

Clinical Communications

Diagnostic accuracy of T2 biomarkers for the prediction of airway eosinophilia in treated smoking asthmatic patients with loss of asthma control

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Clinical Implications

- In current smokers with asthma who are treated by experienced pulmonologists and lose asthma control, a single measurement of blood eosinophils provides useful information for airway eosinophilia.

TO THE EDITOR:

Current smoking represents an important factor for poor asthma control.¹ Measuring and assessing airway inflammation with induced sputum in a clinical setting requires technical support and is considered to be a time-consuming method. In patients with mild to moderate asthma and low smoking status, blood eosinophils showed a higher accuracy for predicting airway eosinophilia compared with the fraction of exhaled nitric oxide (FENO) and serum periostin.² There is some evidence that concomitant local and airway eosinophilia is linked to poor asthma control, but this association may lack strong sensitivity.^{3,4} To our knowledge, there has never been a detailed investigation regarding the association between airway eosinophilia, either pure and/or mixed, and various biomarkers expressing T2 response in a population of current smokers with asthma with loss of asthma control. Furthermore, this is the first study that validates externally various T2 biomarkers in a population of noncontrolled smokers with asthma managed in tertiary asthma clinics.

Consecutive patients previously characterized as well controlled, all current smokers with a smoking habit of at least 10 pack years, were followed at a specialized asthma clinic from 2014 to 2018. All asthmatic subjects were optimally treated according to their attending physicians with inhaled steroids plus another controller at least for 6 months. Compliance was assessed through the electronic prescription system. Patients with severe refractory asthma, chronic obstructive pulmonary disease, or any other respiratory disease, as well as patients with malignancy, heart, renal, liver, or collagen disease were excluded. Patients with a respiratory tract infection or an asthma exacerbation in the past 8 weeks were also excluded. At the time of visit, patients were evaluated for asthma control using the Asthma Control Test. If Asthma Control Test score was less than 20, the patients were characterized as not well-controlled. Then blood samples were assessed for blood eosinophils as percentage and absolute count, and serum periostin, total

IgE, and FENO were also assessed. Within 48 hours, a sputum sample was performed for % cells and measurement of IL-13 in sputum supernatant. Spirometry and baseline demographic characteristics were also recorded. Airway eosinophilia was defined as *pure* when greater than or equal to 3% eosinophils and less than 60% neutrophils were observed and *mixed* when greater than or equal to 3% eosinophils and greater than or equal to 60% neutrophils were observed.

Detailed methods and statistical analysis are provided in this article's Online Repository at www.jaci-inpractice.org.

From 187 screened patients, 91 presented with loss of control during follow-up. Using sputum analysis, 47 patients were characterized as having airway eosinophilia, either pure (35) or mixed (12). Demographic characteristics of study participants are provided in Table E1 in this article's Online Repository at www.jaci-inpractice.org.

Table I presents the diagnostic performance characteristics in predicting airway eosinophilia for the various study biomarkers. The strongest performance was that of blood eosinophils as absolute counts (a cutoff point of 145 cells/ μ L had 94% sensitivity, 89% specificity, with area under the curve value of 0.92; $P < .001$; Figure 1, A). Similar performance was observed for % blood eosinophils (cutoff value 2% had 87% sensitivity, 86% specificity, with area under the curve value of 0.91; $P < .001$; Figure 1, B). We observed similar statistically significant performances in both pure and mixed eosinophilia, with lower area under the curve values in the latter. FENO and IL-13 had a modest performance for the whole group, which improved for the pure eosinophilic profile, whereas they failed to predict airways eosinophilia in the mixed profile. None of the other biomarkers was able to predict airways eosinophilia.

The importance of the current study is the following clinically meaningful message: In current smokers with asthma who are treated by experienced pulmonologists and lose asthma control, a single measurement of blood eosinophils provides useful information for airway eosinophilia, both pure and in the coexistence of neutrophils. Notable strengths include the assessment of patients during a time of instability and the focus on a high-risk group in whom biomarker information might be particularly useful. This single measurement could lead clinicians to follow different treatment strategies to regain disease control.

The importance of monitoring and managing asthma on the basis of sputum cell counts has been extensively proven but at the same time the process requires a dedicated laboratory, is time-consuming, and may cause some discomfort to patients. Many studies have addressed a similar issue using cohorts at a prospective and retrospective manner. However, most of them did not focus on asthma control and were characterized by heterogeneity, either around treatment strategies and/or in the study population,⁵ and, interestingly, none of them focused on smokers with asthma. A study with similar design to ours tried to identify biomarkers for predicting sputum eosinophilia in different adult asthma phenotypes.⁶ The study showed similar diagnostic accuracy for both blood eosinophils and FENO. The above results were not confirmed in a subpopulation of current

TABLE I. Diagnostic performance characteristics in predicting airway eosinophilia

Group	Cutoff point	Sensitivity/specificity	PPV/NPV	AUC 95% CI	P value
All (n = 47)	Blood eosinophils >145 cells/ μ L	94/89	88/92	0.92 (0.85-0.97)	<.001
	Blood eosinophils >2%	87/86	86/87	0.90 (0.84-0.91)	<.001
	FENO >11 ppb	61/70	67/64	0.76 (0.65-0.81)	.032
	Periostin >32 ng/mL	41/73	60/55	0.56 (0.46-0.66)	.318
	IL-13 >78 pg/mL	80/48	60/70	0.62 (0.51-0.71)	.028
	IgE >111 IU/mL	47/68	59/56	0.51 (0.41-0.61)	.877
Pure (n = 35)	Blood eosinophils >155 cells/ μ L	94/77	71/95	0.87 (0.79-0.93)	<.001
	Blood eosinophils >3%	80/82	73/87	0.85 (0.76-0.92)	<.001
	FENO >11 ppb	88/68	62/90	0.82 (0.72-0.89)	<.001
	Periostin >18 ng/mL	88/25	41/77	0.51 (0.40-0.61)	.898
	IL-13 >79 pg/mL	85/48	50/84	0.69 (0.59-0.78)	.008
	IgE >111 IU/mL	42/65	59/56	0.51 (0.41-0.61)	.844
Mixed (n = 12)	Blood eosinophils >144 cells/ μ L	100/52	23/100	0.65 (0.55-0.75)	.045
	Blood eosinophils >2 %	91/56	23/97	0.69 (0.58-0.78)	.023
	FENO >16 ppb	91/24	15/94	0.53 (0.42-0.63)	.735
	Periostin >36 ng/mL	50/67	18/89	0.57 (0.47-0.68)	.390
	IL-13 >43 pg/mL	100/33	18/100	0.62 (0.51-0.72)	.138
	IgE >90 IU/mL	50/66	18/89	0.53 (0.42-0.63)	.716

AUC, Area under the receiver operating characteristic curve; NPV, negative predictive value; PPV, positive predictive value.

and ex-smokers. The latter may be due to heterogeneity of treatment strategies as well as due to high variability in asthma control. There is accumulating evidence that smoking may modify airways inflammation in patients with asthma by enhancing neutrophilic inflammation, which may reduce the diagnostic accuracy for FENO. This was not confirmed in our study, but only in the pure eosinophilic profile where FENO showed similar predictive performance to blood eosinophils. Similar results were also observed for blood eosinophilia, which was less diagnostic in patients with mixed profile than in patients with pure eosinophilic inflammation.

Regarding the remaining biomarkers, we found that neither IgE nor periostin was predictive of airway eosinophilia. Finally, the diagnostic performance of IL-13 in the pure eosinophilic profile partly supports our previous findings where sputum IL-13 was superior to both sputum eosinophils and FENO levels for the identification of well-controlled asthma.⁷

A potential issue is that loss of asthma control may be attributed to the impaired efficacy of inhaled corticosteroids in current smokers with asthma.⁸ However, most of our patients were additionally treated with long-acting β -agonists, which may partially overcome the above-diminished effect.⁹ A possible

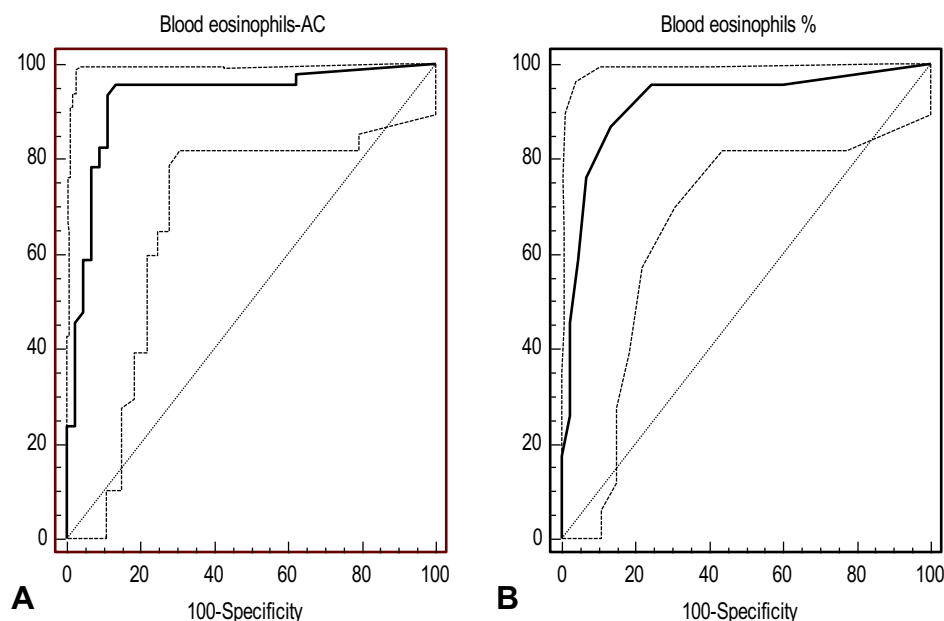


FIGURE 1. Receiver operating characteristic curves for absolute count (AC) and percentage of blood eosinophils for the prediction of airway eosinophilia in current smokers with asthma and loss of asthma control. **A**, Absolute count. **B**, Percentage.

limitation of our study is the fluctuation of asthma control over time. However, the prospective design and the fact that we used data only for patients with confirmed loss of control may overcome this limitation.

In summary, we suggest that measuring blood eosinophils in properly treated smokers with asthma with loss of asthma control can provide valuable information about airway eosinophilia. This information is clinically relevant and may be helpful in the management of smoking asthmatic patients.

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Conflicts of interest: K. Kostikas was a Novartis employee until October 31, 2018.

The rest of the authors declare that they have no relevant conflicts of interest.

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ONLINE REPOSITORY

METHODS

Subjects

Diagnosis of asthma was established according to the Global Initiative for Asthma guidelines.^{E1} All patients signed an informed consent (Ethics approval: 501/7-14 Attiko Hospital).

Sputum induction

Sputum was induced, as previously described, using all the modifications for safe measurements according to the underlying asthma severity.^{E2,E3} Sputum was processed using selected plugs as previously described.^{E4} Cell counting was performed by an observer blind to the clinical characteristics of the subjects. At least 500 inflammatory cells were counted in each sample. A sample was considered adequate when the patient was able to expectorate at least 2 mL of sputum and the slides contained less than 10% squamous cells on differential cell counting. Total cell count expressed as the number of cells $\times 10^6$ and the percentage (%) of sputum inflammatory cells were used for analysis. Sputum supernatants were kept at -70°C for further measurement of cytokines.

Blood measurements

Blood was drawn for the measurement of periostin and blood eosinophils (% and absolute counts).

Lung function

FEV₁ and forced vital capacity were measured using Master Screen Body (Viasys Healthcare, Jaeger, Hoechst, Germany) according to the American Thoracic Society guidelines.^{E5} All values are postbronchodilation.

FENO measurement

FENO was measured using a portable NO analyzer (NIOX MINO, Aerocrine; Sweden) as previously described.^{E6}

Atopic status

A positive skin prick test (mean wheal diameter of ≥ 3 mm) result to any of 20 common aeroallergens (including mites, grasses, trees, fungus, domestic animals) guided by clinical symptoms was used to confirm atopy.

Mediator assays

Serum periostin was measured by an ELISA (R&D, Minneapolis, Minn; duoset Elisa development system, cat. DY3548). The lowest detection level of periostin is 0.975 ng/mL in diluted samples (1:25). The limit of detection was determined by adding 3 SDs to the mean value of 30 zero standard measurements. IL-13 was measured using an ELISA kit (R&D Systems; with a detection limit of 13.2 pg/mL, cat.D1300B).

Statistical analysis

Normally distributed data are presented as mean $\pm$ SD, skewed data as median (interquartile ranges), and categorical data as n (%). Normality of distribution was checked with Kolmogorov-Smirnov test. Differences in numerical variables between 2 groups were evaluated with unpaired *t* tests or Mann-Whitney *U* tests for normally and skewed data, respectively, whereas comparisons of proportions were performed using chi-square tests. For the assessment of the performance, different biomarkers as predictors of an eosinophilic profile either in the whole population as well as in the pure and nonpure eosinophilic profile receiver operating characteristic curves were created. Areas under the receiver operating characteristic curves with 95% CIs and their differences from 0.5 were calculated. Sensitivities, specificities, and positive and negative predictive values were calculated for the optimal cutoff points. Statistical analysis was performed with GraphPad Prism 5 (GraphPad Software Inc, La Jolla, Calif) and MedCalc 9 (MedCalc Software, Mariakerke, Belgium).

RESULTS

Demographic characteristics of study participants are provided in Table E1.

TABLE E1. Subjects' characteristics and inflammatory variables in all patients as well as in those with pure and mixed eosinophilic profile

Characteristic	Whole study population (n = 91)	Pure eosinophilic profile (n = 35)	Mixed eosinophilic profile (n = 12)	P value*
Age (y)	50 (41-59)	51 (37-58)	50 (33-56)	.786
Sex: F/M	37/54	9/35	5/12	.082
Atopy	56/91	21/35	7/12	.325
Smokers	91	35	12	ND
Pack years	31 (19-50)	30 (16-50)	22 (16-50)	.990
Duration of asthma (y)	23 (14-30)	23 (12-30)	23 (14-30)	.878
BMI (kg/m ²)	27 ± 4	26 ± 3	26.5 ± 4	.678
FEV ₁ % pred	83 ± 15	84 ± 13	85 ± 14	.566
FEV ₁ /FVC	72 ± 9	73 ± 7.5	72 ± 8	.499
ACT score	18 (16-20)	17.5 (15-20)	18 (16-20)	.504
Sputum eosinophils %	5 (2-7)	6 (4-7)	5 (4-7)	.553
Sputum neutrophils %	49 ± 13	43 ± 11	64 ± 4	<.001
FENO (ppb)	13 (10-21)	17 (13-22)	15 (12-21)	.045
Serum periostin (ng/mL)	26 (19-38)	27 (21-38)	28 (21-42)	.525
Blood eosinophils % and absolute count	3 (2-7)	6 (4-7)	5 (4-7)	.251
	156 (46-205)	235 (180-288)	198 (173-285)	.049
Sputum IL-13 (pg/mL)	98 (73-156)	120 (90-171)	111 (87-165)	.409
IgE (IU/mL)	114 (88-567)	121 (80-603)	121 (80-603)	.991
Treatment regimens				
ICS	47	35	47	
LABA	44	32	12	ND
LTRAs	19	17	2	

ACT, Asthma Control Test; BMI, body mass index; F, female; FVC, forced vital capacity; ICS, inhaled corticosteroid; LABA, long-acting β_2 -agonist; LTRA, leukotriene receptor antagonist; M, male; ND, not done.

P values showed differences between pure and mixed eosinophilic profiles.

Normally distributed data are presented as mean ± SD, whereas skewed data are presented as median (interquartile ranges).

Bold indicates statistical significance ($P < .05$).

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