporium* species). Histamine (10 mg/mL) and its diluent were used as positive and negative controls, respectively. A positive test result was set as the larger of 2 perpendicular diameters of greater than 3 mm of the control.

nonparametric values. The Pearson coefficient was used to test correlations. Overall predicted trajectory was estimated by using linear mixed models. Data were analyzed with SAS, version 9.1, software (SAS Institute, Inc, Cary, NC).

Of all 61 subjects (37 with severe asthma and 24 with moderate asthma), sputum eosinophil counts of 3% or greater were noted in 28 (46%) subjects on the first visit, which persisted only in 9 (32%) subjects on subsequent visits. Sputum eosinophil counts of less than 3% were noted in 33 (54%) subjects on the first visit, which persisted in 13 (39%) subjects. Sputum neutrophil counts of 65% or greater were noted in 14 (23%) subjects on the first visit, which persisted in 2 (14%) subjects. In the 37 subjects with severe asthma, 19 (51%) subjects had sputum eosinophil counts of 3% or greater on initial visits, which persisted in 6 (32%) subjects (see Fig E1 in this article's Online Repository at www.jacionline.org). Eighteen (49%) subjects had sputum eosinophil counts of less than 3% on initial visits, which persisted in 7 (35%) subjects (see Fig E1). Ten (27%) subjects had sputum neutrophil counts of 65% or greater on initial visits, which persisted in 2 (20%) subjects (see Fig E2 in this article's Online Repository at www.jacionline.org). Variations in subjects with sputum neutrophil counts of less than 65% on initial visits are also depicted in Fig E2. In the 24 subjects with moderate asthma, 9 (38%) subjects had sputum eosinophil counts of 3% or greater on initial visits, which persisted in 3 (33%) subjects (see Fig E3 in this article's Online Repository at www.jacionline.org). Fifteen (62%) subjects had sputum eosinophil counts of less than 3% on initial

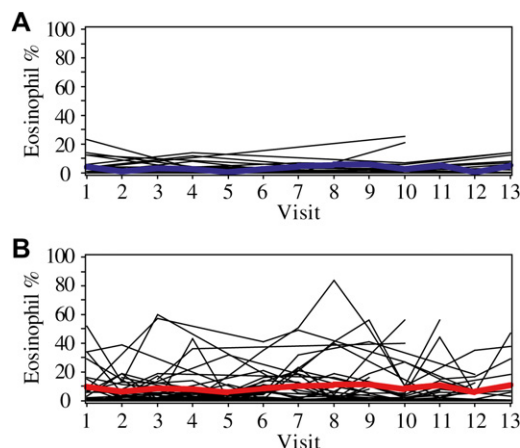


FIG 1. Overall trajectory of percentage eosinophils for subjects with moderate (A) and severe (B) asthma.

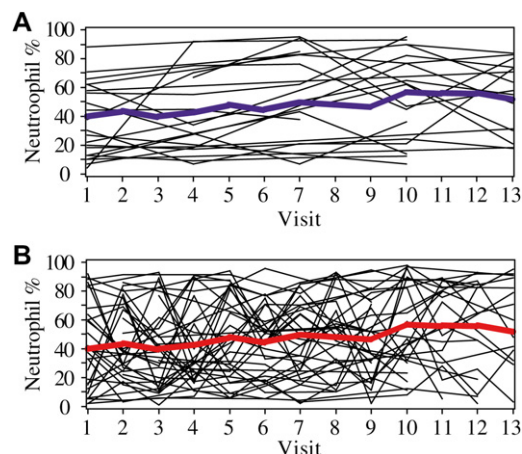


FIG 2. Overall trajectory of percentage neutrophils for subjects with moderate (A) and severe (B) asthma.

TABLE II. Subjects' coefficients of variation for eosinophils and neutrophils

	Eosinophils (%)	Neutrophils (%)	Absolute eosinophil count	Absolute neutrophil count
Subjects with severe asthma (n = 37)	125 ± 71 (44-346)	62 ± 31 (9-152)	147 ± 66 (52-350)	130 ± 41 (71-216)
Subjects with moderate asthma (n = 24)	121 ± 50 (42-224)	42 ± 28 (5-88)	139 ± 48 (60-224)	104 ± 42 (5-171)

The values are presented as means ± SDs (ranges) of the coefficient of variation ($SD \div \text{mean} \times 100$) for percentages and absolute counts (set as the number of cells $\times 10^6/\text{g}$ sputum).

visits, which persisted in 6 (40%) subjects (see Fig E3). Four (16%) subjects had sputum neutrophil counts of 65% or greater on initial visits, which persisted in 1 (4%) patient (see Fig E4 in this article's Online Repository at www.jacionline.org). Variations in subjects with sputum neutrophil counts of less than 65% on initial visits are also depicted in Fig E4.

Sputum eosinophil and neutrophil counts were highly variable in both groups (Figs 1 and 2, respectively), even after adjusting for body mass index, sex, and steroid use. However, the coefficient of variation was lower for neutrophil counts (Table II), indicating a lesser variability. There was a poor correlation between variability in airway inflammation and FEV₁, FEV₁/forced vital capacity ratio, asthma score, or exacerbation.

Eosinophilia was present in 24% of the 573 visits, neutrophilia in 18%, and both in 6%. Exacerbations represented 48 (9%) visits; 62% were neutrophilic, 33% were eosinophilic, and 5% were both. For the scheduled visits (without exacerbations), eosinophilia was present in 33%, neutrophilia in 24%, and both in 9%. One subject with severe asthma and 2 subjects with moderate asthma had low eosinophil and neutrophil counts on all visits. There were only 3 exacerbations in the moderate asthma group. The number of visits with sputum neutrophilia correlated with asthma score (averaged over 12 months: $R = 0.38$, $P = .009$), FEV₁ ($R = -0.43$, $P = .004$), and FEV₁/forced vital capacity ratio ($R = -0.32$, $P = .03$). The number of visits with sputum eosinophilia correlated only with FEV₁ ($R = -0.32$, $P = .03$).

A cross-sectional study by Wenzel et al⁸ demonstrated increased airway neutrophil counts in subjects with severe asthma. Here we show that this inflammatory phenotype is unstable in the majority of subjects studied longitudinally. A significant correlation between sputum eosinophilia ($\geq 2\%$) and postbronchodilator FEV₁ was reported.⁹ Our study, similar to that of Simpson,² did

not reveal a significant correlation between sputum eosinophil counts of 3% or greater and clinical severity.

In summary, this prospective longitudinal study assessed airway inflammation in subjects with moderate and severe asthma. All subjects were taking corticosteroids, although those with severe disease received higher doses. With this enrollment, the confounding effects of steroids on sputum infiltrates are minimized. However, we cannot exclude that oral/inhaled corticosteroids, compliance, or other intervention have influenced our results. Each patient was investigated over a 1-year period on a regular basis and during exacerbations. Our findings demonstrate the sputum inflammatory profile commonly changes in subjects with moderate and severe asthma. Stable phenotypes were noted in only one third of subjects.

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REFERENCES

1. Pin I, Gibson PG, Kolendowicz R, Girgis-Gabardo A, Denburg JA, Hargreave FE, et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax* 1992;47:25-9.
2. Simpson JL. Inflammatory subtypes in asthma: Assessment and identification using induced sputum. *Respirology* 2006;11:54-61.
3. D'Silva L, Cook RJ, Allen CJ, Hargreave FE, Parameswaran K. Changing pattern of sputum cell counts during successive exacerbations of airway disease. *Respir Med* 2007;101:2217-20.
4. Proceedings of the ATS Workshop on Refractory Asthma. Current understanding, recommendations, and unanswered questions. *Am J Respir Crit Care Med* 2000; 162:2341-51.
5. Shannon J, Ernst P, Yamauchi Y, Olivenstein R, Lemiere C, Foley S, et al. Differences in airway cytokine profile in severe asthma compared to moderate asthma. *Chest* 2008;133:420-6.
6. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999;14: 902-7.
7. Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears MR, Hargreave FE. Induced sputum cell counts in healthy adults. *Am J Respir Crit Care Med* 2000; 161:475-8.
8. Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999;160:1001-8.
9. Brinke A, Zwinderman AH, Sterk PJ, Rabe KF, Bel EH. Factors associated with persistent airflow limitation in severe asthma. *Am J Respir Crit Care Med* 2001;164: 744-8.

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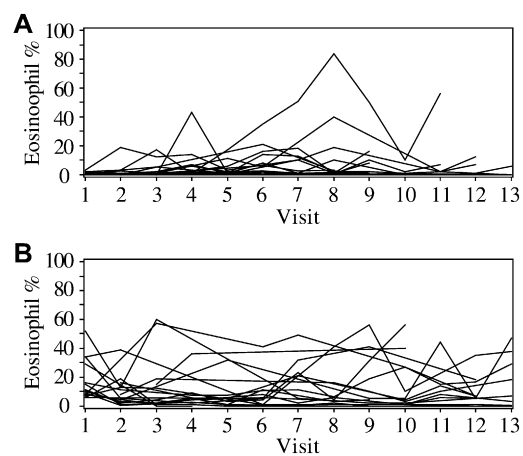


FIG E1. Overall trajectory of percentage eosinophils for subjects with severe asthma and sputum eosinophil counts of less than 3% (**A**) and 3% or greater (**B**) on the initial visits.

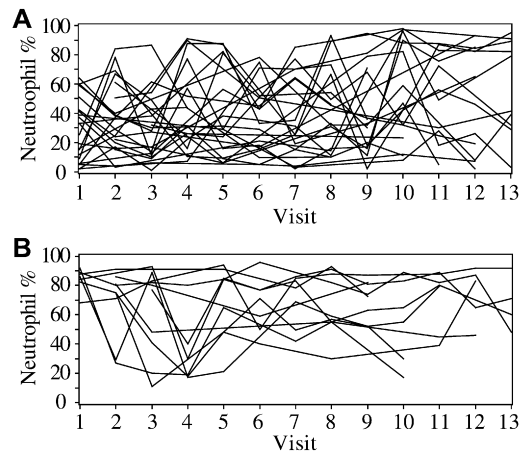


FIG E2. Overall trajectory of percentage neutrophils for subjects with severe asthma and sputum neutrophil counts of less than 65% (**A**) and 65% or greater (**B**) on the initial visits.

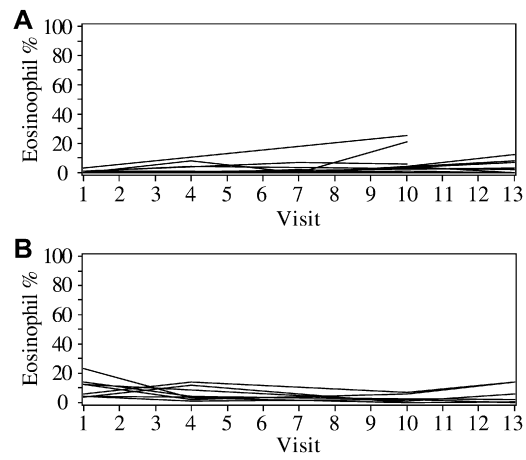


FIG E3. Overall trajectory of percentage eosinophils for subjects with moderate asthma and sputum eosinophil counts of less than 3% (**A**) and 3% or greater (**B**) on the initial visits.

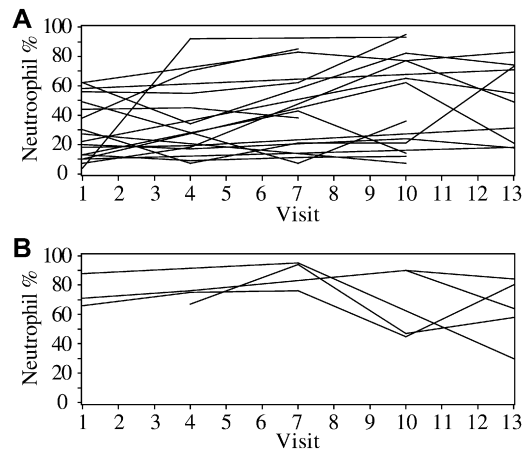


FIG E4. Overall trajectory of percentage neutrophils for subjects with moderate asthma and sputum neutrophil counts of less than 65% (**A**) and 65% or greater (**B**) on the initial visits.