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ORIGINAL RESEARCH

Characteristics of Induced-Sputum Inflammatory Phenotypes in Adults with Asthma: Predictors of Bronchial Eosinophilia

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Purpose: The objectives of this study were, for patients attending a specialist asthma clinic at a tertiary care hospital, to determine, from sputum induction (SI), proportions of bronchial inflammatory phenotypes, demographic, clinical and functional characteristics of each phenotype, and the most accessible non-invasive inflammatory marker that best discriminates between phenotypes.

Patients and Methods: Included were 96 patients with asthma, attending a specialist asthma clinic at a tertiary care hospital, who underwent testing as follows: SI, spirometry, fractional exhaled nitric oxide (FeNO), blood eosinophilia, total immunoglobulin E (IgE), and a skin prick test.

Results: SI phenotypes were 46.9% eosinophilic, 33.3% paucigranulocytic, 15.6% neutrophilic, and 4.2% mixed. No significantly different clinical or functional characteristics were observed between the phenotypes. A positive correlation was observed between SI eosinophilia and both emergency visits in the last 12 months ($p = 0.041$; $r = 0.214$) and FeNO values ($p = 0.000$; $r = 0.368$). Blood eosinophilia correlated with SI eosinophilia ($p = 0.001$; $r = 0.362$) and was the best predictor of bronchial eosinophilia, followed by FeNO, and total blood IgE (area under the receiver operating characteristic curve (AUC-ROC) 72%, 65%, and 53%, respectively), although precision was only fair.

Conclusion: In consultations for severe asthma, the most frequent phenotype was eosinophilic. Peripheral blood eosinophilia is a reliable marker for discriminating between different bronchial inflammatory phenotypes, is useful in enabling doctors to select a suitable biologic treatment and so prevent asthma exacerbation, and is a better predictor of bronchial eosinophilia than FeNO and IgE values.

Keywords: asthma, sputum induction, phenotype, eosinophilia

Introduction

Sputum induction (SI), the gold standard for evaluating bronchial inflammation in patients with asthma, is a non-invasive, standardized, and validated test[1,2] that distinguishes between 4 bronchial inflammatory phenotypes: eosinophilic, paucigranulocytic, neutrophilic, and mixed.[3] This technique, however, is not available in all hospitals as it requires trained personnel and a suitable infrastructure; therefore, other more accessible markers are used in current clinical practice, such as eosinophil count in peripheral blood and fractional exhaled nitric oxide (FeNO) measurement.

Especially important for severe asthma is phenotype identification, as it enables an individualized approach to treatment.[4,5] Several studies have confirmed that eosinophilic airway inflammation predicts response to anti-inflammatory treatment with both inhaled corticosteroids[6,7] and biologics.[8–13] Indeed, the main clinical guidelines for asthma management propose using SI to evaluate severe asthma[14,15] and to manage severe uncontrolled asthma treatment

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if the patient is clinically followed up in suitably equipped centers.[14] However, the fact that SI is laborious and requires experienced personnel explains current attempts to identify non-invasive markers that discriminate between different bronchial inflammatory phenotypes in a simple and cost-effective way. While peripheral blood eosinophilia is a marker that predicts airway eosinophilia,[16] a common cut-off point has not been established, and correlations between blood and SI eosinophilia vary widely.[16–18] Studies have also reported a relationship between blood eosinophilia and the risk of severe exacerbations, decreased lung function, responsiveness to corticosteroid treatment, and predicted efficacy of some biologic treatments.[10–13,19–21] While FeNO is a non-invasive marker that reflects eosinophilic inflammation,[22,23] certain variables may modify its value,[24,25] and its correlation with bronchial and peripheral blood eosinophilia is also variable.[26–29]

Our study objectives, for a population of patients with asthma attending a specialist asthma clinic attached to a tertiary care hospital, were as follows: (a) to determine, from an SI test, the different proportions of bronchial inflammatory phenotypes and their demographic, clinical, and functional characteristics and (b) to identify an accessible non-invasive inflammatory marker used in routine clinical practice that discriminates between the different bronchial inflammatory phenotypes.

Materials and Methods

Our cross-sectional descriptive study included 96 patients, aged 18–80 years old. Patients were consecutively enrolled from our tertiary care university hospital's severe asthma outpatient unit (located in Spain) for evaluation in 2018 and 2019. All the patients complied with asthma diagnostic criteria according to Global Initiative for Asthma (GINA) guidelines.[15] Excluded were smokers and patients who had experienced respiratory infections or required oral corticosteroids in the previous month.

Demographic, clinical, and functional data were collected for the 96 patients, and on the same day, the following procedures were carried out: skin prick test, total blood immunoglobulin E (IgE), absolute eosinophil count, SI inflammatory cell count, and forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), and FeNO measurements. Patients were also asked to complete the Asthma Control Test (ACT)[30] and Asthma Quality of Life Questionnaire (AQLQ).[31] The skin prick test, performed for common local aeroallergens according to the standard procedure,[32] was considered positive when papule diameter was >3 mm. Total blood IgE was determined using the enzyme-linked immunosorbent assay (ELISA) method (UNICAP, Pharmacia, Uppsala, Sweden) and was considered increased for values >160 IU/mL. Used to assess asthma control was the ACT, a self-assessment questionnaire validated in Spanish,[30] with good control considered to be >20 points. Quality of life (QoL) was assessed using the self-administered AQLQ, likewise validated in Spanish.[32] Spirometry measurements were made with a Datospir-600 device (Sibelmed SA, Barcelona, Spain) by an experienced technician and following the 2005 recommendations of the American Thoracic Society/European Respiratory Society (ATS/ERS);[33] FEV1 >80% was considered to be in the reference range of the theoretical value.[34] FeNO, following ATS/ERS 2005 recommendations[35] and using an electrochemical analyzer (NO Vario Analyzer, Filt Lungen- und Thoraxdiagnostik GmbH, Berlin, Germany), was measured at a flow of 50 mL/s and was considered to be significantly increased when values were >50 ppb.[36] Sputum samples were obtained and processed according to the method described by Djukanović et al,[1] and patients were classified according to cell counts as follows: eosinophilic if eosinophils ≥3%, neutrophilic if neutrophils ≥61%, paucigranulocytic if neutrophils <61% and eosinophils <3%, and mixed if neutrophils ≥61% and eosinophils ≥3%.[6] An absolute eosinophil count of ≥300 cells/μL was taken to define blood eosinophilia.[14]

Ethical and Legal Aspects

The study complied with the principles of the Declaration of Helsinki (18th World Medical Assembly, 1964) and was approved by the Clinical Research Ethics Committee of Hospital Santa Creu i Sant Pau in Barcelona. The patients provided their written consent prior to participation in the study and all study data were anonymized.

Statistical Analysis

Categorical variables were expressed as absolute and relative frequencies and quantitative variables as mean and standard deviation (SD) values. Between-group comparisons were analyzed using analysis of variance (ANOVA) for quantitative variables, and the chi-square or McNemar test for categorical variables, as appropriate. The area under the receiver operating characteristic curve (AUC-ROC) of the biomarkers used to detect the eosinophilic inflammatory phenotype was calculated using a combined impact model (general linear model; GLM), and Pearson's test was used for correlation analyses of the studied population.

The results were considered significant for $p < 0.05$. The analysis was performed with SPSS version 26.0 (SPSS Inc., Chicago, IL, USA).

Results

SI Inflammatory Phenotype Proportions and Characteristics

Of the 96 patients who underwent the SI test, almost half were eosinophilic (n = 45; 46.9%), around a third were paucigranulocytic (n = 32; 33.3%), and the remainder were neutrophilic (n = 15; 15.6%) or mixed (n = 4; 4.2%).

Demographic, clinical, and functional characteristics are summarized in Table 1. Overall mean age was 50 years. No significant differences were observed regarding sex, body mass index (BMI), asthma severity, disease control, emergency

Table 1 Demographic, Clinical, and Functional Characteristics for 4 Inflammatory Phenotypes Identified by SI (n = 96)

	Eosinophilic (n=45)	Paucigranulocytic (n=32)	Neutrophilic (n=15)	Mixed (n=4)	p
Age, mean (SD) years	52.1 (14.7)	51.2 (14)	53.2 (17.9)	57 (26.2)	0.900
Women, %	71.1%	62.5%	46.7%	25%	0.143
Childhood asthma diagnosis, %	24.4%	21.9%	20%	25%	0.984
Severe persistent asthma, %	53.3%	34.4%	33.3%	50%	0.674
GINA 2021 asthma treatment steps, %	STEP 1–2: 6.7%	STEP 1–2: 12.9%	STEP 1–2: 15.9%	STEP 1–2: 50%	0.671
	STEP 3: 28.9%	STEP 3: 22.5%	STEP 3: 21%	STEP 3: 0%	
	STEP 4: 35.5%	STEP 4: 38.8%	STEP 4: 21%	STEP 4: 0%	
	STEP 5: 28.8%	STEP 5: 25.8%	STEP 5: 21%	STEP 5: 50%	
Poor asthma control (ACT <20), %	15.6%	12.5%	6.7%	50%	0.371
ED visits in previous 12 months, mean (SD)	1.4 (2.2)	1.1 (1.7)	1.3 (2.6)	3.5 (5.7)	0.291
AQLQ, mean (SD)	3.3 (2.3)	3.1 (3.2)	1.8 (2.1)	8.1	0.162
BMI, mean (SD) kg/m²	27.1 (4.2)	27.6 (5.6)	24.8 (3.6)	24.8 (5.7)	0.201
Nasal polyposis, %	24.4%	9.4%	13.3%	0%	0.250
Rhinitis, %	68.9%	68.8%	53.3%	25%	0.243
FEV1, mean (SD) %	83.7 (21)	103.9 (98.9)	80.9 (17.5)	77.5 (16)	0.433
BDT, %	26.7%	15.6%	33%	50%	0.327
Prick test +, %	68.9%	71.9%	60%	100%	0.476
FeNO, mean (SD) ppb	46.1 (37.2)	33.4 (26.5)	29.8 (27.4)	30.2 (12.9)	0.207

(*Continued*)

Table 1 (Continued).

	Eosinophilic (n=45)	Paucigranulocytic (n=32)	Neutrophilic (n=15)	Mixed (n=4)	p
Total IgE, mean (SD) IU/mL	460.4 (770.8)	276 (301.4)	241.9 (503)	239.2 (349.1)	0.454
Blood eosinophils, mean (SD) cells/μL	360 (300)	230 (100)	230 (100)	320 (800)	0.057[a] 0.027[b]
Eosinophils, mean (SD) %	5.4 (4)	3.5 (2.1)	3.5 (1.9)	3.7 (1)	0.041[a] 0.021[b]
Dose of inhaled corticosteroids, %	Medium 37.8% and high 28.9%	Medium 37.5% and high 28.1%	Medium 20% and high 40%	High 75%	0.587
Eosinophils, mean (SD) %	12.4 (12.3)	0.79 (0.7)	1.17 (0.9)	9.2 (5.8)	0.000
Neutrophils, mean (SD) %	38 (17.7)	36.6 (18)	76.2 (7.6)	72 (3.4)	0.000
Macrophages, mean (SD) %	48.35 (18.7)	59.8 (17.8)	19.8 (8.7)	17 (8.5)	0.000
Lymphocytes, mean (SD) %	0.89 (0.6)	0.93 (0.6)	0.91 (0.4)	1.58 (1.4)	0.207

Notes: [a]Significance comparing the 4 groups. [b]Significance comparing eosinophilic, paucigranulocytic, and neutrophilic phenotype groups.
Abbreviations: ACT, asthma control test; AQLQ, Asthma Quality of Life Questionnaire; BDT, bronchodilator test; BMI, body mass index; ED, emergency department; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; GINA, Global Initiative for Asthma; IgE, immunoglobulin E; SD, standard deviation; SI, sputum induction.

department (ED) visits in the past 12 months, QoL as measured by AQLQ, bronchial obstruction (FEV1), associated rhinitis or nasal polyposis, total blood IgE, FeNO, or inhaled corticosteroid dose. In contrast, significant differences were observed between the different phenotypes in peripheral blood eosinophil percentages and counts, which were significantly higher in the eosinophilic group.

Asthma Severity

In the analysis by asthma severity, the predominant inflammatory phenotypes were as follows: paucigranulocytic with intermittent asthma, 46.6%; eosinophilic with mild persistent asthma, 45%; eosinophilic with moderate persistent asthma, 36%; and eosinophilic with severe persistent asthma, 57.14% (p = 0.674).

Variable Correlations for the Studied Population

For the 96 patients, positive correlations were observed between SI eosinophilia and ED visits in the previous 12 months (p = 0.041; r = 0.214), between SI eosinophilia and FeNO values (p = 0.000; r = 0.368), and between SI eosinophilia and peripheral blood eosinophilia (p = 0.001; r = 0.362). We interpreted discriminatory capacity as follows (see Figure 1): r = 0.5, equivalent to a coin toss; r = 0.5–0.6, poor; r = 0.6–0.75, fair; r = 0.75–0.9, good; and r = 0.9–0.97, very good.

SI Eosinophilia ≥3% Detection in the Combined Model

The AUC-ROC values that detected SI eosinophilia ≥3% were as follows: absolute blood eosinophils (EOS), 72% (p = 0.000); FeNO, 65% (p = 0.014); and total blood IgE, 53% (p = 0.590).

Discussion

In patients attending our specialist asthma clinic at a tertiary care hospital, the eosinophilic bronchial inflammatory phenotype was predominant, and there were no significant differences in clinical and functional characteristics for the various bronchial inflammatory phenotypes. Furthermore, our study supports the following findings: (a) peripheral blood eosinophilia is a marker that enables the eosinophilic inflammatory phenotype to be differentiated from other bronchial inflammatory phenotypes, although note that precision is only fair according to the AUC-ROC; and (b) while positive

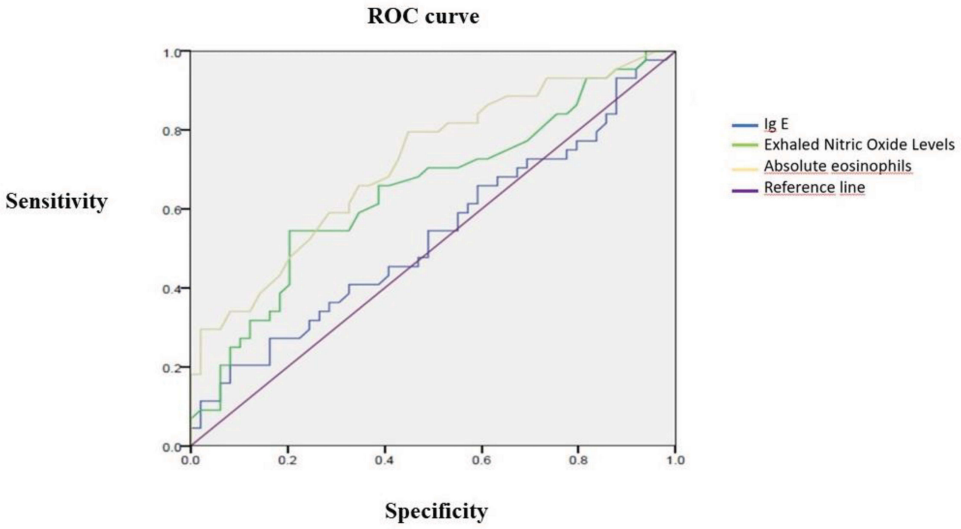

Figure 1 AUC-ROC plot from EOS, FeNO, and total IgE values in a combined model (n=96).
Abbreviations: AUC-ROC, area under the receiver operating characteristic curve; EOS, absolute eosinophils; FeNO, fractional exhaled nitric oxide; IgE, immunoglobulin E.

correlations exist between SI eosinophilia and both FeNO and blood eosinophil values, they were not strong, suggesting a possible activation of various inflammatory pathways in patients with asthma. This would point to the need for a more comprehensive approach to asthma management that goes beyond mere biomarker threshold positivity.

Our AUC-ROC value for detecting SI eosinophilia ≥3% was slightly higher than documented in the literature. Hastie et al[39] reported a value of 69% for detection of SI eosinophilia ≥2% and concluded that while there was an association between blood eosinophilia and SI eosinophilia, precision in terms of correct classification of patients with and without an eosinophilic phenotype was poor, and, furthermore, that this poor precision persisted when biomarkers such as FeNO and IgE were also considered.

The usefulness of blood eosinophilia is supported by several studies. Wagener et al[16] showed that blood eosinophilia was an accurate biomarker of eosinophilic airway inflammation in 2 independent cohorts of patients with asthma (AUC 89%; $p < 0.001$; sensitivity 78% and specificity 91%), while Schleich et al[3] reported that blood eosinophilia ≥220/mm3 enabled SI eosinophilia ≥3% to be detected with 77% sensitivity and 70% specificity (AUC 0.79, $p < 0.0001$).

There is no consensus regarding the blood eosinophilia cutoff value to define the eosinophilic phenotype, and, although current evidence points to 150–300 cells/μL, that range of values is still a matter of debate.[16,18] Note that, in patients with severe asthma, part of the variability reported in different studies is explained by possible variations in blood and SI eosinophilia depending on doses of inhaled or systemic corticosteroids.[4,37] Variability may also result from factors such as smoking (OR = 6.44; $p = 0.013$) and having had a recent asthma exacerbation (OR = 5.84; $p = 0.022$).[38]

While we found positive correlations between SI eosinophilia and both ED visits in the previous 12 months ($p = 0.041$; $r = 0.214$) and FeNO values ($p = 0.000$; $r = 0.368$) and between SI eosinophilia and peripheral blood eosinophilia ($p = 0.001$; $r = 0.362$), none of those correlations were sizeable, indicating no linear relationship between the variables. Another study found better correlation for patients with asthma when the comparison was based on ≥300 cells/μL in peripheral blood and SI eosinophilia ≥2% ($p = 0.0002$; $r = 0.5235$).[40]

The growing importance attached to blood eosinophilia is because it is the most relevant marker for both the choice of, and response to, biologic treatments for severe uncontrolled eosinophilic asthma. The fact that the vast majority of studies use peripheral blood eosinophilia and not SI eosinophilia as the biomarker of choice for a biologic is because not all hospitals have the facilities necessary for SI cell counting.[9,41]

Peripheral blood eosinophilia has been demonstrated to be a marker of a better response to biologics. For instance, it is the key biomarker for measuring response to mepolizumab, a monoclonal antibody against interleukin-5 (IL-5), with exacerbations greatly reduced (73%) in patients with blood eosinophils ≥500/μL.[9,41] Efficacy of another intravenously administered antibody against IL-5, reslizumab, has also been demonstrated for patients with blood eosinophilia ≥400/

μL.[42] Finally, benralizumab, an antibody that acts against the IL-5 receptor through apoptosis of eosinophils and basophils, has been shown to reduce exacerbation rates in patients with blood eosinophilia ≥300/μL.[9,20]

Blood eosinophilia can also predict response to treatment with both inhaled and systemic corticosteroids[14] and can help adjust oral corticosteroid dosage for patients with severe asthma, as demonstrated by Wark et al,[43] who reported that blood eosinophilia maintained at <200 cells/μL prevented exacerbations, improved asthma control, and enabled lower oral corticosteroid doses.

In our series, the FeNO value was less correlated with SI eosinophilia (AUC-ROC 65%) than in the review by Korevaar et al,[44] which included 12 studies with a combined sensitivity of 66% and specificity of 76% in detecting SI eosinophilia ≥3% (AUC 0.74; 95% confidence interval (CI): 0.70–0.78). This difference is possibly explained by the multiple factors that modify FeNO values, such as allergic rhinitis, upper and lower respiratory tract viral infections, age, tobacco use, and atopy;[25–28] it may also be due to the higher sensitivity but lower specificity of FeNO, resulting in high negative predictive values but low positive predictive values for eosinophilic inflammation.[45]

However, it is important to understand the complexity of inflammatory mechanisms in type 2 inflammation in different patients, so it is recommended to simultaneously measure several biomarkers (EOS, IgE, FeNO) to identify potential targets for treatment with biologics. A post-hoc analysis of the QUEST study[46] that assessed dupilumab efficacy by biomarker subgroups, as defined by GINA,[15] found that reference blood eosinophilia and FeNO levels clearly pointed to similar disease severity at the outset in all subpopulations (EOS ≥150 cells/μL, FeNO ≥20 ppb, and both EOS ≥150 cells/μL and FeNO ≥20 ppb).[47] In a study of 110 patients published by our working group, we reported a dissociation between increased FeNO (≥50 ppb) and SI eosinophilia in 42% of patients; that study identified 2 groups with discordant values: a younger group mainly associated with a paucigranulocytic phenotype and atopy, with high FeNO and no SI eosinophilia, and with better FEV1, and an older group mainly associated with a non-allergic eosinophilic phenotype, with low FeNO and high SI eosinophilia, and accounting for more ED visits in the previous 12 months.[48] Those data support the existence of different activation patterns in underlying inflammatory pathways in patients with asthma, suggesting the need for a more comprehensive and more personalized approach to management that goes beyond mere biomarker threshold positivity.

Use of IgE as a biomarker of eosinophilia is poorly supported. In our study, total IgE was the weakest predictor of SI eosinophilia, corroborating other studies[45–47] reporting low sensitivity, specificity, and AUC values for IgE. Demarche et al,[49] in particular, indicated that IgE alone does not adequately predict SI eosinophilic status. Westerhof et al[17] proposed joint use of blood eosinophilia and FeNO to improve airway predictions of eosinophilia (AUC 0.87; $p = 0.027$), while Demarche et al[49] proposed the joint use of blood eosinophilia, FeNO, and IgE, as an approach that, in their study, identified 58% of patients with a high or low probability of having SI eosinophilia ≥3%, and that correctly classified 87% of those patients.

Regarding the distribution of inflammatory phenotypes in our study, the eosinophilic phenotype predominated (almost half), followed by the paucigranulocytic phenotype (around a third); this finding corroborates another large series,[3] but contradicts other studies that reported predominance of the paucigranulocytic phenotype.[50] The difference is possibly explained by the fact that the studies in which the paucigranulocytic phenotype predominated were of patients whose asthma was less severe than that of patients recruited in specialist asthma clinics.

We found no significant differences in clinical and functional characteristics between the bronchial inflammatory phenotypes. This finding differs from that of Schleich et al,[3] who reported that the eosinophilic phenotype was associated with atopy, bronchial hyperresponsiveness, poorer control, a reduced FEV1/FVC ratio, increased FeNO and IgE values, and nasal polyposis.[51] The difference may reflect sample size: 96 in our series compared to 508 in the study by Schleich et al.[3] Note that there is probably a significant overlap in biomarker positivity in patients with asthma[52] that may suggest no differentiating characteristics according to inflammatory phenotype. This issue needs to be addressed through more studies, as relevant pathogenic knowledge is required for an era of biologic monoclonals and more personalized medicine.

Another result to highlight from our study was the positive correlation between SI eosinophilia and ED visits in the previous 12 months, possibly comparable to the poorer asthma control of the eosinophilic phenotype reported by

Schleich et al,[3] and in line with the established fact that eosinophilia is a predictor of exacerbations in patients with asthma.[52]

As limitations, our cross-sectional descriptive study may incur possible selection bias; all our patients were required to have undergone specific testing for inclusion, and were patients with predominantly moderate-severe persistent asthma attending a specialist clinic in a tertiary care hospital. Another limitation is the small sample size compared to other studies (for instance, those by Schleich et al[3] and Abdo et al[53]), and, within our sample, the fact that the patients with a mixed inflammatory phenotype were so few that we were unable to characterize them; note, however, that the mixed phenotype prevalence rate in our study reflects that of other studies.[3] A strength of our study is that inflammatory phenotypes were identified on the basis of SI, and comparisons were possible with biomarkers used in typical asthma consultations, such as peripheral eosinophilia and FeNO.

The main conclusions of our study of 96 patients with asthma attending our specialist asthma clinic at a tertiary care hospital are as follows: (a) the predominant bronchial inflammatory phenotype was eosinophilic, and there were no significant differences in clinical and functional characteristics between the 4 different bronchial inflammatory phenotypes; and (b) peripheral eosinophilia detected SI eosinophilia ≥3% with greater diagnostic accuracy than markers such as FeNO and total IgE and was also the only marker that distinguished the eosinophilic phenotype from the other inflammatory phenotypes.

While the SI cell count is the gold standard for non-invasive evaluation of bronchial inflammation in patients with severe asthma and a useful test to guide the choice of biologic treatment, our study would suggest peripheral blood eosinophilia as an alternative when this test is not available, given that, as a good marker for detection of the eosinophilic inflammatory phenotype, it can be potentially useful for doctors to select a suitable biologic treatment and prevent asthma exacerbations.

Abbreviations

ACT, Asthma Control Test; ANOVA, analysis of variance; ATS, American Thoracic Society; AQLQ, Asthma Quality of Life Questionnaire; AUC, area under the curve; BMI, body mass index; CI, confidence interval; ED, emergency department; ELISA, enzyme-linked immunosorbent assay; EOS, absolute eosinophils; ERS, European Respiratory Society; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; GEMA, Spanish Asthma Management Guidelines; GINA, Global Initiative for Asthma; IgE, immunoglobulin E; IL-5, interleukin-5; QoL, quality of life; ROC, receiver operating characteristic; SD, standard deviation; SI, sputum induction.

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Disclosure

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attendance expenses for conferences from AstraZeneca, Chiesi, and Novartis, has acted as a consultant for ALK, AstraZeneca, Boehringer, Merck Sharp & Dohme, MundiPharma, and Sanofi, and has received funds/grants for research projects from several state agencies, non-profit foundations, and AstraZeneca, Chiesi and Menarini. EFMM and ABS and SSM declare no conflicts of interest in this work.

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