Eosinophil Count in Induced Sputum Could Be More Sensitive Than In Peripheral Blood to Phenotype Patients with Severe Eosinophilic Asthma

Caballero ML[1,2,3*], Dominguez-Ortega J[1,2,3*], Nin-Valencia AR[1], Sánchez-Ocando H[1], Barranco P[1,2,3]

[1]Department of Allergy, La Paz University Hospital, Madrid

[2]La Paz Hospital Institute for Health Research (IdiPAZ) Madrid

[3]CIBER of Respiratory Diseases CIBERES, Madrid

*These authors contributed equally to this work.

Corresponding author

María Luisa Caballero

Department of Allergy, La Paz University Hospital, Institute for Health Research (IdiPAZ), Paseo de la Castellana 261, 28046 Madrid, Spain

Email: mlcsoto@hotmail.com

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Severe asthma is a heterogeneous disease characterized by the need of 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. An accurate phenotyping contribute 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 clinical diagnostic criteria to identify EA [2].However, since induced sputum (IS) technique has been considered the reference standard for phenotyping asthma according to the inflammatory profile [3],the IS eosinophil count could add useful information for a more accurate diagnosis.

The aim of this study was to investigate if EA patients can be diagnosed using a unique criteria of having ≥300 eosinophils/μL in peripheral blood or if the IS eosinophil count could add relevant information to ensure diagnosis.

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

Clinical information was collected from the patients' medical charts regarding allergy sensitization, nasal polyps, bronchiectasis, smoking status, systemic corticosteroid

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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 by Wright-Giemsa staining for a differential cell count after mucolysis of the sputum samples with dithiothreitol, as described [4]. An IS eosinophil count ≥3%wasrequiredto diagnose EA [5].

Data obtained in the study were analyzed with SAS 9.3 statistical program. For variables association chi-square test, Fisher's exact test, Mann-Whitney U test, student's t-test and the Pearson correlation were used when appropriate. ROC curves were also performed. P-values <0.05 were considered to be indicative of statistically significant differences.

Fifty-five patients with severe asthma and reliable results in the differential cell count in IS were included in the study. The clinical characteristics of the patients are summarized in Table I. Thirty-three patients (60%) showed an IS eosinophil count ≥3%,but eight of them (24.2%) had an eosinophil count of less than 300 cells/μL in peripheral blood (Table I).On the other hand, twenty patients (36.4%) had received oral corticosteroids (OCS) in the last 3 months, mainly for uncontrolled asthma (90%). Among these, thirteen patients (65%) had an IS eosinophil count ≥3%and four of them (30.8%) showed an eosinophil count in peripheral blood of less than 300 cells/μL. Non-significant association was found between an IS eosinophil count ≥3% with allergen sensitizations, nasal polyps, bronchiectasis or smoking status, while these clinical features, except smoking status, were more frequently associated with higher values of eosinophils in peripheral blood and ECP. Finally, the patients with IS eosinophil count ≥3%had higher FeNO levels (*P*=0.002), but no significant association was found with ECP.

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This study has the limitation of the small sample size. In addition, several patients had received OCS in the last three months, which could affect the blood eosinophil count [6].Despite that, and focusing in 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 a majority of patients, but this criterion fails to identify many patients (almost a quarter) as having true eosinophilic asthma (as confirmed by IS).This finding can have therapeutic implications for the prescription of biological therapy, since only mepolizumab is indicated with a blood eosinophil count ≥150 eosinophils/μL, limiting the prescription of benralizumab (≥300 eosinophils/μL) or reslizumab (≥400 eosinophils/μL) in some patients with confirmed severe eosinophilic asthma[7,8].

Moreover, low blood eosinophil numbers alone might not accurately reflect the absence of airway eosinophilia [9]. In fact, many situations may influence the eosinophil counts. Rakowski et al [10], retrospectively observed variability of blood eosinophil levels over time in patients displaying levels that exceeded a threshold of 300 eosinophils/μL and revealed some groups of patients who may or may not ever reach a defined blood eosinophils threshold. Our study reflects that 24.2% of patients with EA according to the ISeosinophil counts, did not reach a count of 300 eosinophils/μL in peripheral blood. Conversely, in this group of patients, the more the eosinophils increase in peripheral blood, the more the specificity to detect IS-eosinophilia rises. The ROC curve analysis showed that a cut-off of 465 eosinophils/μL in peripheral blood gave a sensitivity of 66.7% (IC95%=(49.6-80.2)) and specificity of 86.4% (IC95%=(66.7-95.3)).

Regarding other biomarkers, in our study FeNO appeared to be the second-best predictor for IS-eosinophilia. In an ROC AUC, with a cut-off of 36 particles per billion, the sensitivity showed a 71.9% (IC95%=(54.6-84.4)) with the specificity of 71.4%

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(IC95%=(50.1-86.2)).Furthermore, we found an association between higher levels of eosinophils in peripheral blood and serum-ECP. Although ECP can correlate with severity of asthma and peripheral blood eosinophilia, interestingly, we have not observed a correlation between eosinophil counts in IS and serum-ECP or any comorbidity, including allergic sensitization, bronchiectasis or nasal polyps.

In conclusion, this study reinforce the need to perform IS in those patients with severe asthma not treated with OCS in the last three months to properly define their inflammatory profile.

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Conflicts of interest

The authors have not conflicts of interest to declare.

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Tables and Figures

Table 1. Clinical dataof the 55studied patients with severe asthma and reliable results in the differential cell count in induced sputum.

Age (y)	51.96 ±13.93
Sex	
Female	38 (69.1%)
Male	17 (30.9%)
Allergen 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 eosinophils/μL in peripheral blood	8/33 (24.2%)
Eosinophil count ≥ 3% in IS with oral corticosteroids	13/20 (65.0%)
Not reaching 300 eosinophils/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*=0.002)
ECP	NS

IS, induced sputum; **FeNO,**fractional exhaled nitric oxide; **ECP,** eosinophil cationic protein; **NS,**non-significant.

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