

Consistency of sputum eosinophilia in difficult-to-treat asthma: A 5-year follow-up study

To the Editor:

Several inflammatory phenotypes have been described in patients with difficult-to-treat asthma, including the eosinophilic and noneosinophilic phenotypes.¹ Eosinophilic airway inflammation that persists despite vigorous anti-inflammatory treatment has been associated with more severe asthma, frequent exacerbations,² and loss of lung function.³ Notably, persistent sputum eosinophilia has therapeutic consequences, because it allows tailored steroid therapy² as well as targeted treatment with new biologicals in severe refractory asthma.^{4,5}

We investigated whether eosinophilic airway inflammation is a consistent feature over the years and, if so, which clinical characteristics are associated with permanent eosinophilic inflammation. In addition, because airway eosinophilia has been reported to be associated with elevated levels of nitric oxide in exhaled air (FeNO), we assessed the stability of this marker.

Data were collected from 136 adult patients with difficult-to-treat asthma who participated in a longitudinal follow-up study. Characteristics of patients and methods are described elsewhere in detail.^{3,6,7}

In 1998 and 1999 (visit 1), patient characteristics (sex, age, atopic status, asthma onset and duration, medication use), prebronchodilator and postbronchodilator FEV₁, bronchial hyperresponsiveness to histamine, eosinophils in peripheral blood and induced sputum, the fraction of exhaled nitric oxide (FeNO at 100 mL/s, NOA 270B; Sievers, Boulder, Colo), and computed tomography scan of the paranasal sinuses were assessed. Five years later (visit 2), patients were re-examined with respect to medication use, lung function (prebronchodilator and postbronchodilator FEV₁), eosinophils in peripheral blood and induced sputum, and FeNO. Patients had to be clinically stable without asthma exacerbations for at least 1 month before their visits to the clinic. The same standardized methods for the different measurements were used as those at baseline. During the 5-year interval, patients were followed and treated according to usual care by their individual chest physicians.

Permanent sputum eosinophilia was defined as sputum eosinophils $\geq 2\%$ on both time points, based on normal values in healthy adults. We analysed differences between patients with and without permanent sputum eosinophilia, stability of sputum eosinophils and FeNO over 5 years, associations between the presence of permanent sputum eosinophilia and clinical or inflammatory characteristics, and the relationship between sputum eosinophils and FeNO.

Of 136 patients enrolled at baseline, 101 agreed to participate in the follow-up study 5 years later. From 66 patients, adequate baseline sputum samples were available. In 44 (67%) patients, a second adequate sputum sample could be obtained 5 years later. Median follow-up was 5.8 (range, 5.2-6.4) years.

At baseline, 20 patients (45%) had sputum eosinophils $\geq 2\%$. Of these patients, 14 (70%) had sputum eosinophils $\geq 2\%$ at the second visit. Ninety-six percent (23 of 24) without initial eosinophilia ($<2\%$) were also noneosinophilic at follow-up (Fig 1).

Sputum eosinophils were relatively stable, showing an intra-class correlation coefficient Ri of 0.67 (range, 0.47-0.81). The

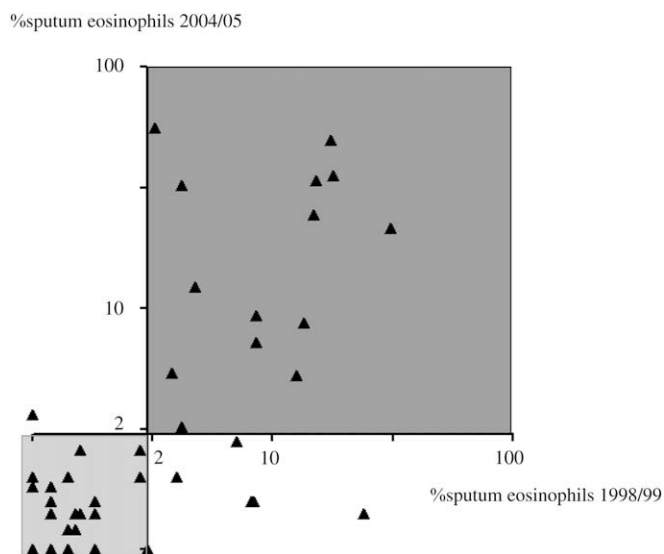


FIG 1. Persistence of the (non-) eosinophilic phenotype. Sputum eosinophil percentages at baseline vs 5 years later. Dark gray box represents data of patients with persistent eosinophilia in sputum. Light gray box represents data of patients with persistent noneosinophilia. Other boxes represent incongruent data between 2 time points. Axes have a logarithmic scale.

coefficient of repeatability (Bland & Altman) was 0.80 for log sputum eosinophil percentage, also indicating low variability of sputum eosinophils over time (see this article's Fig E1 in the Online Repository at www.jacionline.org).

Patients with permanent sputum eosinophilia had later onset of asthma, were less often atopic, had higher blood eosinophils and computed tomography sinus scores, and had a lower FEV₁ than patients without permanent sputum eosinophilia (see this article's Table E1 in the Online Repository at www.jacionline.org). There was no association between changes in inhaled or oral steroid dose and changes in sputum eosinophil percentage over time ($P > .14$).

Odds ratios for clinical or inflammatory factors potentially associated with permanent sputum eosinophilia are shown in Table I. Extensive sinus disease (computed tomography sinus score ≥ 12) appeared to be the only independent factor associated with permanent sputum eosinophilia (odds ratio, 9.2; 95% CI, 1.1-75.4).

The percentage of eosinophils in induced sputum was weakly correlated with FeNO at baseline and 5 years later ($r = 0.38$, $P = .01$; and $r = 0.41$, $P = .007$, respectively). Compared with eosinophils, FeNO was equally reproducible between 1998 to 1999 and 2004 to 2005, with a Ri of 0.71 (range, 0.52-0.84) and a coefficient of repeatability of 0.26 (graph not shown).

This study shows that the presence of sputum eosinophilia in patients with difficult-to-treat asthma is a consistent feature over a period of 5 years in the vast majority of patients and that the percentage of sputum eosinophils is highly reproducible. Adult-onset asthma, extensive sinus disease, high peripheral blood eosinophil counts, and persistent airflow limitation are associated with permanent sputum eosinophilia, but extensive sinus disease is the only independent factor.

FeNO levels were only weakly correlated with sputum eosinophils, but showed similar consistency over time. This implies that patients with eosinophilic airway inflammation despite vigorous steroid treatment do represent a separate phenotype,

TABLE I. Odds ratios for factors potentially associated with permanent sputum eosinophilia

	Odds ratio	(95% CI)	Adjusted odds ratio	(95% CI)
Asthma onset ≥ 18 y	3.8	(0.95-14.8)		
Atopic	0.3	(0.1-1.0)		
FEV ₁ $\leq 75\%$ predicted	4.3	(1.1-17.1)	3.6	(0.4-31.5)
PC ₂₀ histamine ≤ 1 mg/mL	2.2	(0.3-15.2)		
CT sinus score ≥ 12	11.2	(1.7-72.3)	9.2	(1.1-75.4)
FeNO ≥ 11 parts per billion	1.8	(0.5-6.9)		
Blood eosinophils $\geq 0.45 \cdot 10^9/L$	13.3	(2.6-68.6)	2.8	(0.3-28.7)

PC₂₀histamine, Provocative concentration of histamine causing a 20% reduction in FEV₁. CT, Computed tomography.

and that FeNO may provide additional information on the inflammatory process in the airways.

This study is the first follow-up study in patients with difficult-to-treat asthma, showing that the eosinophilic phenotype is consistent over time. One earlier observational study in patients with asthma of varying severity showed that 10 of 11 patients classified as having eosinophilic asthma remained in that classification on the second visit after 5 years.⁸ Interestingly, in the current study, the absence of sputum eosinophilia seemed to be even more consistent. Persistent noneosinophilia might be caused by adequate suppression of airway inflammation by inhaled steroid treatment, whereas other steroid-unresponsive factors such as predominant neutrophilic inflammation still cause symptoms. Taken together, although the precise mechanisms underlying the eosinophilic as well as the noneosinophilic phenotypes remain unresolved, both phenotypes seem to be clinically distinct and stable over time.

More frequent measurements of sputum eosinophils over time might have strengthened our finding that sputum eosinophils are highly reproducible; however, the strength of our study is that measurements were performed during the natural course of the disease, without planned interventions, and despite variation in exposure to asthma triggers in between and preceding the 2 measurements. There was no correlation between changes in sputum eosinophils and inhaled or oral corticosteroid dose, indicating that anti-inflammatory treatment was not an explanatory factor.

Remarkably, exhaled nitric oxide levels were as reproducible as sputum eosinophil percentages, but the association between sputum eosinophils and exhaled nitric oxide in our study was weak. The latter finding confirms earlier observations in patients with severe asthma, showing that FeNO and sputum eosinophil levels are not simply interchangeable.⁹ Although permanent eosinophilic inflammation seems to be related to chronic rhinosinusitis, persistent airflow limitation, and uncontrolled disease, elevated levels of FeNO may provide additional information about chronic injuring processes in the airways that ultimately lead to loss of lung function.⁷

The mechanisms of persistent eosinophilic airway inflammation are not clear. Intense inflammatory processes caused by relative steroid resistance in the airways or inflamed tissue in parts of the body that cannot be easily reached by inhaled corticosteroids such as the paranasal sinuses and the peripheral airways have been postulated. In this study, severe sinus disease was the only independent factor associated with permanent sputum eosinophilia. Several studies support a causal relationship

between the 2 conditions,¹⁰ although the precise mechanisms of this interaction are still obscure.

Recently, specific therapy targeted at reducing circulating and tissue eosinophils has shown promising results for patients with asthma with persistent eosinophilia. It has been demonstrated that the novel humanized mAb against IL-5, mepolizumab, reduces exacerbations, improves asthma-related quality-of-life scores, and allows prednisone sparing in patients with refractory asthma and sputum eosinophilia.^{4,5} The next step will be to demonstrate that this new drug also improves chronic rhinosinusitis and prevents progressive loss of lung function in severe refractory asthma.

In conclusion, sputum eosinophilia is a consistent feature over time in patients with difficult-to-treat asthma despite treatment with high doses of corticosteroids. Persistent sputum eosinophilia should be considered the key characteristic of a specific asthma phenotype characterized by adult onset of the disease, absence of atopy, extensive sinus disease, and persistent airflow obstruction. Patients with this asthma phenotype have severe exacerbations and loss of lung function and are therefore the ideal candidates for targeted therapy with new biologicals aimed at reducing eosinophil recruitment and activation.

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Pulmonary nontuberculous mycobacterial infections in hyper-IgE syndrome

To the Editor:

Nontuberculous mycobacteria (NTM) are commonly isolated from patients with airway clearance defects, such as cystic fibrosis (CF) and primary ciliary dyskinesia (PCD), and from asthenic postmenopausal women with scoliosis, pectus abnormalities, and mitral valve prolapse.¹⁻³ Patients with autosomal dominant signal transducer and activator of transcription 3 (*STAT3*) deficiency (hyper-IgE syndrome [HIES] or Job syndrome) have recurrent pyogenic respiratory infections, frequently with pneumatoceles and bronchiectasis. Once pneumatoceles, bronchiectasis, or both are present, the predominant infections change from *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae* to *Pseudomonas aeruginosa* and *Aspergillus fumigatus*.^{4,5} NTM have not been considered typical pathogens in HIES. We reviewed NTM infections in a large cohort of patients with HIES.

Patients given diagnoses of HIES based on clinical scores and expert opinion of the investigators and confirmed by means of *STAT3* sequencing were followed in an institutional review board–approved study.⁶

Microbiology records from April 1977 to November 2007 were queried for lower respiratory tract specimen mycobacterial smear and culture results. Mycobacteriology was recorded with other respiratory organisms and specimen source. We identified 3 groups: patients with positive NTM cultures and who met the American Thoracic Society (ATS) disease criteria [NTM+/ATS(–)], patients with positive NTM cultures but who did not meet the ATS criteria [NTM+/ATS(–)], and patients with negative NTM cultures [NTM–/ATS(–)].⁷ We reviewed age, pulmonary function test results, and chest computed tomographic (CT) scans (at or nearest to the time of the first positive culture for NTM+ and last available for NTM–) for each patient. Thoracolumbar radiographs (not available for 1 patient) determined the degree of scoliosis. Each CT scan was scored for bronchiectasis, pneumatoceles or cysts (combined), nodules, and alveolar infiltrates.

Sputum was processed with N-acetyl-L-cysteine sodium hydroxide, concentrated by means of centrifugation for smear staining with auramine-rhodamine, and cultured on solid and liquid media. Accuprobe (GenProbe, San Diego, Calif), molecular analysis (16S, hsp65, secA sequencing) or (before 2005) HPLC and biochemical testing were used to identify organisms.³

Summary statistics are presented as means $\pm$ SDs. Continuous variables were compared by using 1-way ANOVA, and frequencies of categorical variables were compared with the χ^2 test.

Of the 62 patients with HIES followed, 32 (51%) had at least 1 specimen submitted for mycobacterial testing (17 female and 15 male patients; age, 2–56 years [31 ± 14 years] at the time of culture; HIES clinical score, 52–100 [79 ± 12]). *STAT3* mutation

was found in 30 of 32 patients with mycobacterial testing; DNA was unavailable for 2 deceased patients.

Nine (28%) of 32 patients grew NTM on at least 1 occasion (Table I). Five (16%) NTM+ patients met ATS criteria for pulmonary disease [NTM+/ATS(+)]: *Mycobacterium avium* grew in 3 patients, *Mycobacterium kansasii* grew in 2 patients, and *Mycobacterium massiliense*, *Mycobacterium mucogenicum*, and *Mycobacterium intracellulare* grew in 1 patient each. More than 1 species grew in 3 patients. NTM+/ATS(+) patients had 16 ± 9.5 specimens submitted for mycobacteriology, with 2 to 11 positive cultures (5.6 ± 4.5) each. They grew up to 10 other potential pathogens; *A. fumigatus* and *P. aeruginosa* were most common. Four patients did not meet ATS criteria [NTM+/ATS(–)]: 2 grew *M. avium*, 1 grew *Mycobacterium chelonae*, and 1 grew *Mycobacterium abscessus*. Cultures from NTM+/ATS(–) patients grew *A. fumigatus*, *S. aureus*, and *H. influenzae* in 2 patients each. NTM–/ATS(–) patients had 1 to 46 specimens (7.5 ± 11.8) submitted for mycobacteriology. Up to 17 other organisms grew, with *H. influenzae* most frequent (43%).

There were proportionately more female patients in the NTM+/ATS(+) group (80%, $P < .05$) than in the NTM+/ATS(–) or NTM–/ATS(–) groups (none and 57%, respectively). All NTM+/ATS(+) patients had at least 10° of scoliosis, as did 3 of 4 NTM+/ATS(–) patients; 63% of NTM–/ATS(–) patients had scoliosis (Table I). Percent predicted FEV₁ was the same [NTM+/ATS(+) patients, $54\% \pm 22\%$; NTM+/ATS(–) patients, $46\% \pm 25\%$; and NTM–/ATS(–) patients, $67\% \pm 21\%$; $P = .15$].

Bronchiectasis and cysts were identified in every patient in whom NTM grew (Fig 1). Nodules were seen in 4 of 5 NTM+/ATS(+) patients but in only 1 of the 4 NTM+/ATS(–) patients. Among NTM– patients, 4 (17%) of 23 had normal lungs on CT scans, bronchiectasis was found in 10 (43%), cysts and nodules were found in 8 (35%) patients each, and alveolar infiltrates were found in 7 (30%) patients. The prevalence of bronchiectasis and cysts was significantly different among the 3 groups ($P < .05$).

Treatment for NTM was initiated in all 5 NTM+/ATS(+) patients and none of the NTM+/ATS(–) patients. All who were treated showed microbiologic, radiologic, or both improvement. Patient 1 successfully cleared *M. avium* complex and continues on treatment for *M. abscessus*.

NTM pulmonary infections are common in patients with CF (13%) and PCD (10%), presumably because of impaired mucociliary transport.^{1,2} Like CF and PCD, HIES is characterized by bronchiectasis, nodules, and cavities.⁵ Once structural damage occurs in patients with HIES, *Aspergillus* and *Pseudomonas* species become common, as with other causes of bronchiectasis.⁸ Therefore it is not surprising that NTM infection in patients with HIES occurs in the setting of bronchiectasis and cavities at a similar frequency to that in patients with CF and PCD and is associated with increased radiographic severity of lung disease. Although no specific airway clearance defect has been identified in patients with HIES, it is clear that these patients have some abnormality making them susceptible to recurrent lung infections in general, as well as NTM infection and subsequent disease. NTM+/ATS(+) patients were disproportionately female and had significant scoliosis. Although common in Job syndrome (63%), this high frequency of scoliosis and the female predominance have been associated with other pulmonary NTM syndromes.^{3,9}

Although this is a retrospective study without systematic evaluation of the entire cohort of patients with Job syndrome, it

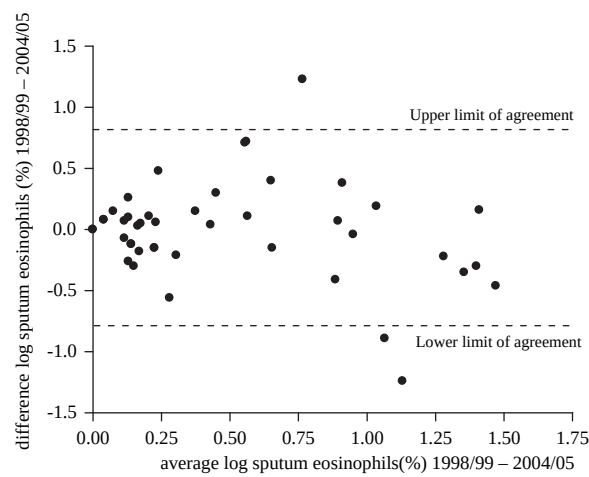


FIGURE E1.

TABLE E1. Characteristics of patients with and without permanent sputum eosinophilia

	No permanent eosinophilia (n = 30)	Permanent eosinophilia (n = 14)	P value
Age (y)	47.8 (10.7)	53.3 (13.3)	.15
Sex (% female)	60	50	.53
Asthma onset (y)*	16 (0.5-54)	36.5 (0.5-60)	.03
Asthma duration (y)*	29 (6-58)	14.5 (4-53)	.10
Atopy (%)	73%	43%	.05
Chronic oral steroids (%)	27%	36%	.72
ICU admission ever (%)	3%	36%	.009
≥3 Exacerbations/y (%)	50%	63%	.67
pb FEV ₁ (% predicted)*	83.0 (41.7-121.7)	59.8 (35.8-111.9)	.02
PC ₂₀ histamine*	1.38 (0.02-8.0)	0.18 (0.02-1.7)	.07
CT sinus score*	3 (0-24)	12 (0-29)	.02
FeNO (ppb)*	9.3 (2.0-72.0)	17.3 (6.3-69.7)	.06
Blood eosinophils (10 ⁹)*	0.14 (0.01-0.75)	0.66 (0.10-1.12)	.000

CT, Computed tomography; ICU intensive care unit; pb, postbronchodilator;

PC₂₀histamine, provocative concentration of histamine causing a 20% reduction in FEV₁.

Values in mean (SD) or median* (range). Permanent sputum eosinophilia is defined as sputum eosinophils ≥2% on both time points.