A Large Subgroup of Mild-to-Moderate Asthma Is Persistently Noneosinophilic

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***Rationale*: Airway eosinophilia is typical of asthma, and many controller treatments target eosinophilic disease. Asthma is clinically heterogeneous, however, and a subgroup of people with asthma do not have airway eosinophilia. The size of this subgroup is uncertain because prior studies have not examined repeated measures of sputum cytology to determine when people with asthma have intermittent versus persistent sputum eosinophila and when they are persistently noneosinophilic.**

***Objectives*: To determine the prevalence and clinical characteristics of the noneosinophilic asthma phenotype.**

***Methods*: We analyzed sputum cytology data from 995 subjects with asthma enrolled in clinical trials in the Asthma Clinical Research Network where they had undergone sputum induction and measures of sputum cytology, often repeatedly, and assessment of responses to standardized asthma treatments.**

***Measurements and Main Results*: In cross-sectional analyses, sputum eosinophilia (≥2% eosinophils) was found in only 36% of subjects with asthma not taking an inhaled corticosteroid (ICS) and 17% of ICS-treated subjects with asthma; an absence of eosinophilia was noted frequently, even in subjects with asthma whose disease was suboptimally controlled. In repeated measures analyses of people with asthma not taking an ICS, 22% of subjects had sputum eosinophilia on every occasion (persistent eosinophilia); 31% had eosinophilia on at least one occasion (intermittent eosinophilia); and 47% had no eosinophilia on every occasion (persistently noneosinophilic). Two weeks of combined antiinflammatory therapy caused significant improvements in airflow obstruction in eosinophilic asthma, but not in persistently noneosinophilic asthma. In contrast, bronchodilator responses to albuterol were similar in eosinophilic and noneosinophilic asthma.**

***Conclusions*: Approximately half of patients with mild-to-moderate asthma have persistently noneosinophilic disease, a disease phenotype that responds poorly to currently available antiinflammatory therapy.**

Keywords: asthma; eosinophil; noneosinophilic; obesity; neutrophil

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Asthma is a heterogeneous disease and eosinophilic and noneosinophilic subtypes of asthma are well described. In this longitudinal study of 995 patients with asthma, we describe the prevalence of sputum eosinophilia and its association with the clinical features of asthma.

What This Study Adds to the Field

By analyzing repeated measures of sputum cytology in subjects with asthma we uncovered three eosinophilic phenotypes of asthma (persistently eosinophilic, intermittently eosinophilic, and persistently noneosinophilic), and reveal the clinical and treatment response characteristics of these three phenotypes. Approximately half of patients with mild-to-moderate asthma have persistently noneosinophilic disease, a disease phenotype that responds poorly to currently available antiinflammatory therapy.

Asthma is a common disease affecting people across the lifespan; the cost to US society is $56 billion annually (1). Airway eosinophilia is well-described in asthma (2–4), and currently available antiinflammatory treatments for asthma almost exclusively target eosinophilic airway inflammation (5, 6). Many people with asthma respond suboptimally to currently available eosinophil-directed treatments, however, most likely because they do not have airway eosinophilia (7–13).

Increased attention is focusing on the clinical heterogeneity of asthma and the argument that failure to recognize and discover the underlying mechanisms of different asthma subtypes is a major limitation to progress (14). Continued uncertainly about the prevalence of eosinophilic versus noneosinophilic subtypes of asthma and about the relationship between airway eosinophilia and treatment responses relates to limitations in previous studies of these issues. The principal limitation of these studies has been that they have usually lacked repeated measures of airway eosinophils, and they have not assessed treatment responses in subjects whose eosinophil phenotype has been categorized by repeated eosinophil measures. The clinical trials supported by National Heart and Lung Institute's Asthma Clinical Research Network (ACRN) provide a valuable resource to address the limitations of prior studies. For example, ACRN studies have frequently included measures of sputum cytology, often measured repeatedly, and done according to standardized protocols and quality control (15). ACRN studies have also included assessment of treatment responses to periods of combined therapy with inhaled and oral corticosteroids, and leukotriene receptor antagonists. Pooled data from subjects with asthma enrolled in these ACRN studies allowed us to generate

robust estimates for the prevalence of eosinophilic phenotypes of asthma and to evaluate the relationship between these phenotypes and treatment responses. Some of the results of these studies have been previously reported in the form of an oral presentation at the American Thoracic Society 2011 international meeting.

METHODS

Subjects

Data were derived from 995 subjects with asthma enrolled in nine clinical trials conducted by the ACRN that included at least one sputum induction as part of the study protocol (aggregated NCT00000577) (*see* Table E1 in the online supplement) (8, 9, 16–21). All subjects were between the ages of 12 and 70 years, met the criteria for persistent asthma as defined by the National Asthma Education and Prevention Program Guidelines for the Diagnosis and Management of Asthma (22), and were current nonsmokers with a lifetime history of smoking no greater than 10 pack-years. In addition, all subjects had either a positive PC_{20} methacholine of less than or equal to 16 mg/ml when on inhaled corticosteroids (ICS) or less than or equal to 8 mg/ml when not on ICS; or a postbronchodilator increase in FEV_1 of greater than or equal to 12%. Sputum induction data and corresponding clinical data were only included when ICS use or nonuse was known and standardized as part of the study protocol at the time of the sputum induction. Of the 995 subjects, 645 had received their baseline sputum assessment after a standardized run-in on low-dose ICS (ICS-positive), and 350 subjects had not been treated with ICS for at least 2 weeks at the time of baseline sputum assessment (ICS-negative) (*see* Figure E1). For repeated measure subgroups (n = 324), 167 ICS-positive subjects with asthma had received baseline sputum assessment after at least 4 weeks of standardized low-dose ICS, whereas 157 ICS-negative subjects with asthma had not used ICS for at least 8 weeks before baseline sputum assessment (*see* Figure E1). Studies had been reviewed and approved by institutional review boards at all participating ACRN centers, and all subjects provided informed consent.

Sputum Induction

Sputum was collected using methods validated for consistency and quality control, as previously described (15, 23, 24). Briefly, subjects had spirometry performed before and 10 minutes after pretreatment with 360 μg of albuterol to ensure postbronchodilator FEV_1 greater than 60% predicted. A 12-minute sputum induction was then performed, and cell differentials (eosinophils, neutrophils, macrophages, epithelial cells, and lymphocytes) were calculated as the percentage of cells in the whole sputum expectorate. The mean ± SD percentage of squamous cells was 31.6 ± 19% in the ICS-negative group and 35.8 ± 19.4% in the ICS-positive subgroup. The other cell types in the sputum were calculated as the percentage of nonsquamous cells.

The presence or absence of eosinophilic inflammation was determined using a 2% cutoff based on published reference values for eosinophils in induced sputum from healthy subjects (25, 26); subjects with greater than or equal to 2% sputum eosinophils were classified as having sputum eosinophilia, and subjects with less than 2% sputum eosinophils were classified as being noneosinophilic.

Statistics

Data analysis was done with the JMP 8.0.2 statistical software (SAS Institute, Cary, NC). Continuous variables were presented within tables as means and standard deviations when normally distributed, and medians and interquartile ranges when not normally distributed. Categorical data were reported using frequencies and percentages. Preliminary analyses revealed three groups of eosinophilic phenotypes when subjects were evaluated using repeated measures: (1) persistently eosinophilic, (2) intermittently eosinophilic, and (3) persistently noneosinophilic. Comparisons of these subgroups were evaluated using ordinal logistic regression to assess for trends.

Post hoc exploratory hierarchical cluster analysis was performed in the subgroup of subjects with asthma who were persistently noneosinophilic and who had not been treated with inhaled ICSs. Continuous and binary variables were centered and standardized. Ward minimum variance method was used to establish clusters using 15 variables that were available for all subjects. At each step, Ward method combines clusters by merging the pair of clusters that minimizes the sum of squared deviations from the cluster mean, across all clusters.

The sensitivity and specificity of peripheral blood eosinophils and of nitric oxide levels in exhaled breath as biomarkers of sputum eosinophilia were calculated using data from all subjects who had not been treated with ICSs and who had either test done on the same day as a sputum collection; all paired measures available for each subject were included. A receiver operating characteristic curve analysis was used to select cutoff values for peripheral blood eosinophils and fractional exhaled nitric oxide (FE_{NO}) that maximized sensitivity and specificity and to evaluate the sensitivity and specificity of these biomarkers using usual clinical cutoffs.

RESULTS

Cross-sectional Analyses of Sputum Eosinophils and Neutrophils in Initial Induced Sputum Samples

Of the 995 subjects with asthma with induced sputum data available, 645 were being treated with an ICS (ICS-positive) and 350 were not (ICS-negative). Both groups had disease severity characteristic of mild-to-moderate asthma as shown in Table 1. In analyses of single induced sputum samples from these subjects, we found that sputum eosinophilia (≥2% eosinophils) occurs in a minority of both steroid-treated and untreated subjects with asthma. In the ICS-negative subgroup, the distribution of sputum eosinophils was highly skewed (Figure 1A), with 64% of the subjects having fewer than 2% eosinophils. In the ICS-positive subgroup, the distribution of sputum eosinophils was even more highly skewed with 83% of the subjects having fewer than 2% eosinophils (Figure 1B).

To determine if sputum eosinophilia is influenced by degree of asthma control, we analyzed induced sputum cytology in a subset of 486 subjects who had assessment of asthma control after receiving standardized treatment with low-dose ICS for 4–6 weeks as part of ACRN treatment protocols. Asthma control was assessed using FEV_1, daytime symptoms, short-acting β-agonist use, and nocturnal awakenings. We found that 26% of the subjects who achieved good asthma control had sputum eosinophilia compared with 15% of subjects who did not achieve good control ($P = 0.004$) (Figure 1C). Thus, most subjects who did not achieve good asthma control did not have sputum eosinophilia.

Because measurement of sputum eosinophil numbers is not a simple clinical test, we evaluated peripheral blood eosinophil counts and measures of FE_{NO} as more easily measured biomarkers of sputum eosinophilia. ICS-negative subjects had a total of 361 peripheral blood eosinophil measures and 756 FE_{NO} measures paired with sputum eosinophil measures from a corresponding study visit. We found that established clinical cutpoints of peripheral blood eosinophils (>400/μl) and FE_{NO} (≥25 ppb) were specific for sputum eosinophilia, but the

TABLE 1. CLINICAL CHARACTERISTICS OF THE STEROID-NAIVE AND STEROID-TREATED SUBJECTS WITH ASTHMA INCLUDED IN STUDY ANALYSES

	ICS Negative	ICS Positive
N	350	645
Age	30 (25–38)	35 (27–46)
Females, N (%)	198 (56)	421 (65)
FEV_1%	83.8 ± 13.3	82.4 ± 14.8
FVC%	96.5 ± 12.9	94.1 ± 13.7
PC_{20}, mg/ml	0.8 (0.3–1.9)	2.5 (1–5.9)
Albuterol use, puffs per day	0.4 (0–1.4)	0.3 (0–1.2)
ED visit in prior 1 yr, N (%)	62 (18)	162 (25)
Hospitalized in prior 1 yr, N (%)	3 (1)	19 (3)
Prednisone use for asthma in prior 1 yr, N (%)	56 (16)	135 (21)

Definition of abbreviations: ED = emergency department; ICS = inhaled corticosteroid; IQR = interquartile range.

Data are mean ± SD or median (IQR), unless otherwise indicated.

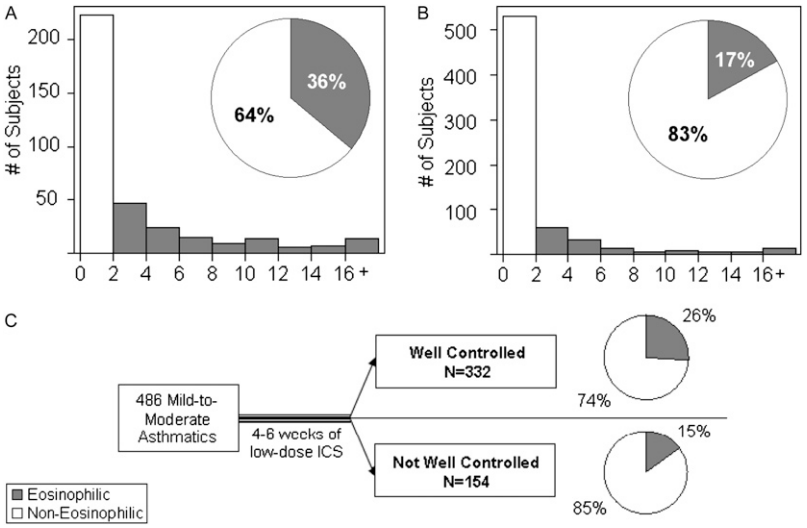


Figure 1. Cross-sectional analysis of sputum eosinophils in mild-to-moderate asthma. (*A*) Frequency distribution of sputum eosinophils in subjects not treated with inhaled corticosteroids (ICS) at baseline assessment (n = 350). (*B*) Frequency distribution of sputum eosinophils in subjects treated with ICS at baseline (n = 645). (*C*) Sputum eosinophils in mild-to-moderate subjects with asthma given standardized low-dose ICS for 4–6 weeks. Asthma control was assessed at the end of the standardized treatment phase. Subjects were considered not well controlled if FEV_1 was less than 70% of predicted, or if daytime symptoms or short-acting β-agonist use were present on greater than or equal to 6 days per week, or if nocturnal awakening was present on greater than or equal to 2 nights per week during the final 2 weeks of low-dose ICS treatment (either beclomethasone, 160 μg, or triamcinolone acetonide, 800 μg daily). Subjects were considered well-controlled if FEV_1 was greater than 70% of predicted and either diurnal variation in peak flow was less than 20% or if daytime symptoms or short-acting β-agonist use were present less than or equal to 5 days per week or nocturnal awakening was present less than or equal to 1 night per week during the final 2 weeks of low-dose ICS treatment. The frequency of sputum eosinophilia was significantly lower in the subgroup with suboptimal asthma control ($P = 0.004$).

sensitivity of both tests was poor (34% and 49%, respectively). Using receiver operating characteristics, the highest achievable combination of sensitivity and specificity for peripheral blood eosinophil count was reached at a derived threshold of 220/μl, which yielded 72% sensitivity and 69% specificity (Figure 2). For FE_{NO}, a value of 20 ppb discriminated those with sputum eosinophilia from those without with a sensitivity of 64% and specificity of 73%. We tested a combined metric using FE_{NO} and peripheral blood eosinophils to evaluate whether these two tests would improve the ability to detect sputum eosinophilia; no improvements in sensitivity and specificity were seen using the combined metric (data not shown).

Neutrophilic subtypes of asthma have been proposed and described (27–29); we therefore also analyzed sputum neutrophils in the ACRN dataset. We found that neutrophils are a very common cell type in sputum in steroid-untreated and steroid-treated subgroups, and frequency distributions of sputum neutrophil percentages approximate normal (Figure 3). We did not find evidence for a distinct or stable neutrophilic subgroup in cross-sectional analyses and so we limited additional studies of neutrophils to evaluation of sputum neutrophil percentages in the different eosinophil phenotypes identified by analysis of repeated sputum inductions (discussed next).

Analyses of Repeated Induced Sputum Samples

The classification of sputum eosinophil phenotypes in cross-sectional analyses could be inaccurate if sputum eosinophilia is intermittent, and thus not necessarily detectable in single sample analysis. We identified 157 ICS-negative subjects and 167 ICS-positive subjects with asthma in the ACRN sputum database who had repeated sputum inductions while assigned to the same therapy over periods as long as 52 weeks (*see* Figure E1). ICS-negative subjects had a mean of 2.7 sputum inductions contributing to the analysis (range, 2–4); the ICS-positive subjects had mean of 2.5 sputum inductions (range, 2–4). Matched clinical data were available for each sputum measure and included spirometry, PC_{20} methacholine, and FE_{NO}. The frequencies of eosinophilic and noneosinophilic phenotypes of asthma in the initial sputum induction from these 324 subjects with repeated measures were similar to the estimates generated from all 995 subjects in the cross-sectional analyses (Figure 4). However, when the definition of sputum eosinophilia included eosinophilia occurring on any one of the repeated sputum analyses, the percentage of subjects who had eosinophilic asthma increased from 36% to 53% in the ICS-negative subgroup and from 19% to 28% in the ICS-positive subgroup (Figure 4). This analysis also revealed that, among subjects with asthma in the ICS-negative subgroup, 22% of subjects had sputum eosinophilia on every occasion (persistent eosinophilia), 31% had eosinophilia on at least one occasion (intermittent eosinophilia), and 47% had less than 2% sputum eosinophils on every occasion (persistently noneosinophilic); the corresponding values for the ICS-positive subgroup were 7%, 20%, and 72%, respectively. The distribution of eosinophilic asthma phenotypes across the age spectrum is shown in Table 2 in the online supplement. In addition, the intraclass correlation coefficient and the within subject variability for the sputum cell counts is provided in Table E3.

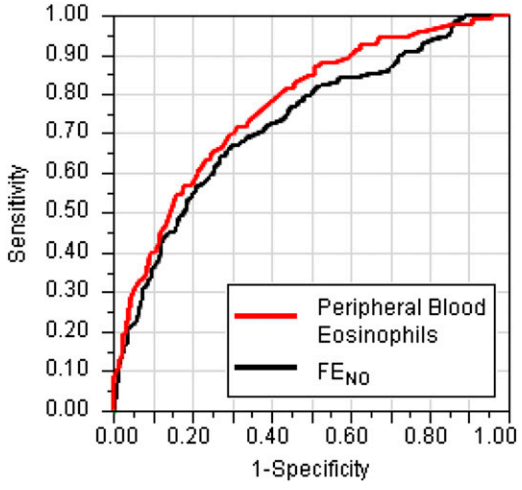


Figure 2. Receiver operating characteristics (ROC) analysis of peripheral blood eosinophils and fractional exhaled nitric oxide (FE_{NO}) as biomarkers of sputum eosinophilia. The *red line* shows the ROC curve for peripheral blood eosinophils, where the highest combination of sensitivity and specificity was reached at a threshold of 220/μl, which yielded 72% sensitivity and 69% specificity (area under the curve, 0.77). The *black line* shows the ROC curve for FE_{NO}, where the highest combination of sensitivity and specificity was achieved at a threshold of 20 ppb, which yielded 64% sensitivity and 73% specificity (area under the curve, 0.72).

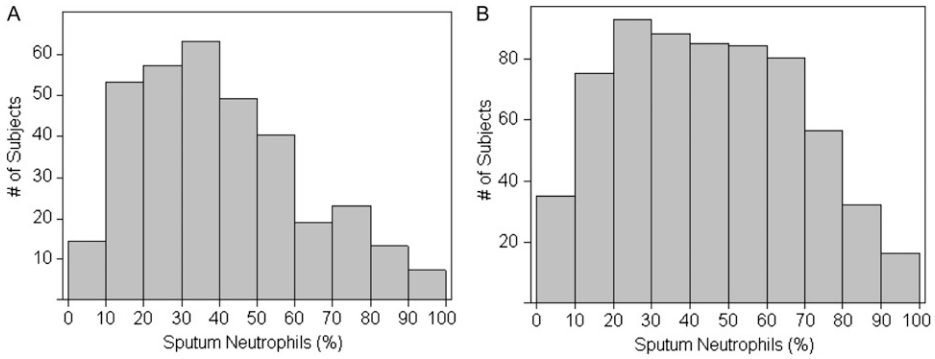


Figure 3. Cross-sectional analysis of sputum neutrophils in mild-to-moderate asthma. (*A*) Frequency distribution of sputum neutrophils in subjects not treated with inhaled corticosteroids (ICS) at baseline assessment (n = 350). (*B*) Frequency distribution of sputum neutrophils in subjects treated with ICS at baseline (n = 645).

To determine whether clinical characteristics differed among the three eosinophil phenotypes, we restricted our analyses to the 157 ICS-negative subjects with repeated measures, reasoning that single measures or steroid treatment could result in misclassification of eosinophil subtypes and confound our analyses. The eosinophilic phenotypes tended to be younger than the patients with persistently noneosinophilic disease, although the proportions of subjects whose asthma had started in childhood were similar in all three subgroups (Table 2). Interestingly, the persistently eosinophilic subgroup was much more likely than the noneosinophilic subgroup to be of normal body weight (Figure 5). Body mass index was normal in 73% of the persistently eosinophilic subgroup compared with only 45% of the noneosinophilic subgroup and 32% of the American population-at-large (30). Although average FEV_1 values were not significantly different among subgroups (Table 2), the percentage of subjects whose FEV_1 was less than 80% predicted was higher in the persistently eosinophilic subgroup (Figure 5). In addition, bronchial hyperresponsiveness differed

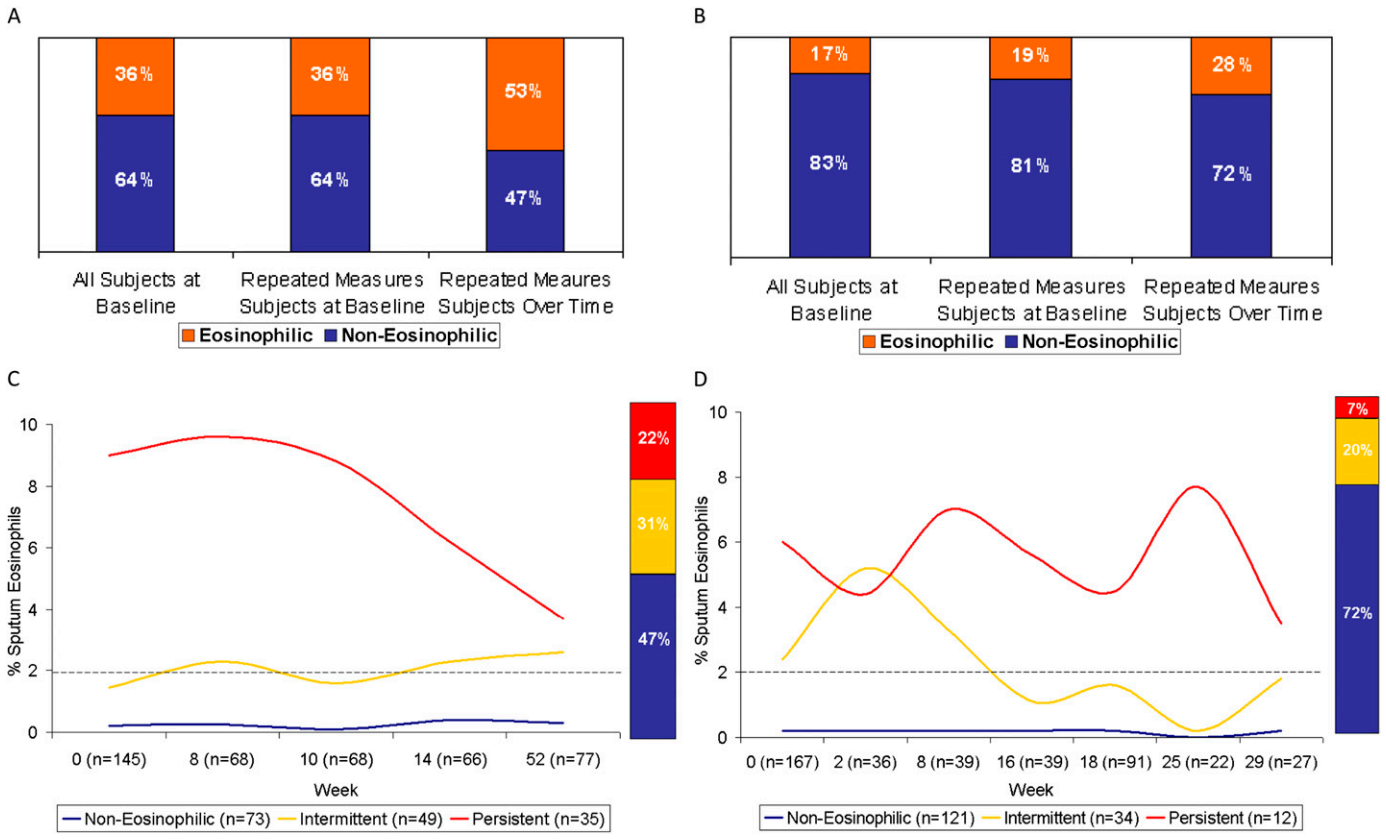


Figure 4. Sputum eosinophil percentage in the inhaled corticosteroid (ICS)− and ICS-positive subjects with asthma assessed by single and repeated measures. (*A*) Subjects with asthma not treated with steroids (ICS−). Frequency of sputum eosinophilia in all subjects at baseline (n = 350) compared with the baseline frequency in the subgroup who had repeated measures (n = 157), and with the frequency of eosinophilia during any one of two to four sputum inductions. (*B*) Subjects with asthma treated with steroids (ICS-positive). Frequency of sputum eosinophilia in all subjects at baseline (n = 645) compared with the baseline frequency in the subgroup who had repeated measures (n = 167), and with the frequency of eosinophilia during any one of two to four sputum inductions. (*C* and *D*) Median sputum eosinophil percentage at different time points in the ICS− (*C*) and ICS-positive (*D*) asthma subgroups. The graphs reveal three subgroups of subjects with asthma: (*1*) those with persistently eosinophilic asthma whose sputum eosinophil percentage is greater than or equal to 2% on every occasion it was measured, (*2*) those with intermittently eosinophilic asthma whose sputum eosinophil percentage is greater than 2% on at least one occasion but can be less than 2% on some occasions, and (*3*) a third subgroup whose sputum eosinophil percentage is persistently less than 2% on all occasions measured. Note that the number of subjects varies at each time point; as indicated in the main manuscript, the number of repeated measures ranged from two to four.

TABLE 2. CLINICAL CHARACTERISTICS OF EOSINOPHILIC SUBTYPES OF MILD-TO-MODERATE ASTHMA

	Persistently Eosinophilic	Intermittently Eosinophilic	Noneosinophilic	*P* Value
N	31	54	73	
Age	31.2 ± 1.8	29.9 ± 10.7	34.4 ± 10.6	0.04
Females, N (%)	20 (65)	37 (69)	42 (58)	0.30
Race, N (%)				0.52
Black	0	12 (22)	9 (12)	
White, Hispanic	26 (84)	38 (70)	52 (71)	
White, non-Hispanic	4 (13)	2 (4)	6 (8)	
Other	1 (3)	2 (4)	6 (8)	
Childhood-onset asthma, N (%)	23 (77)	42 (79)	50 (70)	0.33
History of exacerbation in prior 12 mo, N (%)	7 (23)	25 (46)	19 (26)	0.52
Albuterol use, puffs per day	0.71 (0–3.85)	0.81 (0–2)	0.42 (0.08–1.50)	0.08
FEV_1% predicted	84.4 ± 12.5	87.6 ± 12.5	87.3 ± 10.5	0.39
PC_{20}, mg/ml	0.51 (0.34–0.72)	0.65 (0.30–1.25)	1.72 (0.82–3.83)	<0.001
FE_{NO}, ppb	27 (22.3–42.4)	22.2 (15.5–28.1)	13.2 (10–20.1)	<0.001
Peripheral blood eosinophils, per μl	367 (265–600)	255 (158–321)	150 (115–220)	<0.001
Total IgE, IU	312 (179–477)	201 (76–328)	115 (43–220)	0.02
Sputum eosinophils, %	7.9 (5.5–15.1)	2.7 (1.8–4.9)	0.4 (0.1–0.7)	<0.001
Sputum neutrophils, %	33 (28–47)	36 (25–51)	42 (27–59)	0.15

Definition of abbreviations: FE_{NO} = fractional exhaled nitric oxide; IQR = interquartile range.
Data are mean ± SD or median (IQR), unless otherwise indicated.

among subgroups (Table 2) and the percentage of subjects with severe bronchial hyperresponsiveness was higher in the persistently eosinophilic subgroup (Figure 5). Notably, atopy was not confined to the eosinophilic phenotypes; most of the subjects who were persistently noneosinophilic were also atopic, as evidenced by their IgE and aeroallergen sensitization data (Table 2, Figure 5). Finally, sputum neutrophilia was uncommon in all groups, although it did occur in 21% of the persistently noneosinophilic subgroup (Figure 5).

To determine whether responses to asthma treatment differed among the three eosinophil phenotypes, we studied a subset of the ICS-negative subgroup (n = 77) who had received a period of intense combined asthma therapy in the ACRN IMProving Asthma Control Trial (17). The period of intense combined asthma therapy included 10–14 days of treatment with 0.5 mg of prednisone/kg/day, 800 μg of budesonide twice daily, and 20 mg of zarfirlukast twice daily. Subjects had been without ICS for 10 weeks before this period of intense combined asthma therapy. We found that the 2 weeks of combined antiinflammatory therapy caused significant improvements in airflow obstruction in eosinophilic asthma, but not in persistently noneosinophilic asthma (Figure 6). In contrast, bronchodilator responses to albuterol were similar in eosinophilic and noneosinophilic asthma (Figure 6).

The clinical characteristics of the persistently noneosinophilic subgroup are of interest, because of the possibility that they might suggest specific disease mechanisms. To provisionally explore the clinical heterogeneity apparent in the persistently noneosinophilic subgroup, we performed an unsupervised cluster analysis in the 157 ICS-negative subjects to determine if specific clinical characteristics grouped together. This analysis revealed three clusters (Table 3). One cluster comprised 42% of the 157 subjects and was characterized by younger age, female sex, lean body mass, well-preserved FEV_1, and moderate-to-severe bronchial hyperresponsiveness. It resembles a cluster identified previously by others (31). Another cluster comprised 22% of the subjects and was characterized by older age, female sex, later-onset asthma, high body mass index, low lung function, high sputum neutrophils, low IgE levels, and infrequent skin test reactivity to aeroallergens. The grouping of older heavier females with asthma of late onset has been described previously (31, 32). A third cluster comprised 36% of the subjects and was characterized by younger age, lean body mass, male sex, low FEV_1, childhood-onset asthma, and sensitivity to fungal aeroallergens (especially to *Alternaria*). Interestingly, a previous study has described two subphenotypes of childhood asthma based on the presence or absence of *Alternaria* skin test reactivity at age 6 (33), and a characteristic of children positive for *Alternaria* was their high rate of asthma persistence after age 11.

DISCUSSION

Previous studies have called attention to heterogeneity in eosinophil phenotypes among people with asthma (7–13), but estimates for the prevalence of noneosinophilic asthma in these prior studies were generated from single sputum samples. The importance and novelty of our study is that we show that sputum eosinophilia is persistently absent in a large subgroup of mild-to-moderate asthma when sputum is analyzed repeatedly over time. Subjects whose asthma was persistently noneosinophilic had a very poor response to a period of intense combined treatment with asthma controller treatments, despite showing good responses to bronchodilator therapy. These data suggest that a sizeable subgroup of mild-to-moderate asthma has a disease phenotype that is not the usual eosinophilic, steroid-responsive subtype, but a different subtype whose mechanisms are poorly understood and for which new controller treatments are needed.

Among subjects with asthma with eosinophilia, some had it persistently and others had it intermittently. The clinical characteristics of eosinophilic asthma are most evident in the persistently eosinophilic subtype. Here we found a relatively homogeneous asthma phenotype characterized by normal body weight and greater bronchial hyperresponsiveness. Our data reporting the association of normal body mass index with persistent eosinophilic asthma are novel, and a relative paucity of eosinophils in the airway of overweight or obese people with asthma may contribute to the relative glucocorticoid insensitivity observed in obese people with asthma (34–36). Our findings also support the emerging concept that eosinophils play a role in metabolic homeostasis and prevention of obesity (37). Specifically, studies in mice show that eosinophils in white adipose tissue secrete Th2 cytokines to induce alternative activation of macrophages, promote glucose homeostasis, and prevent obesity.

The intermittently eosinophilic subgroup was larger than the persistently eosinophilic subgroup and was characterized by lower average eosinophil percentages that often fell below the 2% cutoff for eosinophilia in repeated sputum analyses. Importantly, the treatment responses of intermittently eosinophilc

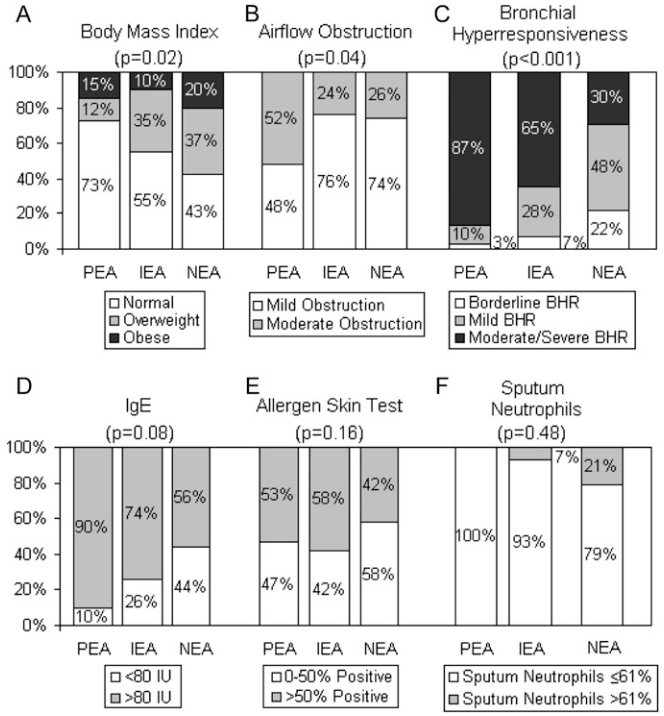
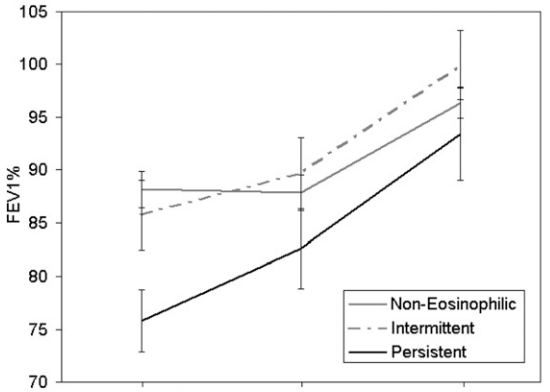


Figure 5. Clinical characteristics of eosinophil phenotypes in 157 inhaled corticosteroids (ICS)− subjects with repeated sputum measures. The histograms show the percentage of subjects in each eosinophil subgroup categorized by abnormalities in body mass index, FEV_1, PC_{20} for methacholine, serum IgE level, aeroallergen skin test reactivity, and sputum neutrophils. The comparative statistics are the *P* values for trend using ordinal logistic regression to assess for trends among the eosinophil subgroups. (*A*) Body mass index categorized as normal (<25 kg/m^2); overweight (25–30 kg/m^2); or obese (>30 kg/m^2). (*B*) FEV_1 categorized as normal (FEV_1 ⩾80%) or abnormal (FEV_1 61–79%). (*C*) PC_{20} for methacholine was categorized as moderately to severely abnormal (PC_{20} <1 mg/ml); mildly abnormal (PC_{20} 1–4 mg/ml); or borderline abnormal (PC_{20} 4–16 mg/ml). (*D*) IgE was categorized as normal (<80 IU) or high (⩾80 IU). (*E*) Allergen skin test reactivity categorized based on the number of positive skin tests to a panel of 10–12 aeroallergens; the two categories were designated as a positive reaction to more than or less than half of the allergens tested. (*F*) Sputum neutrophils categorized as normal (<61% neutrophils) or abnormal (>61% neutrophils). BHR = bronchial hyperresponsiveness; IEA = intermittently eosinophilic asthma; NEA = noneosinophilic asthma; PEA = persistently eosinophilic asthma.



%Δ in FEV_1 (L)	Non-Eosinophilic	Intermittent	Persistent	p
Pre- to Post-PICT	-0.2%	4.7%	8.6%	0.001
Post-PICT to Max Rev	10.1%	12.1%	13.5%	0.32

Figure 6. Change in FEV_1 in a subgroup of the inhaled corticosteroid–negative group (n = 77) who received a period of intense combined therapy (PICT) for 10–14 days. The PICT consisted of 0.5 mg of prednisone/kg/day, 800 μg of budesonide twice per day, and 20 mg of zarfirlukast twice per day. Prebronchodilator FEV_1 was measured before and after the PICT. In addition, maximal bronchodilator reversibility was measured after the PICT and involved measures of spirometry after up to 720 μg of inhaled albuterol, as previously described (17). The data represent means and standard error.

asthma mirrored those of persistently eosinophilic asthma. Thus, intermittently eosinophilic asthma can be considered a less severe form of eosinophilic asthma and not necessarily a distinct pathologic phenotype. The advantage of categorizing intermittent and persistent eosinophil subtypes separately is that this categorization emphasizes that repeated measures of sputum eosinophils are necessary to properly identify eosinophilic asthma. In this regard, it is notable that peripheral blood eosinophils and FE_{NO} cannot be relied on to identify eosinophilic asthma phenotypes, because both of these tests were not sufficiently sensitive as biomarkers of sputum eosinophilia.

Persistently noneosinophilic asthma comprised approximately half of subjects with asthma with mild-to-moderate disease in our study. The median values for sputum eosinophils in this subgroup were consistently closer to 0% than to the 2% cutoff on each of the repeated measure time-points. This provides confidence that these subjects indeed were noneosinophilic and were not a subgroup falling just below the 2% cutoff and thereby possibly explained by measurement variability. We considered the possibility that the low sputum eosinophil percentage could reflect good asthma control in the subjects studied and that estimates could be much higher in patients with less optimal disease control. In analyses to address this possibility, we surprisingly found that only 15% of subjects who did not achieve good control with ICS treatment had sputum eosinophilia; this compared with 26% of subjects with sputum eosinophilia among subjects with asthma who did achieve good asthma control with ICS treatment. We interpret this apparent discrepancy as revealing how the poor asthma control subgroup is enriched in noneosinophilic people with asthma (because steroids do not control asthma in patients with this disease subtype).

The subjects with asthma who were persistently noneosinophilic did not have an FEV_1 response to a 2-week period of intense treatment with oral and ICSs and oral zafirlukast. They had the potential to respond as evidenced by a 10% improvement in FEV_1 with albuterol treatment. Although other studies have shown sputum eosinophils to be important in treatment responses to corticosteroids in asthma (7, 38, 39), and we have shown previously that the Th2-low molecular phenotype of asthma (which has low airway eosinophils) does not respond well to corticosteroids (10), the data we report here provide stronger evidence that noneosinophilic asthma represents a disease phenotype that does not benefit from corticosteroid treatment. The high doses of corticosteroids given in the period of intense combined therapy and the documentation of a bronchodilator response to albuterol show that inadequate steroid dosing and fixed airflow obstruction are not explanations for poor steroid responsiveness in this subgroup. Our careful classification of eosinophil phenotype is also important because we show that it is the persistently noneosinophilic subgroup that does not respond; the intermittently eosinophilic subgroup has treatment responses that mirror those of the persistently eosinophilic subgroup. Additional studies of steroid responsiveness in persistently noneosinophilic asthma are needed and should include evaluation of asthma exacerbation outcomes. However, previous clinical trials of corticosteroids in asthma have also identified sizeable subgroups with poor treatment responses

TABLE 3. CLINICAL CHARACTERISTICS OF THREE CLUSTERS OF PERSISTENTLY NONEOSINOPHILIC SUBJECTS WITH ASTHMA*

Baseline Measure	Cluster 1	Cluster 2	Cluster 3
N	31	16	26
Age	30.8 ± 7.9	46.8 ± 8.2	30.9 ± 8.6
Males, N (%)	8 (26)	6 (38)	16 (62)
FEV_1%	91.7 ± 11.8	85.2 ± 9.1	82.7 ± 9.4
Body mass index	24.7 ± 3.7	31 ± 7.1	27.4 ± 4.5
Childhood-onset asthma, N (%)	17 (59)	8 (50)	24 (92)
PC_{20}, mg/ml	1.12 (0.41–2.73)	3.83 (1.16–6.84)	1.91 (0.57–3.57)
Albuterol use, puffs per day	0.4 (0.1–1.1)	0.2 (0–1)	0.7 (0.1–2.3)
Asthma exacerbation in prior 12 mo, N (%)	7 (23)	4 (25)	9 (35)
Percent positive allergen skin tests, median†	25 (17–42)	29 (17–50)	54 (42–75)
Fungal aeroallergen sensitivity, N (%)‡	12 (39)	6 (38)	19 (73)
Alternaria sensitivity, N (%)	5 (16)	6 (38)	15 (57)
Aspergillus sensitivity, N (%)	7 (23)	2 (13)	6 (23)
Cladosporium sensitivity, N (%)	3 (10)	1 (6)	4 (15)
Sputum neutrophils, %	27.7 (20.1–37.9)	62.1 (55–74.2)	49.4 (31.4–64.3)

Definition of abbreviations: IQR = interquartile range.

Data are mean ± SD or median (IQR), unless otherwise indicated.

*Variables included in the cluster analysis were age; sex; childhood onset; exacerbation history in prior 12 months; body mass index; asthma quality of life questionnaire score; short-acting β-agonist use; percent positive allergen skin tests; fungal aeroallergen sensitivity; FEV_1% predicted; and percent of nonsquamous cell count totals for sputum cell types (macrophages, neutrophils, eosinophils, lymphocytes, and epithelial cells).

†The data refer to the percentage of subjects who were positive per aeraoallergen panel (e.g., the typical patient in cluster 1 had 3 of 12 aeroallergen skin tests react positively, so that the statistic reflects that the median number of positive tests per person was 25%).

‡Specific fungal aeroallergen data were only available for 61 of the 73 subjects.

(9, 40), and we argue that these poor steroid treatment responses are because of asthma phenotypes, such as persistent noneosinophilia, that are not steroid sensitive.

The clinical data from the persistently noneosinophilic subjects with asthma showed that this is not a homogeneous group of patients with obvious clinical identifiers. In preliminary unsupervised analyses to identify possible clusters of clinical characteristics within the persistently noneosinophilic asthma subgroup, we identified sex, body weight, presence or absence of neutrophilia, and sensitivity to fungal aeroallergens as important variables likely to underlie mechanisms of noneosinophilic asthma. Some of these variables have also been associated with noneosinophilic asthma phenotypes in other studies (31, 41). Notably, however, our analysis identified a novel cluster characterized by asthma occurring in nonobese males who develop asthma in childhood and who have sensitivity to *Alternaria*. Male children frequently have sensitization to *Alternaria*, and epidemiologic studies suggest that, unlike other forms of male childhood asthma, it does not remit (42–44). Thus, adult males with asthma and *Alternaria* sensitivity may represent a cohort of males whose asthma persists from childhood. The mechanisms by which *Alternaria* causes sensitization and asthma in males are unknown, but Th17 cells may be involved because they are already implicated in asthma, are known to be involved in fungal immune responses at mucosal surfaces, and do not always use eosinophils as effector cells.

The identification of persistently noneosinophilic asthma as a common phenotype of mild to moderate asthma that responds poorly to currently available asthma controller medications has important implications for future research in asthma. One implication is that clinical studies in subjects with asthma should characterize the eosinophil phenotype of enrolled patients to enable better understanding of disease mechanisms and treatment responses. Another is that *in vitro* and mouse models of asthma, currently heavily weighted toward study of mechanisms of eosinophilic airway disease, need to better emphasize the study of mechanisms of noneosinophilic airway disease.

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