The transition of sputum inflammatory cell profiles is variable in stable asthma patients

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Background: The sputum inflammatory cell profile is an important indicator for classifying asthma phenotypes.

Objective: To investigate if sputum inflammatory cell profile remains stable and there are different characteristics between groups that show different profile over time in stable asthmatic patients.

Methods: A total of 149 asthmatic patients, who were clinically stable at the time of sputum examination and had undergone sputum analysis twice, were subjected to a detailed review. Eosinophilic inflammation was diagnosed when the proportion of the sputum eosinophils was >3%. We divided the patients into 4 groups according to the transition patterns of their sputum profiles: group 1, persistent eosinophilia; group 2, eosinophilic to noneosinophilic; group 3, noneosinophilic to eosinophilic; and group 4, persistent noneosinophilia. The results of the pulmonary function tests and other clinical parameters were compared between these 4 groups.

Results: Thirty-four of the initially eosinophilic asthmatic patients (39.5%; 34 of 86 patients) demonstrated noneosinophilic airway inflammation at their second sputum examination, and 24 of the initially noneosinophilic patients (38.1%; 24 of 63 patients) demonstrated eosinophilic airway inflammation at follow-up. Various clinical parameters, except the blood eosinophil count, demonstrated no significant differences between the eosinophilic and noneosinophilic asthmatic patients or among the 4 groups.

Conclusion: A substantial proportion of asthmatic patients who demonstrate a certain sputum inflammatory cell profile at the initial examination demonstrated profile transition in clinically stable settings over time. The clinical significance of using induced sputum analysis to phenotype stable asthmatic patients requires further evaluation.

Key words: Asthma; Sputum inflammatory cell; Eosinophils; Phenotype

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INTRODUCTION

Asthma is a heterogeneous disease. The phenotypes of asthma are classified according to both clinical and pathological factors. Since chronic airway inflammation is a fundamental feature of asthma, categorizing patients based on their inflammatory cells has also been emphasized.

To evaluate airway inflammation, invasive methods such as bronchial biopsy and bronchoalveolar lavage have been applied to asthmatic patients [1]. However, bronchoscopy is not only potentially hazardous but also hard to perform. Therefore, noninvasive methods for assessing airway inflammation have been employed, and analyzing induced sputum is the most commonly used test for asthma.

The simple and practical classification of asthma involves 2 categories: eosinophilic asthma (EA) and noneosinophilic asthma (NEA). In addition, 4 subtypes have also been proposed—eosinophilic, neutrophilic, paucigranulocytic, and mixed cellularity—depending on the presence or absence of sputum eosinophilia and neutrophilia [2]. Although the exact clinical implications for each inflammatory asthma phenotype should be further clarified, identifying these phenotypes may be useful in clinical practice, particularly for the eosinophilic subtype that is usually a good predictor of response to corticosteroids [3, 4]. Furthermore, many novel biologics for severe asthma target the eosinophilic phenotype by blocking the cytokines that are directly linked to eosinophilic infiltration, such as interleukin (IL)-4 [5], IL-5 [6-9], and IL-13 [10-13].

When interpreting the results of induced sputum analysis, there are several factors that can influence cellular profiles. Most importantly, corticosteroid-based medications are potentially critical since they could contribute to increased airway neutrophilia [14] and reduced eosinophilia [15]. Moreover, there is the possibility that the inflammatory cellular phenotypes for each patient do not remain stable over time.

Determination of the inflammatory phenotype before starting medications in newly diagnosed asthmatic patients helps to predict response to certain therapeutic options. However, there certainly exists an unmet need of evaluating the profiles of asthmatic patients on maintenance therapy in order to provide insights into the clinical course of the disease and modulate asthma medications according to the results of the sputum analysis. Nevertheless, repetitive sputum analysis is not routinely performed in clinical practice, nor has the stability of airway inflammatory patterns been extensively explored. Only a few studies have investigated, and the results of these reports are controversial [2, 16-20].

In our current study, we investigated if inflammatory subtypes are stable over time in patients with stable asthma and evaluated the differences in the clinical characteristics between groups that demonstrate different longitudinal transition patterns in terms of their sputum inflammatory cell profiles.

MATERIALS AND METHODS

Study patients and design

A total of 149 asthmatic patients (>18 years) receiving inhaled corticosteroids (ICS) who had undergone sputum analysis twice at the out-patient clinic of a tertiary referral hospital were included. The interval between the 2 induced sputum examinations varied among the patients (mean, 29.6 months; 95% confidence interval, 26.74–32.47). During the interval, patients have taken ICS and other asthma medication regularly.

All of the patients were diagnosed with asthma based on either the presence of airway hyperresponsiveness (provocative concentration of methacholine causing a 20% fall in forced expiratory volume in 1 second [PC20] < 16 mg/mL on methacholine bronchial provocation test) or positive bronchodilator test according to the American Thoracic Society criteria. All patients were clinically stable without asthma exacerbation at the moment of sputum examination. Patients with any symptoms of respiratory infection within 4 weeks before sputum examination, other pulmonary diseases, or significant comorbidity were excluded. This study was approved by the Institutional Review Board of Asan Medical Center (approval number: 2014-0971) and obtained signed informed consent from all patients.

All patients were subjected to a detailed retrospective review. Demographic data and various clinical information, such as atopic status, age at asthma onset, duration of asthma, ICS treatment duration, compliance, asthma control status, results of the pulmonary function tests, PC20 values, blood eosinophil counts, total IgE, and the results of the induced sputum analysis, were obtained at both sputum examinations. Before sputum induction all subjects were asked to fill up in a self-administered asthma control test questionnaire. Patients were evaluated adherence to ICS by using count of inhaler prescribed according to the medical
records, overall clinical judgment of physician, and inhaler technique assessment. An individual’s baseline compliance to ICS (range, 1–3) was based on count of inhaler prescribed according to the medical records, overall clinical judgment of physician, and inhaler technique assessment before the sputum induction; higher scores denote more compliance.

The data were analyzed and compared between the phenotypes and study groups that were classified according to the results of the induced sputum analysis, as described below.

Sputum induction and analysis

Sputum was induced using aerosolized 0.9% saline, followed by a 3%–5% NaCl solution through a nebulizer [21]. Patients were asked to blow their nose, rinse their mouth, and swallow water to minimize contamination by postnasal drip and saliva. The expectorated sputum was processed according to the protocol previously described [22]. The slides were stained with Giemsa, and differential cell counts were expressed as the percentage out of nonsquamous cells. Those with significant squamous cell contamination (>80%) were excluded from further analyses [23].

Classifications of the sputum inflammatory cellular patterns

A sputum eosinophil percentage ≥3% was used to define eosinophilic inflammation. The normal range for the sputum eosinophil count was determined using the cutoff point for the 95th percentile used to define healthy control groups [2]. A sputum neutrophil percentage ≥40% was considered neutrophilic inflammation as referenced by a previous report [24]. Based on these criteria, patients were divided in 2 different ways: (1) Patients were classified into 4 phenotypes—eosinophilic, neutrophilic, mixed granulocytic, or paucigranulocytic inflammation—by the eosinophil and neutrophil counts; and (2) Patients were divided into 2 phenotypes—EA or NEA—according to the presence of eosinophilic inflammation, regardless of the presence or absence of sputum neutrophilia.

Subjects with sputum neutrophil proportion ≥40% and <3% sputum eosinophil proportion were classified as neutrophilic inflammation and those with an eosinophil proportion ≥3% and <40% neutrophils were classified as eosinophilic inflammation. Subjects had both increased neutrophils and eosinophils and were classified as mixed granulocytic inflammation. A further group had normal levels of both neutrophils and eosinophils were classified as paucigranulocytic inflammation.

The eosinophilic phenotype consists of all subjects with more than 3% eosinophils; eosinophilic inflammation + the mixed granulocytic inflammation. And noneosinophilic phenotype consists of all subjects with less than 3% eosinophils; neutrophilic inflammation + the paucigranulocytic inflammation.

In addition, we also divided the patients into 4 groups according to the transition patterns of their sputum profiles: group 1, eosinophilic to eosinophilic phenotype; group 2, eosinophilic to noneosinophilic phenotype; group 3, noneosinophilic to eosinophilic phenotype; and group 4, noneosinophilic to noneosinophilic phenotype.

Statistical analysis

The results for the continuous variables were expressed as the mean ± standard deviation. Categorical data were reported using frequencies and percentages. The Kruskal-Wallis test was used to assess the different subgroups of patients with asthma. The Spearman rank correlation coefficient was used to assess the association between the blood cell counts and sputum cell counts. Results were considered significant when \( p < 0.05 \). Data analysis was performed using IBM SPSS Statistics ver. 20.0 (IBM Co., Armonk, NY, USA).

RESULTS

Comparison of the clinical characteristics of different asthma phenotypes, as defined using sputum eosinophils and neutrophils

The proportions of each inflammatory phenotype at the initial sputum examination were as follows: eosinophilic inflammation (n = 32, 21.5%), neutrophilic inflammation (n = 35, 23.5%), mixed granulocytic inflammation (n = 53, 35.6%), and paucigranulocytic inflammation (n = 29, 19.5%). There was no significant difference in the clinical characteristics between the 4 phenotypes (Table 1).

At the second sputum examination, asthmatic patients were again divided into 4 phenotypes: EA (n = 17, 11.4%), neutrophilic asthma (n = 58, 38.9%), mixed granulocytic inflammation (n = 59, 39.6%), or paucigranulocytic inflammation (n = 15, 10.1%). The clinical features of the 4 inflammatory phenotypes also demonstrated no significant differences.

Next, we compared the clinical characteristics of EA and NEA at the time of both sputum examinations. At the initial sputum examination, EA comprised 57.0% (n = 85) and NEA comprised
43.0% (n = 64) of the study population. At the second sputum examination, EA comprised 51.0% (n = 76) and NEA comprised 49.0% (n = 73). EA demonstrated a significantly higher level of blood eosinophils. There were no significant differences in other clinical characteristics between EA and NEA patients, as shown in Table 2. No differences were observed for ICS treatment duration (EA, 4.96 ± 6.43 years; NEA, 4.89 ± 6.02 years), ICS dose or compliance to ICS therapy.

There was a significantly positive relationship between the blood eosinophil counts and percentage of sputum eosinophils (r = 0.536, p < 0.001 at the initial examination; and r = 0.594, p = 0.002 at the second examination).

**Clinical characteristics of 4 groups defined by the different transition patterns of the sputum inflammatory cell profiles**

The changes in the sputum eosinophil and neutrophil counts between the first and second sputum examinations were remarkably variable (Fig. 1). The proportions of the each group were 34.9% (n = 52), 22.8% (n = 34), 16.1% (n = 24), and 26.2%
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(n = 39) for groups 1, 2, 3, and 4, respectively. The percentage of the patients who demonstrated sputum profile transition reached 39.9% of all patients (58 of 149 patients). Thirty-four of the initially EA patients (39.5%; 34 of 86 patients) were NEA at their second sputum examination. Twenty-four of the initially NEA patients (38.1%; 24 of 63 patients) demonstrated eosinophilic airway inflammation at the follow-up examination (Fig. 2). The initial blood eosinophil counts were significantly higher in group 1, which demonstrated persistent sputum eosinophilia, in comparison with the other groups. No other significant differences in the clinical features or laboratory findings obtained at the initial examination were found among the 4 groups. No differences were observed for ICS treatment duration, ICS dose or compliance to ICS therapy and forced expiratory volume in 1 second predicted level at the second examination among the four groups (Table 3).

**DISCUSSION**

In our study, a substantial number of asthmatic patients demonstrated certain sputum inflammatory cellular patterns that transitioned over time, in clinically stable settings. No factors other than blood eosinophil counts were found to predict persistence

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**Table 2. Clinical characteristics of eosinophilic and noneosinophilic asthma classified on the initial examination and the second examination**

| Variable                      | Eosinophilic asthma Initial exam | Eosinophilic asthma Second exam | Noneosinophilic asthma Initial exam | Noneosinophilic asthma Second exam | p-value
|-------------------------------|----------------------------------|---------------------------------|-------------------------------------|-----------------------------------|--------
| No. of patients               | 85 (57.0)                        | 76 (51.0)                       | 64 (43.0)                           | 73 (49.0)                         | 0.830  0.638
| Age (yr)                      | 49.6 ± 12.9                      | 50.0 ± 14.0                     | 51.8 ± 14.9                         | 51.1 ± 13.7                       | 0.037  0.154
| Sex                           | Male:female 43:50                | Male:female 45:38               | Male:female 43:54                   | Male:female 43:50                 | 0.337  0.250
| Asthma onset (y)              | 45.5 ± 14.4                      | 45.4 ± 14.8                     | 47.8 ± 15.7                         | 47.4 ± 15.1                       | 0.435  0.464
| ICS treatment duration (yr)   | 4.96 ± 6.43                      | 5.01 ± 5.66                     | 4.89 ± 6.02                         | 4.85 ± 6.82                       | 0.575  0.250
| Compliance                    | 2.41 ± 0.69                      | 2.30 ± 0.73                     | 2.41 ± 0.63                         | 2.41 ± 0.67                       | 0.903  0.469
| Asthma control (ACT)          | 21.36 ± 4.20                     | 21.68 ± 3.09                    | 20.89 ± 3.17                        | 20.43 ± 4.38                      | 0.154  0.247
| Nonsmoker (%)                 | 56.6                             | 54.8                            | 57.1                                | 59.7                              | 0.805  0.356
| Body mass index (kg/m²)       | 23.9 ± 2.6                       | 23.8 ± 3.0                      | 23.8 ± 4.8                          | 23.9 ± 4.3                        | 0.987  0.960
| Atopy (%)                     | 51.5                             | 52.6                            | 43.1                                | 43.3                              | 0.373  0.318
| Interval of sputum analysis (mo)| 276 ± 18.2                      | 276 ± 16.3                      | 32.2 ± 16.8                         | 31.7 ± 19.0                       | 0.116  0.158
| FEV₁ (% predicted)            | 74.8 ± 23.5                      | 76.6 ± 22.5                     | 74.7 ± 18.9                         | 79.6 ± 20.5                       | 0.990  0.395
| Prebronchodilator FEV₁/FVC ratio| 70.9 ± 14.9                     | 66.5 ± 15.1                     | 70.0 ± 11.9                         | 71.9 ± 12.7                       | 0.703  0.021
| FVC (% predicted)             | 88.4 ± 19.1                      | 93.5 ± 19.0                     | 88.3 ± 19.1                         | 90.0 ± 17.8                       | 0.988  0.245
| Blood neutrophil %            | 55.5 ± 12.5                      | 51.0 ± 12.0                     | 57.7 ± 12.4                         | 55.5 ± 10.2                       | 0.314  0.034
| Blood eosinophil %            | 7.2 ± 8.3                        | 8.1 ± 7.6                       | 3.6 ± 2.9                           | 4.3 ± 3.7                         | 0.001  0.001
| Sputum neutrophil %           | 50.1 ± 31.1                      | 59.7 ± 26.5                     | 48.3 ± 39.9                         | 70.4 ± 31.4                       | 0.765  0.026
| Sputum eosinophil %           | 26.8 ± 26.2                      | 18.1 ± 17.5                     | 0.4 ± 0.7                           | 0.7 ± 0.7                         | 0.000  0.000
| Immunoglobulin E              | 391 ± 574.6                      | 356 ± 389.3                     | 368 ± 411.4                         | 405 ± 266.2                       | 0.846  0.521
| C-reactive protein (mg/dL)    | 0.2 ± 0.4                        | 0.2 ± 0.2                       | 0.2 ± 0.3                           | 0.7 ± 2.6                         | 0.951  0.228
| ICS (mcg)                     | 750.8 ± 574.4                    | 742.4 ± 603.9                   | 740.6 ± 603.2                       | 766.9 ± 634.3                     | 0.741  0.626

Values are presented as mean ± standard deviation unless otherwise indicated.
ICS, inhaled corticosteroids; ACT, asthma control test; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity.
The patients were divided into 2 phenotypes—eosinophilic asthma or noneosinophilic asthma—according to the presence of eosinophilic inflammation, regardless of the presence or absence of sputum neutrophilia.
Defining asthma phenotypes is pivotal for implementing individualized treatments to each asthmatic patient. Clearly, the airway inflammatory pattern in steroid-naïve asthmatic patients could provide valuable information for determining the future clinical course and responsiveness to treatment. In particular, airway eosinophilia is a good predictor of response to inhaled steroids [3] and the likelihood of benefiting from higher doses of systemic corticosteroid treatment [4]. Trials that used sputum eosinophil percentages to guide treatment and predict asthma control report positive results [8, 10, 17, 25-27]. Furthermore, many novel biologics for severe asthma target eosinophilic phenotypes. On the other hand, trials that used sputum neutrophils to manage refractory asthma have reported mixed effectiveness of antibiotics [28-30]. Considering the clinical importance of the inflammatory patterns of asthma, defining airway inflammation phenotypes is pivotal for managing asthma patients. Despite the clinical usefulness of sputum examination for determining inflammatory phenotypes, it is unclear if the results of induced sputum analyses alone are sufficient and reliable for determining the true phenotypes.

Our results demonstrated a wide range of percentages for the sputum eosinophils and neutrophils, and more than half of all patients (n = 85, 57.0%) had eosinophilic inflammation, although all of our study patients were quite clinically stable and receiving the appropriate ICS treatment. Interestingly, various clinical parameters demonstrated no significant difference between eosinophilic and NEA as classified according to the results of the induced sputum analysis. These results suggest that the classification of airway inflammatory phenotypes in stable asthmatic patients receiving ICS may not significantly anticipate different clinical courses or prognoses. The phenotypes that were classified before ICS treatment would be more important for predicting the prognosis of asthma. In our study, appropriate ICS therapy was maintained for all subjects throughout the 2 study visits and, therefore, the possibility of the steroid medication

![Fig. 1. Transitions of the sputum eosinophil and neutrophil percentages between the first and second sputum examinations. (A) Sputum eosinophil percentages at each sputum examination. (B) Sputum neutrophil percentages at each sputum examination.](image)

![Fig. 2. Transitions of the sputum inflammatory cell profiles in eosinophilic and noneosinophilic asthmatic patients. EA, eosinophilic asthma; NEA, noneosinophilic asthma.](image)
Table 3. Clinical characteristics of the 4 asthma patient groups showing different sputum inflammatory cell profile transition patterns

| Variable                      | Group 1 (n = 52) | Group 2 (n = 34) | Group 3 (n = 24) | Group 4 (n = 39) | p-value |
|-------------------------------|------------------|------------------|------------------|------------------|---------|
| Age (yr)                      | 49.3 ± 12.4      | 50.1 ± 13.6      | 51.5 ± 17.1      | 51.9 ± 13.8      | 0.810   |
| Sex                           |                  |                  |                  |                  | 0.992   |
| Male:female                   | 25:27            | 12:22            | 13:11            | 16:23            |         |
| Male ratio                    | 48.1             | 35.2             | 54.2             | 41.0             |         |
| Asthma onset (yr)             | 44.2 ± 13.5      | 47.6 ± 15.5      | 48.5 ± 4.7       | 47.3 ± 15.0      | 0.704   |
| ICS treatment duration (yr)   | 5.12 ± 6.18      | 4.65 ± 6.80      | 4.79 ± 4.44      | 5.03 ± 6.93      | 0.547   |
| Compliance                    | 2.36 ± 0.72      | 2.46 ± 0.65      | 2.35 ± 0.61      | 2.48 ± 0.67      | 0.850   |
| Compliance*                   | 2.30 ± 0.79      | 2.40 ± 0.68      | 2.29 ± 0.61      | 2.43 ± 0.67      | 0.893   |
| Asthma control (ACT)          | 20.88 ± 4.31     | 21.90 ± 3.99     | 21.22 ± 2.59     | 20.78 ± 3.56     | 0.406   |
| Asthma control (ACT)*         | 21.58 ± 3.37     | 21.04 ± 4.03     | 21.93 ± 2.40     | 19.84 ± 4.70     | 0.282   |
| Nonsmoker (%)                 | 54.0             | 61.8             | 56.5             | 579              | 0.669   |
| Body mass index (kg/m²)       | 23.9 ± 2.5       | 23.7 ± 2.7       | 23.8 ± 3.9       | 24.0 ± 5.4       | 0.992   |
| Atopy (%)                     | 52.6             | 48.3             | 55.6             | 38.7             | 0.676   |
| Interval of visit (mos.)      | 26.1 ± 16.1      | 30.8 ± 21.3      | 30.9 ± 16.6      | 32.5 ± 16.9      | 0.340   |
| FEV₁ (% predicted)            | 72.0 ± 29.4      | 79.3 ± 22.8      | 75.0 ± 22.0      | 74.3 ± 17.4      | 0.500   |
| FEV₁ (% predicted)*           | 78.2 ± 22.8      | 86.70 ± 14.6     | 73.4 ± 21.8      | 73.3 ± 23.0      | 0.053   |
| FEV₁/FVC ratio                | 68.2 ± 16.6      | 75.3 ± 10.6      | 68.9 ± 13.7      | 70.4 ± 10.7      | 0.111   |
| FVC (% predicted)             | 87.4 ± 19.2      | 89.8 ± 18.2      | 91.9 ± 20.2      | 88.3 ± 19.1      | 0.630   |
| Blood neutrophil %            | 54.3 ± 13.1      | 575 ± 11.1       | 56.6 ± 14.1      | 58.3 ± 11.6      | 0.491   |
| Blood eosinophil %            | 8.3 ± 9.7        | 5.4 ± 4.7        | 3.8 ± 3.2        | 3.4 ± 2.6        | 0.002   |
| Sputum neutrophil %           | 44.9 ± 30.8      | 573 ± 30.4       | 49.3 ± 40.8      | 49.4 ± 35.0      | 0.457   |
| Sputum eosinophil %           | 32.9 ± 26.7      | 16.7 ± 22.5      | 0.5 ± 0.8        | 0.3 ± 0.6        | 0.000   |
| Immunoglobulin E              | 302.52 ± 275.67  | 590.00 ± 882.60  | 547.67 ± 912.74  | 240.88 ± 317.28  | 0.227   |
| C-reactive protein (mg/dL)    | 0.2 ± 0.4        | 0.2 ± 0.3        | 0.3 ± 0.3        | 0.2 ± 0.2        | 0.968   |
| ICS (mcg)                     | 818.08 ± 630.05  | 635.29 ± 459.44  | 733.33 ± 611.27  | 755.90 ± 610.03  | 0.766   |
| ICS (mcg)*                    | 718.46 ± 596.08  | 755.29 ± 654.01  | 794.17 ± 630.18  | 754.36 ± 616.97  | 0.956   |

Values are presented as mean ± standard deviation unless otherwise indicated.
ICS, inhaled corticosteroids; ACT, asthma control test; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity.
Group 1, eosinophilic to eosinophilic phenotype; group 2, eosinophilic to noneosinophilic phenotype; group 3, noneosinophilic to eosinophilic phenotype; group 4, noneosinophilic to noneosinophilic phenotype.
*These laboratory findings were recorded at the second examination. Other laboratory findings were recorded at the initial examination.

Effect on modulating airway inflammation may be excluded. However, it is still possible that treatment with ICS abolishes the intrinsic airway inflammatory pattern in asthmatic patients. In this study, we evaluated if inflammatory phenotypes are stable over time in the patients with stable asthma and the results were found quite unstable. These results are consistent with the findings of several other studies. One earlier study reported an inconsistency between sputum inflammatory profiles over 5 years [2]. Another prospective study on patients with moderate to severe asthma revealed that the inflammatory phenotype was unstable [16]. Also in line with our current findings, a recent study on the Pan-European BIOAIR cohort found that allocation to clusters changed in 42.3% of patients when stratified according to airway inflammation. This instability has been also found in steroid-naive asthmatic patients. In one study, repeated sputum analyses were performed for the patients not receiving ICS and the results demonstrated that 22% had persistent eosinophilia, 31% had eosinophilia on ≥1 occasion,
and 47% had persistent noneosinophilia [19]. Another study has reported that 35% of steroid-naive asthmatics demonstrated changes in their inflammatory phenotypes [18]. Moreover, phenotypic variability was reported that it is not influenced by changes in the corticosteroid dose [17]. In contrast, another study reported that the majority of adult patients with difficult-to-treat asthma demonstrated consistent sputum analysis results over a 5-year period and that the percentage of sputum eosinophils was highly reproducible [20]. The inconsistent findings from the different studies described above might be due to the small number of patients, differences in asthma severity, possible effects of varying doses of corticosteroid therapy, and variable confounding factors such as exposure to tobacco smoke. Taken together, the evidence suggests that various factors possibly influence airway inflammation and the potential variability of the inflammatory patterns should be considered when defining inflammatory phenotypes in asthmatic patients. This also indicates that the inflammatory cell type alone is not sufficient to predict treatment outcomes.

Finally, we attempted to determine if there were any unique characteristics of the groups that showed inflammatory phenotype transitions. Only few studies have investigated the clinical factors affecting airway inflammatory pattern transition in asthma and one study has reported that there were no clinical differences between the different inflammatory patterns [17]. In our study, interestingly, a high blood eosinophil count was the only factor for predicting the sustainability of EA, and no other clinical differences were found. The blood eosinophil count has been reported to exhibit good correlation with sputum eosinophils in asthmatic patients [31], which is associated with disease severity and asthma phenotypes [32, 33]. Blood eosinophils can be used to predict and direct anti-inflammatory therapy, for which there is preliminary evidence for asthma [8, 9]. A recent study reported that using a cutoff value of 0.45 × 10^9 cells/L for blood eosinophilia can usefully predict airway eosinophilia in patients with severe asthma [34]. In another study of mild to moderate asthma, as well as severe asthma, blood eosinophils demonstrated the highest accuracy for identifying sputum eosinophilia. The blood eosinophil count can be used as an easy-to-measure biomarker for sputum eosinophil percentage in patients with asthma, and can also have practical advantages for guiding novel anti-inflammatory therapies [35]. On the other hand, a recent study by the Severe Asthma Research Program demonstrated poor correlations between blood and sputum eosinophils [36], thereby raising controversy. Since blood eosinophils generally transmigrate quickly into tissues in response to localized inflammation, the association between the blood eosinophils and airway inflammation can be transient [37] and may lack strong positive correlation [36]. Although the correlation between the magnitude of blood eosinophils and airway eosinophilia could be modest, it can be clearly assumed that the use of blood eosinophils facilitates the individualized treatment and management of asthma.

The limitations of our study include the fact that the data were retrospectively analyzed so that the overall asthma control status could not be clearly defined and the time interval between the 2 sputum examinations was variable. Furthermore, it was hard to evaluate the clinical implications of each airway inflammatory pattern on the long-term clinical courses by using only our current findings. Prospectively designed studies with more frequent sputum eosinophil measurements are needed to verify our results and elucidate the meaning of each inflammatory cell transition in asthmatic patients. Another limitation was the relatively small number of study participants. It will be important in the future to include larger patient cohorts and undertake prospective analyses to determine the possible mechanism of phenotype instability in asthmatic patients.

In conclusion, a substantial proportion of asthmatic patients who demonstrate certain sputum inflammatory cell profiles at the initial examination will develop profile transitions over time in clinically stable settings. The results of our present study suggest that a single sputum sample assessment cannot reliably distinguish between EA and NEA and may not help guide clinical decisions in asthmatic patients. Further prospective studies are needed to search for valuable clinical factors to improve asthma management plan for inflammatory phenotype-based therapeutic strategies.

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REFERENCES

1. Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, Chu HW. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. Am J Respir Crit Care Med 1999;160:1001-8.

2. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. Respiratory 2006;11:54-61.

3. Pavord ID, Brightling CE, Woltmann G, Wardlaw AJ. Non-eosinophilic corticosteroid unresponsive asthma. Lancet 1999;353:2213-4.

4. ten Brinke A, Zwenderman AH, Sterk PJ, Rabe KF, Bel EH. “Refractory” eosinophilic airway inflammation in severe asthma: effect of parenteral corticosteroids. Am J Respir Crit Care Med 2004;170:601-5.

5. Wenzel S, Wilbraham D, Fuller R, Getz EB, Longphre M. Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies. Lancet 2007;370:1422-31.

6. Flood-Page P, Swenson C, Faiferman I, Matthews J, Williams M, Brannick L, Robinson D, Wenzel S, Busse W, Hansel TT, Barnes NC, International Mepolizumab Study Group. A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. Am J Respir Crit Care Med 2007;176:1062-71.

7. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O’Connor BJ, Walls CM, Mathur AK, Cowley HC, Chung KF, Djukanovic R, Hansel TT, Holgate ST, Sterk PJ, Barnes PJ. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. Lancet 2000;356:2144-8.

8. Ortega HG, Liu MC, Pavord ID, Brusselle GG, Fitzgerald JM, Chetta A, Humbert M, Katz LE, Keene ON, Yancey SW, Chanez P, MENS Investigators. Mepolizumab treatment in patients with severe eosinophilic asthma. N Engl J Med 2014;371:1198-207.

9. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, Ortega H, Chanez P. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet 2012;380:651-9.

10. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsley MV, Arron JR, Harris JM, Scheerens H, Wu LC, Su Z, Mosesova S, Eisner MD, Bohn SP, Matthews JG. Lebrikizumab treatment in adults with asthma. N Engl J Med 2011;365:1088-98.

11. Gauvreau GM, Boulet LP, Cockcroft DW, Fitzgerald JM, Carlsten C, Davis BE, Deschesnes F, Duong M, Durn BL, Howie KJ, Hui L, Kasaian MT, Killian KJ, Strinich TX, Watson RM, Y N, Zhou S, Raible D, O’Byrne PM. Effects of interleukin-13 blockade on allergen-induced airway responses in mild atopic asthma. Am J Respir Crit Care Med 2011;183:1007-14.

12. Piper E, Brightling C, Niven R, Oh C, Faggionti R, Poon K, She D, Kell C, May RD, Geba GP, Molfino NA. A phase II placebo-controlled study of tralokinumab in moderate-to-severe asthma. Eur Respir J 2013;41:330-8.

13. Wenzel S, Ford L, Pearlman D, Spector S, Sher L, Skobieranda F, Wang L, Kirkesseli S, Rocklin R, Bock B, Hamilton J, Ming JE, Radin A, Stahl N, Yancopoulos GD, Graham N, Pirozzi G. Dupilumab in persistent asthma with elevated eosinophil levels. N Engl J Med 2013;368:2455-66.

14. Cox G. Glucocorticoid treatment inhibits apoptosis in human neutrophils. Separation of survival and activation outcomes. J Immunol 1995;154:4719-25.

15. Cowan DC, Cowan JO, Paldy R, Williamson A, Taylor DR. Effects of steroid therapy on inflammatory cell subtypes in asthma. Thorax 2010;65:384-90.

16. Al-Samri MT, Benedetti A, Préfontaine D, Olivenstein R, Lemiére C, Nair P, Martin JG, Hamid Q. Variability of sputum inflammatory cells in asthmatic patients receiving corticosteroid therapy: a prospective study using multiple samples. J Allergy Clin Immunol 2010;125:1161-3.e4.

17. Kupczyk M, Dahlén B, Sterk PJ, Nizankowska-Moqilnicka E, Papi A, Bel EH, Chanez P, Howarth PH, Holgate ST, Brusselle G, Siafakas NM, Gjomarkaj M, Dahlén SE; BIOAIR investigators. Stability of phenotypes defined by physiological variables and biomarkers in adults with asthma. Allergy 2014;69:1198-204.

18. Majewski S, Ciebiada M, Domagala M, Kurmanowska Z, Gorski P. Short-term reproducibility of the inflammatory phenotype in different subgroups of adult asthma cohort. Mediators Inflamm 2015;2015:419039.

19. McGrath KW, Ilicovic N, Boushey HA, Lazarus SC, Sunder-ullah ER, Chinchilli VM, Fahy JV. Asthma Clinical Research Network of the National Heart, Lung, and Blood Institute. A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. Am J Respir Crit Care Med 2012;185:612-9.

20. van Veen IH, Ten Brinke A, Gauw SA, Sterk PJ, Bae CF, Bel EH. Consistency of sputum eosinophilia in difficult-to-treat asthma: a 5-year follow-up study. J Allergy Clin Immunol 2009;124:615-7, 617.e1-2.

21. Pin I, Gibson PG, Kolendowicz R, Girgis-Gabardo A, Denburg JA, Hargrave FE, Dolovich J. Use of induced sputum cell counts to investigate airway inflammation in asthma. Thorax 1992;47:25-9.
22. Gibson PG, Wlodarczyk JW, Hensley MJ, Gleeson M, Henry RL, Cripps AW, Clancy RL. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. Am J Respir Crit Care Med 1998;158:36-41.

23. Fahy JV, Boushey HA, Lazarus SC, Mauger EA, Cherniack RM, Chinchilli VM, Craig TJ, Drazen JM, Ford JG, Fish JE, Israel E, Kraft M, Lemanske RF, Martin RJ, McLean D, Peters SP, Sorkness C, Szefler SJ; NHLBI Asthma Clinical Research Network. Safety and reproducibility of sputum induction in asthmatic subjects in a multicenter study. Am J Respir Crit Care Med 2001;163:1470-5.

24. Hastie AT, Moore WC, Meyers DA, Vestal PL, Li H, Peters SP, Bleecker ER; National Heart, Lung, and Blood Institute Severe Asthma Research Program. Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. J Allergy Clin Immunol 2010;125:2038-36.e13.

25. Deykin A, Lazarus SC, Fahy JV, Wechsler ME, Boushey HA, Chinchilli VM, Craig TJ, Dimango E, Kraft M, Leone F, Lemanske RF, Martin RJ, Pesola GR, Peters SP, Sorkness CA, Szefler SJ; Israel E; Asthma Clinical Research Network, National Heart, Lung, and Blood Institute/NIH. Sputum eosinophil counts predict asthma control after discontinuation of inhaled corticosteroids. J Allergy Clin Immunol 2005;115:720-7.

26. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002;360:1715-21.

27. Petsky HL, Cates CJ, Lasserson TJ, Li AM, Turner C, Kynaston JA, Chang AB. A systematic review and meta-analysis: tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils). Thorax 2012;67:199-208.

28. Black PN, Blasi F, Jenkins CR, Scicchitano R, Mills GD, Rubinfeld AR, Ruffin RE, Mullins PR, Dangain J, Cooper BC, David DB, Allegra L. Tial of roxithromycin in subjects with asthma and serological evidence of infection with Chlamydia pneumoniae. Am J Respir Crit Care Med 2001;164:536-41.

29. Simpson JL, Powell H, Boyle MJ, Scott RJ, Gibson PG. Clarithromycin targets neutrophilic airway inflammation in refractory asthma. Am J Respir Crit Care Med 2008;177:148-55.

30. Sutherland ER, King TS, Icicovic N, Ameredes BT, Bleecker E, Boushey HA, Calhoun WJ, Castro M, Cherniack RM, Chinchilli VM, Craig TJ, Denlinger L, DiMango EA, Fahy JV, Israel E, Jarjour N, Kraft M, Lazarus SC, Lemanske RF Jr, Peters SP, Ramsdell J, Sorkness CA, Szefler SJ, Walter MJ, Wasserman SJ, Wechsler ME, Chu HW, Martin RJ; National Heart, Lung and Blood Institute's Asthma Clinical Research Network. A trial of clarithromycin for the treatment of suboptimally controlled asthma. J Allergy Clin Immunol 2010;126:747-53.

31. Schleich FN, Manise M, Sele J, Henket M, Seidel L, Louis R. Distribution of sputum cellular phenotype in a large asthma cohort: predicting factors for eosinophilic vs neutrophilic inflammation. BMC Pulm Med 2013;13:11.

32. Bousquet J, Chanez P, Lacoste JY, Barnéon G, Ghavanian N, Enander I, Venge P, Ahlstedt S, Simony-Lafontaine J, Godard P, et al. Eosinophilic inflammation in asthma. N Engl J Med 1990;323:1033-9.

33. Nadif R, Siroux V, Oryszczyn MP, Ravault C, Pison C, Pin I, Kauffmann F. Epidemiological study on the Genetics and Environment of Asthma (EGEA). Heterogeneity of asthma according to blood inflammatory patterns. Thorax 2009;64:374-80.

34. Fowler SJ, Tavernier G, Niven R. High blood eosinophil counts predict sputum eosinophilia in patients with severe asthma. J Allergy Clin Immunol 2015;135:822-4.e2.

35. Wagener AH, de Nijs SB, Lutter R, Sousa AR, Weersink EJ, Bel EH, Sterk PJ. External validation of blood eosinophils, FE(NO) and serum perisin as surrogates for sputum eosinophils in asthma. Thorax 2015;70:115-20.

36. Hastie AT, Moore WC, Li H, Rector BM, Ortega VE, Pascual RM, Peters SP, Meyers DA, Bleecker ER; National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Biomarker surrogates do not accurately predict sputum eosinophil and neutrophil percentages in asthmatic subjects. J Allergy Clin Immunol 2013;132:72-80.

37. Hogan SP, Rosenberg HF, Moqbel R, Phipps S, Foster PS, Lacy P, Kay AB, Rothenberg ME. Eosinophils: biological properties and role in health and disease. Clin Exp Allergy 2008;38:709-50.