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ORIGINAL ARTICLE

Mixed Sputum Granulocyte Longitudinal Impact on Lung Function in the Severe Asthma Research Program

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Abstract

Rationale: Some reports indicate longitudinal variability in sputum differential cell counts, whereas others describe stability. Highly variable sputum eosinophil percentages are associated with greater lung function loss than persistently elevated eosinophil percentages, but elevated neutrophils are linked to more severe asthma.

Objectives: To examine sputum granulocyte stability or variability longitudinally and associations with important clinical characteristics.

Methods: The SARP III (Severe Asthma Research Program III) cohort underwent comprehensive phenotype characterization at baseline and annually over 3 years. Adult subjects with acceptable sputum levels were assigned to one of three longitudinal sputum groups: eosinophils predominantly <2%, eosinophils predominantly ⩾2%, or highly variable eosinophil percentages (>2 SDs determined from independent, repeated baseline eosinophil percentages). Subjects were similarly assigned to one of three longitudinal neutrophil groups with a 50% cut point.

Measurements and Main Results: The group with predominantly <2% sputum eosinophils had the highest lung function (prebronchodilator FEV_1% predicted, $P < 0.01$; FEV_1/FVC ratio, $P < 0.001$) at baseline and throughout 3 years compared with other eosinophil groups. Healthcare use did not differ, although the highly variable eosinophil group reported more asthma exacerbations at Year 3. Longitudinal neutrophil groups showed few differences. However, a combination of predominantly ⩾2% eosinophil and ⩾50% neutrophil groups resulted in the lowest prebronchodilator FEV_1% predicted ($P = 0.049$) compared with the combination with predominantly <2% eosinophils and<50% neutrophils.

Conclusions: Subjects with predominantly ⩾2% sputum eosinophils in combination with predominantly ⩾50% neutrophils showed greater loss of lung function, whereas those with highly variable sputum eosinophils had greater healthcare use.

Keywords: eosinophils; neutrophils; longitudinal inflammation; exacerbations; healthcare use

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This article has a related editorial.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

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At a Glance Commentary

Scientific Knowledge on the Subject: Longitudinal assessment of airway inflammation in a majority of patients with asthma shows mainly stable granulocyte numbers, supporting the utility of cross-sectional sputum observations toward understanding ongoing inflammatory pathology in an individual. However, patients with highly variable eosinophils over time require greater healthcare resources for control.

What This Study Adds to the Field: Combined elevated eosinophils and elevated neutrophils in sputum are associated with greater lung function loss over time than either high eosinophils or high neutrophils alone, indicating that overlapping inflammatory pathways may have a greater detrimental effect.

Reports assessing longitudinal stability of airway inflammation in subjects with asthma present widely differing results (1–8). Longitudinal persistence of elevated eosinophils in both moderate and severe asthma ranges from 7% (3) to 76% (6) of subjects in subsequent visits, but time intervals for studies vary greatly, ranging from 1 month (9) to 5 years or longer (1, 5), with inclusion criteria also varying greatly. Newby and colleagues reported that those subjects with severe asthma and variable sputum eosinophilia showed approximately twice the loss of lung function over 8 years than the persistently noneosinophilic and persistently eosinophilic asthma groups that did not differ in declines; however, no difference in asthma exacerbations was observed in these groups (5).

More recent cluster analyses combining fractional exhaled nitric oxide and blood eosinophil biomarkers with clinical variables reported good longitudinal stability of distinct clinical and biomarker phenotypes for four clusters identified in the ADEPT (Airway Disease Endotyping for Personalized Therapeutics) severe asthma cohort examined over 1 year (7). The sputum inflammation in the four ADEPT clusters mirrored inflammatory stratifications reported by Simpson and colleagues (10), Hastie and colleagues (11), and Kupczyk and colleagues (4): paucigranulocytic, primarily neutrophilic, primarily eosinophilic, or mixed granulocytic groups. Baseline assignment for the SARP III (Severe Asthma Research Program III) cohort into these four categories explored changes in clinical characteristics over 3 years of follow-up (12) but did not address whether changes in sputum inflammation occurred or potentially altered group assignment and clinical outcomes over time. Denlinger and colleagues (13), reporting on the SARP III cohort alternatively stratified by a longitudinal change in post-bronchodilator FEV_1% predicted into severe decline, mild decline, no change, and improvement groups, found that the severe decline group had the highest sputum eosinophil percentage before triamcinolone administration.

Conflicting observations of sputum granulocyte variability over time in previous reports (1–8) prompted the examination of the SARP III cohort for stable versus variable airway eosinophils or neutrophils and the impact of stable or variable inflammation on important clinical outcomes. Although the focus has generally been on eosinophilic inflammation (1, 5, 6), more severe asthma has been associated with neutrophils (14) and severe exacerbations with proinflammatory and type 1 mediators in sputum (15). Increasing evidence suggests that different inflammatory pathways may be present simultaneously (16, 17). Therefore, we have examined both eosinophils and neutrophils for longitudinal variation associated with pulmonary function and healthcare use and investigated the potential overlap between longitudinal groups (16), which may result in worsening asthma. Some of the subjects and data in this study have been previously examined in a different stratification scheme based prospectively only on baseline characteristics rather than on the assignment based on longitudinal characteristics, as investigated here (12), and have been separately presented as a conference abstract for eosinophil groups only, which were stratified by more stringent criteria than those used in this study (18).

Methods

Subjects

Adult subjects with an acceptable baseline sample and at least two to three longitudinal sputum samples ($N = 206$) were recruited at seven clinical sites and signed an informed consent form approved by institutional review boards at each center and by the NHLBI Data Safety Management Board (www.clinicaltrials.gov). Patients, 62% with severe asthma and 38% with nonsevere asthma as defined by the International European Respiratory Society/American Thoracic Society guidelines (19), were comprehensively characterized by the SARP III longitudinal protocol at baseline (20). Briefly, nonsmoking subjects (<10 pack-years) meeting American Thoracic Society criteria for asthma underwent spirometry; testing for bronchodilator reversibility after controller medication withhold; testing for bronchial responsiveness to methacholine; ImmunoCAP (Thermo Fisher Scientific) tests for 15 allergens; testing for total serum IgE; blood collection for DNA genome-wide association studies; plasma, serum, exhaled NO, and urine collection; sputum induction; and questionnaires that addressed medical history, symptoms, quality of life, medications, and healthcare use. Repeat clinical assessment was obtained at 1, 2, and 3 years (more detailed information is provided in the online supplement).

Sputum Induction and Processing

Methods for induction and whole sputum sample processing were those employed by ACRN (Asthma Clinical Research Network), AsthmaNet (NHLBI programs), and previous SARP cross-sectional observation studies (11, 21). Adult subjects at clinical sites who were eligible for induction (post-bronchodilator FEV_1% predicted ⩾50%, or if less, collection of a spontaneous sample) had sputum samples collected at baseline, Year 1, Year 2, and Year 3 visits. Thus, four determinations were available for sputum cell differentials, but subjects were included if they had acceptable sputum differentials at baseline and at least two additional annual visits, in accord with the finding that three visits determine the inflammation subtype with 93% sensitivity and 100% specificity (22). Differential counts for acceptable sputum samples (<80% squamous cells) were obtained on at least 500 nonsquamous cells by the central slide-reading center.

Analyses and Statistics

Each subject was assigned to only one of three groups on the basis of longitudinal sputum eosinophil percentages over 3 years: the predominantly low-eosinophil group (all, or at least two of three or three of four,

acceptable samples with <2% eosinophils), predominantly high-eosinophil group (all, or at least two of three or three of four, acceptable samples with ⩾2% eosinophils), and highly variable eosinophil group (individual subject's eosinophil variation both above and below 2% and >2 SDs). Subjects were similarly assigned to only one of three groups on the basis of longitudinal sputum neutrophil percentages over 3 years: predominantly low neutrophils (all, or at least two of three or three of four, acceptable samples with <50% neutrophils), predominantly high neutrophils (all, or at least two of three or three of four, acceptable samples with ⩾50% neutrophils), and highly variable neutrophils (individual subject's neutrophil variation both above and below 50% and >2 SDs). Subjects remained in their group for analyses of clinical characteristics at baseline and annual visits. Subjects were excluded ($n = 197$) if they had no baseline sputum differential or if they were placed on biologic therapy at any time during the study (anti-IgE, anti-IL5, or multiple biologics). The clinical characteristics for excluded subjects have been previously reported (12). Further information on the subject stratification is in the online supplement.

Demographic and biomarker information for continuous data are presented as the mean ± SD, or as the median and quartiles when distribution was markedly skewed, and as the numerator n (and percentage positive) for categorical variables. Continuous variables were tested by using ANOVA or a Kruskal-Wallis test corresponding to their presentation as either the mean or median, whereas categorical variables were tested by using a chi-square test (version 9.4; SAS Institute). To adjust for multiple tests, the P values have been adjusted to preserve the overall false discovery rate (FDR) at which the 0.05 threshold was accepted as significant. Only variables with a significant FDR-adjusted P value were further explored by *post hoc* pairwise tests with Sidak correction to pairwise P values.

Results

Clinical Characteristics of Cohort Stratified into Longitudinal Groups: Predominantly Low, Predominantly High, or Highly Variable Sputum Eosinophils

The cohort of SARP III subjects with acceptable baseline sputum determinations and at least two additional annual sputum determinations over Years 1, 2, or 3 were stratified into three groups: predominantly low, predominantly high, or highly variable sputum eosinophils (Figure 1). The baseline clinical characteristics of these groups (presented in Table 1) show that 25% of the cohort had predominantly high eosinophils, whereas 59% had predominantly low eosinophils and 16% had highly variable eosinophils over the 3 years. The proportions of SARP III subjects in these longitudinal groups are generally comparable to those reported for other studies (*see* Table E1 in the online supplement) and indicate that a majority with low or high sputum eosinophils are repeatedly stable over time.

There were no differences for age, sex, race, Hispanic ethnicity, or severity across groups. At baseline and for the following 3 years, the group with predominantly low eosinophils had better lung function measures for the prebronchodilator FEV_1 (liters and percent predicted) and prebronchodilator FEV_1/FVC ratio than the highly variable and predominantly high-eosinophil groups.

Measures for healthcare use generally did not differ across the three longitudinal eosinophil groups, but the highly variable eosinophil group had higher proportions of subjects reporting unscheduled doctor's visits, emergency department visits, and hospitalizations in the past year. The highly variable eosinophil group's number of exacerbations at Year 3 was significantly greater than that of the stable eosinophil groups (Table 1 and Figure 2). In addition, the highly variable eosinophil group generally indicated more use of controller medications, including inhaled corticosteroid use in the past 3 months and leukotriene receptor antagonist use in the past 3 months by Year 3 (Table 2). Despite these indications of potentially less well-controlled asthma in the highly variable eosinophil group, there was no difference in Asthma Control Test (ACT) scores compared with the predominantly low or predominantly high-eosinophil groups (Table 1).

Clinical Characteristics of Cohort Stratified into Longitudinal Neutrophil Groups: Predominantly Low, Predominantly High, or Highly Variable Sputum Neutrophils

In contrast to the results for stable or variable sputum eosinophil groups, the cohort stratified into predominantly low, predominantly high, or highly variable sputum neutrophil groups (Figure E1) had few demographic or clinical differences (Table E2). Age and years since diagnosis were significantly greater for the predominantly high-neutrophil group. However, lung function, healthcare use measures, and medication use did not differ across stable or variable neutrophil groups (Tables E2 and E3).

Clinical Characteristics of Combined Eosinophil and Neutrophil Longitudinally Stable Groups

Previous analyses of the cross-sectional SARP I and II cohorts have shown that elevated sputum eosinophils combined with elevated neutrophils were associated with lower lung function and greater healthcare requirements (11). In addition, analyses of sputum molecular constituents and other clinical biomarkers indicate an overlap of different inflammatory pathways (16, 17). Therefore, we have examined whether individuals with longitudinal predominantly low or high eosinophils combined with longitudinal predominantly low or high neutrophils showed differences in important clinical characteristics. The highly variable eosinophil group and highly variable neutrophil group were not included because of very small numbers in the resulting subgroups (*see* Table E4).

The clinical characteristics of the combined, longitudinal, predominantly low- or high-eosinophil and neutrophil groups, presented in Table 3, show that the predominantly high-eosinophil group combined with the predominantly high-neutrophil group had the lowest lung function for the prebronchodilator FEV_1% predicted and FEV_1/FVC ratio and had the maximum FEV_1 response to albuterol. There was also a gradual decline in the prebronchodilator FEV_1% predicted over the 3 years for the predominantly high-eosinophil group compared with no change or slight improvement in the predominantly low-eosinophil groups, with differences becoming significant in Years 2 and 3 (Figure 3). Healthcare use measures did not differ across the four groups (Table 3).

Discussion

As indicated in earlier reports, subjects followed longitudinally have varying

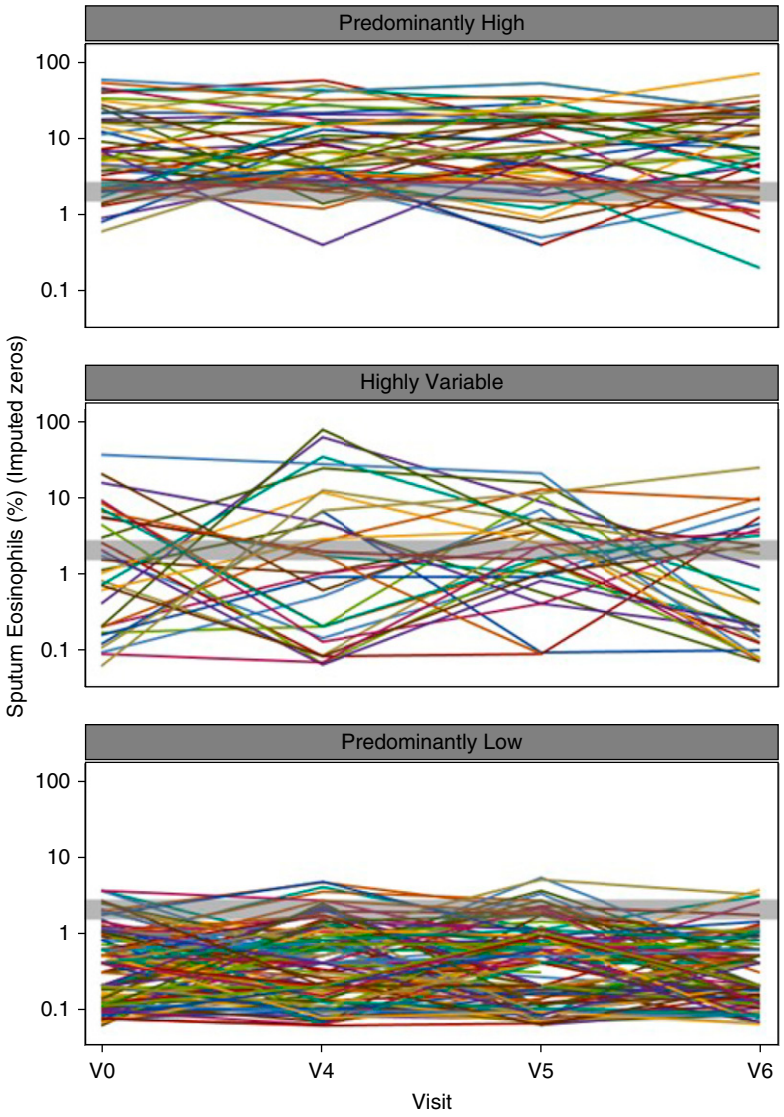

Figure 1. The graphs show eosinophil percentages at baseline (V2) and subsequent annual visits (V4, V5, and V6 for Years 1, 2, and 3, respectively) for longitudinal groups of subjects having predominantly high (top), highly variable (middle), and predominantly low (bottom) sputum eosinophils. The horizontal gray bar in each graph indicates 2% eosinophils. V = visit.

patterns of sputum inflammation, with some being apparently stable at later follow-up and with others showing substantial variability (1–8). The SARP cohort assigned to one of three longitudinal sputum eosinophil groups, from baseline through three subsequent annual visits, had two predominantly stable groups with low or high eosinophils and one highly variable eosinophil group (>2 SDs). A major proportion of our subjects having "stable" eosinophilic inflammation, either high or low, corresponds to similar majorities reported earlier (1, 3–6, 9). Differences regarding longitudinal stability of asthma endotype clusters have been reported for participants stratified by inflammatory parameters combined with clinical variables (7, 23), but those represent an alternative approach to our stratification based on longitudinal inflammatory characteristics alone. We observed that subjects with predominantly high sputum eosinophils throughout had a loss of lung volumes over time, but unlike Newby and colleagues (5), we did not observe a greater loss of lung volumes for the longitudinal, highly variable eosinophil group. The SARP III highly variable eosinophil group actually had a modest improvement in lung function measures over the 3 years. As an additional contrast to the highly variable eosinophil group in the report by Newby and colleagues (5), the SARP III cohort's highly variable eosinophil group reported significantly more exacerbations by Year 3 than the predominantly low-eosinophil group. This higher exacerbation rate occurred despite the reported greater use of inhaled and oral corticosteroids together with other additional controller medications in the highly variable eosinophil group. Thus, the SARP III longitudinal highly variable sputum eosinophil group can be characterized as "labile," requiring greater healthcare resources without having a significant loss of lung function, unlike the more stable, predominantly high-eosinophil group. These results suggest that the longitudinal, highly variable eosinophil group had poorer control of asthma over the 3 years of the SARP study. This conclusion is similar to that of another study, but that study combined subjects with intermittent and persistent eosinophilia (24), thus differing from the SARP cohort. Nevertheless, no differences were found in SARP ACT scores in the longitudinal sputum eosinophil groups at baseline or throughout the 3 years. In addition to showing a longitudinal loss of lung function, the predominantly high-eosinophil group had higher albuterol bronchodilator responses throughout the study, a characteristic found in steroid-resistant, type 2–high asthma in this cohort (25) but contrasting with another study group stratified by the outcome of loss of reversibility, which showed a decline in the FEV_1% predicted over 10 years (26).

Because of the association of more severe asthma with sputum neutrophils (14), the SARP cohort was alternatively stratified by longitudinal sputum neutrophil percentages: predominantly high, predominantly low, and highly variable. Although greater age and longer duration of asthma were associated with the predominantly high neutrophils, there were no differences between stable or variable neutrophil groups for lung function, nor were there any differences in healthcare use or medication use across the longitudinal sputum neutrophil groups. These observations suggest neutrophils alone have less impact on progression to a more severe asthma phenotype.

However, previous cross-sectional observations for elevated sputum

Table 1. Clinical Characteristics for Subjects Stratified Longitudinally by Sputum Eos Remaining Predominantly Low (<2%), Predominantly High (≥2%), or Highly Variable (>2 SDs, Ranging from <2% to ≥2%) over 3 Years

Characteristics for Eos Groups	Predominantly Low Eos	Highly Variable Eos (*>2 SDs*)	Predominantly High Eos	*P* Value across 3 Eos Groups
n at baseline	122	32	52	—
n at Year 3 with sputum	113	31	49	—
Age at baseline, yr	48.2 ± 15.1	49.1 ± 15.1	49.1 ± 14.5	0.912
Years since diagnosis of asthma	28.1 ± 15.9	22.6 ± 14.6	29.2 ± 17.2	0.202
Years since onset of asthma symptoms	32.2 ± 16.3	24.5 ± 15.1	32.3 ± 17.0	0.100
BMI, kg/m^2				
Baseline	31.8 ± 8.1	32.2 ± 8.0	30.6 ± 8.9	0.343
Year 3	32.1 ± 7.9	32.2 ± 8.3	29.7 ± 7.5	0.141
Sex, M, *n* (%)	42 (34.4)	8 (25)	20 (38.5)	0.445
Race, %				
White	73	75	59.6	0.200
Black	17	18.8	21.2	0.828
Other	9.8	6.3	19.2	0.169
Hispanic ethnicity, *n* (%)	2 (1.6)	1 (3.1)	2 (3.8)	0.662
Stage 3 severe (defined by meds and control), *n* (%)				
Baseline	55 (45.1)	21 (65.6)	27 (51.9)	0.160
Year 3	41 (36.3)	13 (41.9)	19 (38.8)	0.838
ACT/CACT (6–11) score				
Baseline	17.9 ± 4.3	17.8 ± 4.6	17.3 ± 4.1	0.588
Year 3	19.4 ± 4.0	18.3 ± 5.3	18.7 ± 3.5	0.408
Pre-BD FEV_1, L				
Baseline	2.49 ± 0.94	2.04 ± 0.76	2.14 ± 0.75	**0.028***
Year 3	2.42 ± 0.91	2.09 ± 0.80	2.04 ± 0.73	**0.042**†
Pre-BD FEV_1% pred, L				
Baseline	78.5 ± 17.8	68.9 ± 19.5	71.0 ± 19.6	**0.023**†
Year 3	79.3 ± 18.7	72.3 ± 19.3	70.4 ± 20.2	**0.025**†
Post-BD FEV_1% pred, L				
Baseline	87.9 ± 17.0	82.1 ± 18.5	85.7 ± 19.1	0.304
Year 3	87.6 ± 18.3	82.2 ± 17.4	84.9 ± 20.4	0.250
Pre-BD FEV_1/FVC ratio				
Baseline	0.71 ± 0.10	0.67 ± 0.12	0.66 ± 0.09	**0.002**†
Year 3	0.71 ± 0.10	0.68 ± 0.11	0.65 ± 0.08	**0.002**†
Maximum FEV_1 albuterol response (absolute change in percent pred)				
Baseline	9.0 ± 6.1	13.1 ± 7.6	14.8 ± 9.3	**<0.001***†
Year 3	8.3 ± 6.0	9.9 ± 6.7	14.4 ± 7.9	**<0.001**†‡
Triamcinolone response: absolute change in pre-BD FEV_1% pred	2.0 ± 6.7	2.6 ± 9.4	4.4 ± 9.7	0.200
Pre-BD FEV_1% pred change from baseline				
Year 1	0.4 ± 7.4	−0.7 ± 11.5	−1.2 ± 11.2	0.291
Year 3	1.8 ± 9.7	1.5 ± 13.4	−1.1 ± 10.2	0.201
Post-BD FEV_1% pred change from baseline				
Year 1	−0.3 ± 7.1	1.0 ± 9.3	−1.1 ± 8.9	0.552
Year 3	0.6 ± 8.7	1.3 ± 10.6	−0.4 ± 9.8	0.552
$F_{E_{NO}}$, ppb§				
Baseline	20.0 (14.0–30.0)	19.0 (12.0–43.0)	36.0 (20.0–53.0)	**<0.001**†
Year 2	19.0 (14.0–30.0)	26.0 (13.5–46.5)	49.5 (28.0–80.0)	**<0.001**†‡
Blood eosinophil count, cells/μl§				
Baseline	160 (108–280)	202 (111–340)	429 (274–631)	**<0.001**†‡
Year 3	144 (84–276)	280 (162–336)	392 (271–550)	**<0.001***†‡
Blood neutrophil count, cells/μl§				
Baseline	3,865 (3,100–4,921)	4,000 (3,092–5,214)	5,180 (3,954–6,616)	**0.034***
Year 3	3,792 (2,856–5,092)	4,293 (3,341–5,649)	3,626 (2,583–4,392)	0.101
Total sputum cell count, count × 10^4/ml§				
Baseline	97.4 (46.9–174.8)	98.3 (55.6–199.0)	136.8 (69.2–205.1)	0.286
Year 3	93.5 (30.8–183.0)	103.2 (50.9–423.9)	130.0 (64.6–195.7)	0.186
Sputum eosinophils, %§				
Baseline	0.4 (0.0–0.8)	1.1 (0.1–5.4)	5.2 (2.2–16.7)	**<0.001***†‡
Year 3	0.2 (0.0–0.6)	0.4 (0.0–3.4)	6.2 (2.2–19.5)	**<0.001**†‡

(Continued)

Table 1. (*Continued*)

Characteristics for Eos Groups	Predominantly Low Eos	Highly Variable Eos (*>2 SDs*)	Predominantly High Eos	*P* Value across 3 Eos Groups
Sputum neutrophils, %[§]				
Baseline	51.9 (31.0–71.7)	50.4 (35.9–74.6)	48.6 (34.1–68.0)	0.883
Year 3	59.1 (41.3–74.8)	69.1 (55.9–88.4)	57.7 (45.4–70.1)	**0.043**[*‡]
Total IgE at baseline, U/ml[§]	114.9 (31.2–257.2)	103.6 (31.4–440.0)	186.7 (89.6–588.1)	**0.025**[†]
Number of positive specific IgE results (of 15 tests) at baseline	4.1 ± 3.5	3.5 ± 3.9	5.0 ± 4.6	0.325
At least one positive specific IgE result at baseline, *n* (%)	99 (82.5)	24 (75)	37 (71.2)	0.242
Unscheduled visit in past 12 mo, *n* (%)				
Baseline	48 (39.3)	14 (43.8)	15 (28.8)	0.307
Year 3	21 (18.6)	10 (32.3)	12 (24.5)	0.251
Emergency dept visit in past 12 mo, *n* (%)				
Baseline	17 (13.9)	9 (28.1)	9 (17.3)	0.199
Year 3	4 (3.5)	4 (12.9)	3 (6.1)	0.178
Hospitalized in past 12 mo, *n* (%)				
Baseline	7 (5.7)	5 (15.6)	2 (3.8)	0.140
Year 3	1 (0.9)	1 (3.2)	1 (2)	0.616
Number of exacerbations in past yr				
Baseline	0.9 ± 1.5	2.0 ± 2.8	1.3 ± 1.7	0.200
Year 3	0.4 ± 1.0	1.0 ± 1.5	0.8 ± 1.8	**0.019**[*]

Definition of abbreviations: ACT = Asthma Control Test; BD = bronchodilator; BMI = body mass index; CACT = Childhood ACT; dept = department; Eos = eosinophil(s); Fe_{NO} = fractional exhaled nitric oxide; meds = medications; pred = predicted.
Baseline and Year 3 data for these groups are shown. Categorical variables have the numerator *n* (percentage positive); *n* denominators are baseline and Year 3 numbers for all groups. Other variables are shown as mean ± SD unless otherwise indicated. Adjusted *P* value for false discovery rate in bold font denotes $P < 0.05$.
[*]Low Eos versus variable Eos, $P < 0.05$.
[†]High Eos versus low Eos, $P < 0.05$.
[‡]High Eos versus variable Eos, $P < 0.05$.
[§]Median (interquartile range).

eosinophils and neutrophils revealed the lowest lung function and greater healthcare resource requirements for combined increased eosinophils and neutrophils (11, 27), which prompted examining combinations of the longitudinal groups. The lowest lung function was previously observed for a high-eosinophil and high-neutrophil group (11, 28), but in the present study, the high-eosinophil and high-neutrophil group had longitudinal decline over the course of this study, confirming the earlier associations of reduced lung function with combined increased eosinophils and neutrophils in cross-sectional studies (11, 28). Thus, we would conclude that longitudinal stability, overlap, and interaction of increased eosinophils and neutrophils, representing different inflammatory pathways, are more detrimental in terms of decline in lung function than any single inflammatory pathway in the progression of severe asthma. Confirming the longitudinal trajectory of overlapping inflammation are previous reports identifying similar patterns of interacting inflammatory pathways in cross-sectional analyses (16, 17).

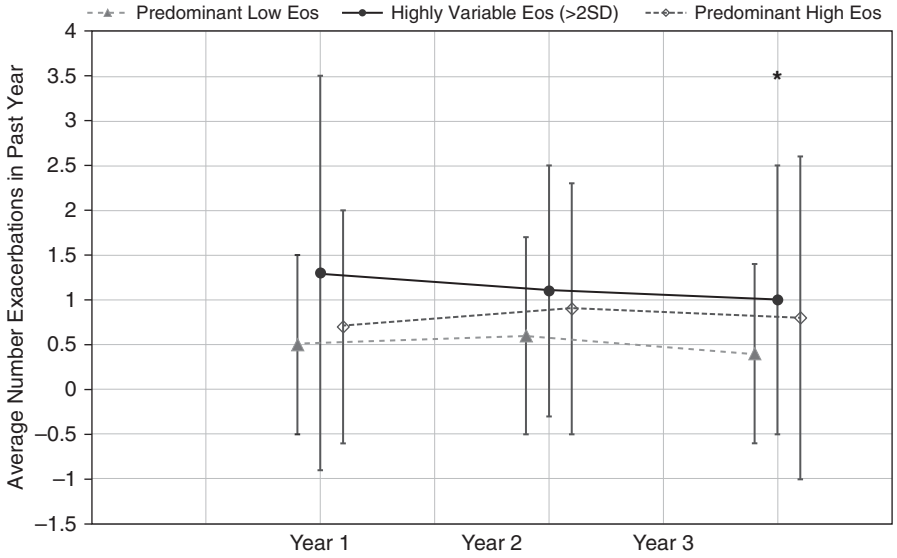

Figure 2. Number of exacerbations in the past year for the longitudinal groups with predominantly low, predominantly high, and highly variable Eos, staggered on the graph to show the ±SD error bars. There was a significant difference across the three groups in Year 3. *$P = 0.018$. Eos = eosinophil.

Strengths of this cohort are the comprehensive characterization of subjects longitudinally over 3 years, with the cohort including a majority classified as severe but also including subjects with nonsevere asthma, to capture changes that may occur early in the course of the

Table 2. Controller Medications Including CS for Subjects Stratified Longitudinally by Sputum Eos Remaining Predominantly Low (<2%), Predominantly High (≥2%), or Highly Variable (>2 SDs Ranging from <2% to ≥2%) at Baseline and Year 3

Characteristics for Eos Groups	Predominantly Low Eos	Highly Variable Eos *(>2 SDs)*	Predominantly High Eos	*P* Value* across 3 Eos Groups
n at baseline	122	32	52	—
n at Year 3 with sputum	113	31	49	—
Short-acting β-agonist, current, *n* (%)				
Baseline	106 (86.9)	29 (90.6)	48 (92.3)	0.550
Year 3	95 (84.1)	28 (90.3)	45 (91.8)	0.338
Short-acting anticholinergic, current, *n* (%)				
Baseline	11 (9)	6 (18.8)	1 (1.9)	0.060
Year 3	4 (3.5)	4 (12.9)	2 (4.1)	0.155
Long-acting β-agonist, current, *n* (%)				
Baseline	85 (69.7)	29 (90.6)	38 (73.1)	0.100
Year 3	71 (62.8)	26 (83.9)	32 (65.3)	0.138
Long-acting anticholinergic in past 3 mo, *n* (%)				
Baseline	4 (3.3)	4 (12.5)	2 (3.8)	0.140
Year 3	3 (2.7)	2 (6.5)	2 (4.1)	0.596
Leukotriene receptor antagonist in past 3 mo, *n* (%)				
Baseline	36 (29.5)	10 (31.3)	14 (26.9)	0.905
Year 3	20 (17.7)	11 (35.5)	18 (36.7)	**0.035**†
5-Lipoxygenase inhibitor in past 3 mo, *n* (%)				
Baseline	3 (2.5)	1 (3.1)	2 (3.8)	0.881
Year 3	3 (2.7)	1 (3.2)	2 (4.1)	0.891
Inhaled CS in past 3 mo, *n* (%)				
Baseline	96 (78.7)	31 (96.9)	46 (88.5)	0.056
Year 3	80 (70.8)	30 (96.8)	41 (85.4)	**0.011**‡
High-dose inhaled CS, *n* (%)				
Baseline	64 (52.5)	21 (65.6)	27 (51.9)	0.381
Year 3	44 (38.9)	14 (45.2)	19 (39.6)	0.820
Daily oral CS, current, *n* (%)				
Baseline	6 (4.9)	5 (15.6)	3 (5.8)	0.142
Year 3	4 (3.5)	4 (12.9)	3 (6.1)	0.178
Oral CS in past 12 mo, *n* (%)				
Baseline	50 (41)	15 (46.9)	24 (46.2)	0.740
Year 3	17 (15)	13 (41.9)	15 (30.6)	**0.010**‡
Daily oral CS dose, *n* (%)				
Baseline	7.5 ± 3.3	7.4 ± 2.8	5.0 ± 0.0	0.462
Year 3	6.0 ± 2.7	11.3 ± 4.8	10.0 ± 0.0	0.170

Definition of abbreviations: CS = corticosteroid; Eos = eosinophil.
Categorical variables have numerator *n* (percentage positive); *n* denominators are baseline and Year 3 numbers for all groups.
**P* value in bold font denotes $P < 0.05$.
†High Eos versus low Eos, $P < 0.05$.
‡Low Eos versus variable Eos, $P < 0.05$.

disease. This SARP cohort with sputum was larger than those of other longitudinal studies (*see* Table E1) and therefore may show differences in subgroup analyses missed in smaller cohorts. We acknowledge that 3 years may be insufficient to detect important small changes, which accumulate over a longer period to produce larger effects or may have occurred earlier in the disease process. However, the increasing use of biologics in subjects with more severe or less controlled asthma over the duration of this study introduced a potential modification of inflammation and thus a subsequent impact on clinical outcomes. Therefore, those subjects prescribed biologic therapy at any time during the study were not included in this analysis. Correction of *P* values to preserve the overall FDR provides confidence in the statistical significance attributed to any particular test result. Differences between this and other reports may relate not only to the number of subjects, their severity of asthma, the use of controller medications, and the length of the study but also to the differences in cohort age at enrollment, racial group composition, and factors such as the degree of tobacco exposure among participants (limited here to <5 pack-years for <35 yr old and to <10 pack-years for those >35 yr old).

This study was observational and did not specify treatment algorithms, unlike the longitudinal study of Aziz-Ur-Rehman and colleagues (29), which managed a group of prednisone-dependent patients with asthma longitudinally by maintaining sputum eosinophils below 3%. Therapy in the SARP III cohort was left to the discretion of participants' clinicians, and, in fact, despite >60% of subjects being classified at enrollment as having severe asthma, only a

Table 3. Clinical Characteristics for Subjects in Combined Groups for Predominantly Low Eos and Predominantly Low Neu, Predominantly Low Eos and Predominantly High Neu, Predominantly High Eos and Predominantly Low Neu, and Predominantly High Eos and Predominantly High Neu

Label	Low Sput Eos and Low Sput Neu	Low Sput Eos and High Sput Neu	High Sput Eos and Low Sput Neu	High Sput Eos and High Sput Neu	*P* Value for Four Eos and Neu Groups	*P* Value for Low Eos–Low Neu vs. High Eos–High Neu
n at baseline	46	59	20	28	—	—
n at Year 3	41	55	19	27	—	—
Age at baseline, yr	43.0 ± 13.1	53.8 ± 13.9	45.6 ± 14.6	50.8 ± 14.5	**0.002**	**0.017**
Years since diagnosis of asthma	23.1 ± 12.4	33.3 ± 16.9	26.0 ± 14.6	32.0 ± 18.2	**0.029**	**0.038**
Years since onset of asthma symptoms	27.4 ± 13.5	37.0 ± 17.0	30.0 ± 14.9	34.3 ± 18.2	0.072	—
BMI						
Baseline	32.1 ± 7.5	32.2 ± 8.6	30.6 ± 9.7	30.7 ± 8.4	0.430	—
Year 3	32.5 ± 7.4	32.5 ± 8.3	31.0 ± 8.5	29.5 ± 7.0	0.275	—
Sex, M, *n* (%)	14 (30.4)	23 (39)	6 (30)	14 (50)	0.399	—
Race, %						
White	67.4	78	60	60.7	0.187	—
Black	19.6	13.6	10	25	—	—
Other	13.0	8.5	30	14.3	—	—
Stage 3 severity, *n* (%)						
Baseline	24 (52.2)	26 (44.1)	8 (40)	16 (57.1)	0.549	—
Year 3	15 (36.6)	21 (38.2)	6 (31.6)	12 (44.4)	0.840	—
Ever-smoker *n* (%)	9 (19.6)	12 (20.3)	7 (35)	9 (32.1)	0.412	—
Pre-BD FEV_1% pred						
Baseline	80.3 ± 14.9	77.2 ± 19.1	76.9 ± 18.2	68.2 ± 19.7	0.076	—
Year 3	82.2 ± 15.7	76.8 ± 20.1	75.9 ± 19.9	67.8 ± 19.6	**0.049**	**0.001**
Post-BD FEV_1% pred						
Baseline	91.1 ± 14.2	85.3 ± 17.5	89.6 ± 16.6	84.5 ± 19.9	0.248	—
Year 3	92.3 ± 14.5	83.8 ± 19.4	89.9 ± 18.7	82.7 ± 20.8	0.151	—
Pre-BD FEV_1/FVC						
Baseline	0.71 ± 0.09	0.71 ± 0.10	0.68 ± 0.08	0.64 ± 0.07	**0.005**	**0.001**
Year 3	0.71 ± 0.08	0.70 ± 0.11	0.66 ± 0.10	0.65 ± 0.07	**0.010**	**0.002**
Triamcinolone response: absolute change in pre-BD FEV_1% pred at baseline	2.3 ± 8.3	1.6 ± 5.6	1.7 ± 11.9	5.9 ± 8.2	0.187	—
Maximum FEV_1 albuterol response (absolute change in percent pred)						
Baseline	10.8 ± 6.9	7.3 ± 5.4	12.7 ± 6.8	16.3 ± 11.1	**<0.001**	**0.034**
Year 3	10.2 ± 6.6	7.0 ± 5.7	14.0 ± 6.9	14.9 ± 9.0	**<0.001**	**0.037**
Pre-BD FEV_1% pred change						
Baseline to Year 1	0.2 ± 8.2	0.5 ± 7.3	−6.1 ± 7.7	2.1 ± 12.6	**0.041**	0.624
Baseline to Year 3	1.3 ± 11.2	2.0 ± 9.3	−2.6 ± 10.5	−0.3 ± 10.3	0.392	—
Post-BD FEV_1% pred change						
Baseline to Year 1	−0.1 ± 7.3	−0.3 ± 7.7	−2.4 ± 9.0	−1.5 ± 8.9	0.756	—
Baseline to Year 3	1.1 ± 9.7	0.5 ± 8.3	0.1 ± 11.2	−1.5 ± 8.8	0.451	—

(Continued)

Table 3. (*Continued*)

Label	Low Sput Eos and Low Sput Neu	Low Sput Eos and High Sput Neu	High Sput Eos and Low Sput Neu	High Sput Eos and High Sput Neu	*P* Value for Four Eos and Neu Groups	*P* Value for Low Eos–Low Neu vs. High Eos–High Neu
F_{ENO}*						
Baseline	25.0 (17.0–38.0)	18.0 (14.0–24.0)	39.0 (29.0–59.5)	33.0 (16.0–49.0)	**<0.001**	—
Year 2	19.0 (14.0–28.0)	19.0 (14.0–30.0)	51.0 (28.5–96.0)	40.5 (24.0–80.0)	**<0.001**	**<0.001**
Total Sput cell count, count $\times 10^4$/ml*						
Baseline	97.3 (46.9–163.4)	106.8 (48.3–215.9)	137.4 (100.4–187.5)	131.2 (61.6–230.7)	0.503	—
Year 3	103.3 (38.6–162.6)	93.6 (36.0–189.7)	86.2 (39.9–164.6)	130.5 (87.9–243.0)	0.340	—
Sput Eos, %*						
Baseline	0.4 (0.0–1.0)	0.3 (0.0–0.7)	7.0 (3.8–21.6)	4.5 (2.0–11.7)	**<0.001**	**<0.001**
Year 3	0.2 (0.0–0.5)	0.2 (0.0–0.8)	4.7 (2.1–22.2)	7.6 (2.2–18.9)	**<0.001**	**<0.001**
Sput Neu, %*						
Baseline	31.9 (18.4–41.2)	65.8 (56.5–83.3)	34.1 (22.5–46.3)	65.2 (50.2–78.2)	**<0.001**	**<0.001**
Year 3	42.3 (33.5–60.3)	68.6 (57.5–82.0)	40.3 (26.0–53.1)	66.3 (57.1–74.5)	**<0.001**	**<0.001**
Blood Eos, count*						
Baseline	163 (89–251)	160 (112–281)	422 (236–590)	429 (285–657)	**<0.001**	**<0.001**
Year 3	131 (82–291)	141 (92–246)	312 (184–560)	490 (271–550)	**<0.001**	**<0.001**
Blood Neu, count*						
Baseline	4,067 (2,997–5,280)	3,685 (3,150–4,756)	4,487 (3,131–5,214)	3,920 (3,026–5,360)	0.886	—
Year 3	3,840 (2,915–5,040)	3,422 (2,698–5,092)	4,067 (2,736–5,016)	3,096 (2,520–4,392)	0.659	—
Total IgE baseline geometric mean	96.3 ± 3.7	72.6 ± 5.2	254.2 ± 3.9	174.2 ± 5.1	**0.025**	0.074
Number of positive specific IgE test results of 15	4.0 ± 3.6	4.0 ± 3.4	6.0 ± 5.1	4.6 ± 4.0	0.611	—
At least one positive specific IgE result	38 (84.4)	48 (82.8)	15 (75)	21 (75)	0.669	—
Unscheduled visit in past 12 mo, *n* (%)						
Baseline	21 (45.7)	21 (35.6)	3 (15)	9 (32.1)	0.187	—
Year 3	5 (12.2)	10 (18.2)	5 (26.3)	7 (25.9)	0.448	—
ED in past 12 mo, *n* (%)						
Baseline	9 (19.6)	6 (10.2)	3 (15)	4 (14.3)	0.604	—
Year 3	3 (7.3)	0 (0)	2 (10.5)	1 (3.7)	0.221	—
Hospitalized in past 12 mo, *n* (%)						
Baseline	3 (6.5)	3 (5.1)	1 (5)	0 (0)	0.621	—
Year 3	1 (2.4)	0 (0)	1 (5.3)	0 (0)	0.392	—
Daily OCS, current, *n* (%)						
Baseline	4 (8.7)	2 (3.4)	2 (10)	1 (3.6)	0.534	—
Year 3	3 (7.3)	1 (1.8)	2 (10.5)	1 (3.7)	0.432	—
Daily OCS dose (zero set to missing)						
Baseline	8.3 ± 2.4	6.0 ± 5.7	5.0 ± 0.0	5.0	0.525	—
Year 3	4.7 ± 0.6	10.0	10.0 ± 0.0	10.0	0.196	—
Exacerbations in past yr						
Baseline	1.3 ± 2.1	0.6 ± 0.9	0.9 ± 1.7	1.4 ± 1.8	0.137	—
Year 3	0.4 ± 1.2	0.3 ± 1.0	0.9 ± 2.2	0.9 ± 1.6	0.198	—

Definition of abbreviations: BD = bronchodilator; BMI = body mass index; ED = emergency department; Eos = eosinophil(s); F_{ENO} = fractional exhaled nitric oxide; Neu = neutrophil(s); OCS = oral corticosteroid; pred = predicted; Sput = sputum.

Categorical variables have numerator *n* and (percentage positive); *n* denominators are baseline and Year 3 numbers for all groups. Other variables are shown as mean ± SD unless otherwise indicated. The false discovery rate–adjusted *P* value in bold font denotes $P < 0.05$.

*Median (interquartile range).

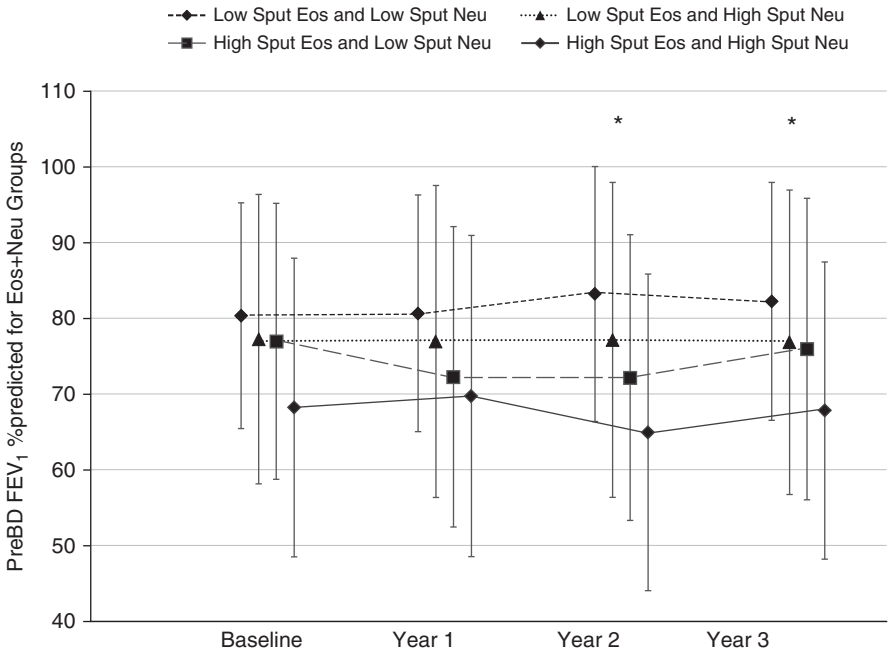

Figure 3. Pre-BD FEV_1% predicted (±SD) from baseline through 3 years for combined groups of predominantly low eosinophils (Eos) and predominantly low neutrophils (Neu), predominantly low Eos and predominantly high Neu, predominantly high Eos and predominantly low Neu, and predominantly high Eos and predominantly high Neu. There was a significant difference between groups by Years 2 and 3. *False discovery rate–adjusted $P = 0.027$ and $P = 0.048$, respectively. BD = bronchodilator; Sput = sputum.

small percentage of subjects were receiving daily oral corticosteroids. We cannot confirm that subjects complied with prescribed controller medications or that they were suboptimally treated. Nevertheless, there is little reason to expect differences in adherence across the groups. Interestingly, the SARP III cohort showed reductions in severity, healthcare use, exacerbations, and controller medication use over the course of the study without apparent change in control as determined by ACT scores. Rather than a concern with suboptimal treatment, these results may suggest the possibility that doses of inhaled corticosteroid could be reduced as observed by others, particularly for noneosinophilic asthma (30).

Conclusions

In summary, subjects with asthma stratified by longitudinal sputum inflammation into stable high inflammation, stable low inflammation, or highly variable groups demonstrate that those with predominantly low sputum eosinophils have higher lung function and retained greater lung function throughout the study. Those subjects with longitudinal, predominantly high sputum eosinophils showed a loss in the prebronchodilator FEV_1% predicted, which was unlike the improvements for longitudinal, predominantly low or highly variable eosinophil groups. However, subjects with highly variable sputum eosinophils reported a greater frequency of asthma exacerbation rates despite additional use of controller medications. Although subjects stratified by longitudinal sputum neutrophils into a stable, high-neutrophil group, a stable low-neutrophil group, or a highly variable group had few demographic and clinical differences, the stable, predominantly high-neutrophil group combined with the predominantly high-eosinophil group resulted in a lower prebronchodilator FEV_1% predicted over the 3 years than that observed for either the predominantly high-eosinophil group or the predominantly high-neutrophil group alone. This further supports the concept of overlapping inflammatory cells and pathways having a greater detrimental effect in the progression of severe asthma. ■

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