Can Sputum Eosinophilia Be a Constant Feature in Severe Refractory Asthmatics? A 3-Year Longitudinal Study

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Key Words

Severe asthma · Sputum eosinophils · Phenotypes

Abstract

In difficult-to-treat asthmatics, uncontrolled despite a high level of therapy and followed for 3 years with a mean number of sputum samples/patient = 10, sputum eosinophilia ($\geq 3\%$) was observed in 87% of all sputum samples. Persistent sputum eosinophilia is a characteristic of severe uncontrolled asthma.

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Typically, sputum in patients with asthma is rich in eosinophils, but variable inflammatory cell profiles have been reported, prompting a classification with 4 phenotypes: eosinophilic, neutrophilic, mixed and paucigranulocytic [1]. These profiles might change with time [2], although other studies have shown a fairly long-lasting stability of the noneosinophilic pattern in mild-to-moderate asthmatic patients over time [3, 4].

Eosinophilic airway inflammation that persists despite intensive antiasthma treatment is associated with severe

asthma with frequent exacerbations requiring therapeutic adjustment with oral corticosteroids and/or high-dose inhaled corticosteroids (HD-ICS) and also new biologicals. While sputum eosinophilia was highly reproducible in two measures in the space of 5 years in patients with difficult-to-treat asthma [5], a longitudinal study performed over 1 year assessing inflammatory cells in the sputum every month in difficult-to-treat asthmatics shows that the sputum inflammatory profile commonly changes, and stable phenotypes were noted in only a minority of the patients [6].

In the present study, we investigated whether eosinophilic airway inflammation in the induced sputum was a consistent feature over time in patients with severe refractory asthma. Data were collected from a group of 21 adult asthmatics (mean age $\pm$ SD: 60.4 ± 9.6 years) with frequent asthma symptoms, daily rescue medication use and ≥ 2 exacerbations requiring oral corticosteroids in the year before the enrollment (mean $\pm$ SD: 2.8 ± 1 exacerbation/year) despite regular treatment with HD-ICS ($\geq 1,500$ mcg daily of BDP equivalent) and long-acting β_2 agonists. In the year before the enrollment, the patients were accurately and frequently examined in our clinic in order to appropriately treat their asthma and comorbid-

ities and check for compliance: only patients with a compliance in the drug assumption of >80% (as derived from their diary card) and whose comorbidities (mainly nasal polyps or chronic rhinosinusitis) were optimally treated were considered. According to this, they represented a very selected population of severe refractory asthma (frequent exacerbations, current symptoms and limitation in daily life, despite HD-ICS/ β_2 agonist treatment according to the algorithm proposed by the U-Biopred study) [7].

At the enrollment, the predicted FEV₁ was 74.3 $\pm$ 16.6%, and the median sputum eosinophil percentage was 21.4% (1.2, 86.5). The patients entered into a longitudinal follow-up study (which is part of our clinical routine) with the aim of optimally treating them according to clinical and functional findings. Hypertonic saline-induced sputum was collected every 4 months, together with spirometry, and the frequency and severity of the asthma symptoms and number of exacerbations (requiring at least a short course of oral corticosteroids) were collected. The methods are described elsewhere in detail [8]. Sputum eosinophilia was defined as sputum eosinophils of $\geq 2\%$, based on normal values in healthy adults [9], while a value of $\geq 3\%$ was considered as clinically significant sputum hypereosinophilia [10]. Sputum samples collected during an acute exacerbation or within 2 weeks from the end of an oral corticosteroid course were excluded.

Patients were followed for 3.0 $\pm$ 1.1 years, with repeated assessment of induced sputum under regular treatment, producing 10.1 $\pm$ 4.2 samples/subject (range: 4–20; samples obtained during or in the 4 weeks following an acute exacerbations were excluded). At the baseline evaluation, only 3 out of 21 subjects showed normal sputum eosinophils (<2%); in these 3 patients, only 6 out of 26 sputum samples obtained during the follow-up still showed normal sputum eosinophils. Out of 208 sputum samples obtained from all 21 patients during the follow-up, 92% showed sputum eosinophils $\geq 2\%$, and 87% of patients $\geq 3\%$. In 16 out of 21 patients, a clinically significant sputum eosinophilia was observed in $\geq 80\%$ of all sputum samples (table 1). There was no correlation between the FEV₁ values or the severity of asthma symptoms at any of the time points and the eosinophil percentage in the sputum collected at the same points. A large intra- and intersubject variability may be observed (fig. 1); however, most measurements confirmed the presence of sputum eosinophilia despite the high level of asthma therapy.

In this study, we showed that the inflammatory phenotype is relatively constant, because in the large major-

Table 1. Percent of sputum samples with eosinophils $\geq 3\%$ for each patient during the observation period

Patients, No.	Observations, n	Time period, years	Samples with eosinophils $\geq 3\%$, %
1	18	5	100
2	10	3	90
3	13	3	79
4	7	2	43
5	16	5	63
6	7	2	71
7	12	4	67
8	9	2	89
9	20	5	95
10	9	3	89
11	4	2	100
12	13	4	92
13	6	2	83
14	6	2	100
15	5	2	80
16	12	4	92
17	7	2	86
18	8	3	100
19	10	3	80
20	10	3	80
21	11	3	100
mean $\pm$ SD	10.1 $\pm$ 4.2	3.0 $\pm$ 1.1	84.7 $\pm$ 14.6

ity of patients studied longitudinally, sputum eosinophil percentages remained greater than normal. Only 3 patients had a low sputum eosinophil percentage at the baseline, but none of them maintained this feature in all of the following samples. On the other hand, only 24% of our patients showed sputum eosinophilia in all sputum samples. According to the recent WHO classification of severe asthma [11], the patients we studied may be included in just 1 of the different subgroups reported in this new classification, in particular in the category of patients with treatment-resistant severe asthma (refractory asthma). This fact may explain the substantial homogeneity of the patients we studied and also the high median sputum eosinophil percentage at their enrollment, while in the literature different subgroups of patients with severe asthma according to the old American Thoracic Society or the new WHO classification were included. Sputum eosinophilia was independent from the day-by-day changes in symptoms and FEV₁, although we must consider that samples collected during or immediately after an acute exacerbation were excluded. The discrepancy between clinical features and biomarkers is well known in asthma, and for that reason, biomarkers may represent

Fig. 1. Single data points of the sputum eosinophil percentages obtained in all patients during the observation period. Cutoffs of 2% (normal threshold) and of 3% (clinically significant threshold) are reported.

new additional information for the management of asthma. Sputum eosinophils and exhaled nitric oxide have been demonstrated to be predictors of a possible loss of asthma control during a step-down strategy or to be associated with a rapid decline of FEV₁. In several studies, some markers of eosinophil activation demonstrated an ability to be influenced by treatments [12, 13].

In summary, this observational longitudinal study assessed the change over time of the airway eosinophilic inflammation in a subgroup of patients with treatment-resistant severe asthma or refractory asthma, despite the fact that they were taking HD-ICS plus other drugs. Each

patient was investigated over a 3-year period on a regular basis, with a mean number of 10 measurements each. Our findings demonstrate that the sputum inflammatory profile remains eosinophilic in the large majority of the samples collected from these patients with uncontrolled asthma and frequent exacerbations.

Acknowledgments

The work has been supported by a project from the Italian Agency for the Drug (AIFA).

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