Eosinophil Count Could Be More Sensitive in Induced Sputum Than in Peripheral Blood for Phenotyping of Patients With Severe Eosinophilic Asthma

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To the Editor:

Severe asthma is a heterogeneous disease characterized by the need for treatment with high doses of inhaled corticosteroids and other controller drugs. It affects 5%-10% of asthmatic patients and comprises various clinical and pathophysiological phenotypes. Accurate phenotyping contributes to the correct selection of the appropriate treatment [1]. Among the various inflammatory phenotypes, eosinophilic asthma (EA) is associated with a good response to inhaled corticosteroids and can benefit from new specific biologics. A cut-off of 300 eosinophils/µL in peripheral blood is widely used as a clinical diagnostic criterion for EA [2]. However, since induced sputum (IS) has been considered the reference technique for phenotyping asthma according to inflammatory pattern [3], the IS eosinophil count could provide a more accurate diagnosis.

The aim of this study was to investigate whether EA patients can be diagnosed using the single criterion of an eosinophil count ≥300/µL in peripheral blood or whether the IS eosinophil count could add relevant information to confirm a diagnosis.

A retrospective cross-sectional study was performed on data from patients with severe asthma not treated with biologics who underwent IS during 2015-2019 and from whom a suitable sputum sample was obtained for a differential cell count. All the patients gave their written informed consent.

The clinical information collected from the patients' medical charts included allergic sensitization, nasal polyps, bronchiectasis, smoking status, systemic corticosteroid treatment, fractional exhaled nitric oxide (FeNO), eosinophil cationic protein (ECP), and the number of eosinophils in peripheral blood. The number of eosinophils in IS was determined using Wright-Giemsa staining for a differential cell count after treatment of the sputum samples with dithiothreitol, as described elsewhere [4]. An IS eosinophil count ≥3% was required to confirm the diagnosis of EA [5].

Data obtained in the study were analyzed using SAS 9.3. The variables were compared using the χ^2 test, Fisher exact test, Mann-Whitney test, t test, and Pearson correlation, as appropriate. Receiver operating characteristic (ROC) curves were also constructed. Statistical significance was set at $P<.05$.

The study population comprised 55 patients with severe asthma and reliable results in the differential cell count in IS. The clinical characteristics of the patients are summarized in the Table. Thirty-three patients (60%) had an IS eosinophil count ≥3%, and 8 of these (24.2%) had an eosinophil count of <300/µL in peripheral blood (Table). A total of 20 patients

Table. Clinical Data of the 55 Study Patients With Severe Asthma and Reliable Results in the Differential Cell Count in Induced Sputum

Mean (SD) age, y	51.96 (13.93)
Sex	
Female	38 (69.1%)
Male	17 (30.9%)
Allergic sensitization	35 (63.6%)
Pollens	26 (47.3%)
Perennial allergens	9 (16.4%)
Others	5 (9.1%)
Polyps	31 (56.4%)
Bronchiectasis	29 (52.7%)
Smoking status	
Current	8 (14.8%)
Ex-smoker	17 (31.5%)
Never	29 (53.7%)
Oral corticosteroids treatment in the last 3 months	20 (36.4%)
Eosinophil count ≥3% in IS	33 (60.0%)
Not reaching 300/µL in peripheral blood	8/33 (24.2%)
Eosinophil count ≥3% in IS with oral corticosteroids	13/20 (65.0%)
Not reaching 300/µL in peripheral blood	4/13 (30.8%)
Eosinophil count ≥3% in IS association with	
Allergen sensitization	NS
Polyps	NS
Bronchiectasis	NS
Smoking status	NS
FeNO (elevation)	(P=.002)
ECP	NS

Abbreviations: IS, induced sputum; ECP, eosinophil cationic protein; FeNO, fractional exhaled nitric oxide; NS, nonsignificant.

(36.4%) had received oral corticosteroids (OCS) in the previous 3 months, mainly for uncontrolled asthma (90%). Among these, 13 patients (65%) had an IS eosinophil count ≥3% and 4 (30.8%) had an eosinophil count in peripheral blood of <300/μL. A nonsignificant association was found between an IS eosinophil count ≥3% and sensitization to an allergen, nasal polyps, bronchiectasis, and smoking. However, these clinical features, except for smoking, were more frequently associated with higher values of eosinophils in peripheral blood and ECP. Finally, the patients with an IS eosinophil count ≥3% had higher FeNO levels (*P*=.002), although no significant association was found with ECP.

This study is limited by its small sample size. In addition, the fact that several patients had received OCS in the previous 3 months may have affected the blood eosinophil count [6]. Nevertheless, focusing on patients not treated with OCS, our results show that using a cut-off of 300 eosinophils/μL in peripheral blood can predict sputum eosinophilia in most patients, although the criterion fails to identify many patients (almost a quarter) as having true EA (as confirmed by IS). This finding may have therapeutic implications for the prescription of biologics, since only mepolizumab is indicated for patients with a blood eosinophil count ≥150/μL, thus limiting the prescription of benralizumab (≥300/μL) and reslizumab (≥400/μL) in some cases of confirmed severe EA [7,8].

Moreover, low blood eosinophil counts alone might not accurately reflect the absence of airway eosinophilia [9]. In fact, many situations can influence eosinophil counts. Rakowski et al [10] retrospectively observed variability in blood eosinophil levels over time in patients with >300/μL and identified groups of patients who may or may not ever reach a defined blood eosinophil threshold. According to the IS eosinophil counts, we found that 24.2% of patients with EA did not reach a count of 300/μL in peripheral blood. Conversely, in this group of patients, the more the eosinophil count increases in peripheral blood, the greater the specificity for detection of eosinophilia by IS. The ROC curve analysis showed that a cut-off of 465/μL in peripheral blood yielded a sensitivity of 66.7% (95%CI, 49.6-80.2) and a specificity of 86.4% (95%CI, 66.7-95.3).

Regarding other biomarkers, FeNO appeared to be the second-best predictor for detection of eosinophilia by IS. ROC analysis based on a cut-off of 36 ppb revealed a sensitivity of 71.9% (95%CI, 54.6-84.4) and a specificity of 71.4% (95%CI, 50.1-86.2). Furthermore, we found an association between higher eosinophil levels in peripheral blood and serum ECP. Although ECP can correlate with severity of asthma and peripheral blood eosinophilia, interestingly, we did not observe a correlation between eosinophil counts in IS and serum ECP or any comorbidity, including allergic sensitization, bronchiectasis, and nasal polyps.

In conclusion, this study reinforces the need to perform IS in patients with severe asthma who have not been treated with OCS during the previous 3 months in order to properly define their inflammatory pattern.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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