Blood Eosinophils, Fraction of Exhaled Nitric Oxide, and Serum Eosinophil Cationic Protein as Surrogate Markers for Sputum Eosinophils in Asthma: Influence of Treatment With Inhaled Corticosteroids

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Bronchial asthma is a heterogeneous disease that includes various phenotypes with differences in clinical expression and response to therapy. Among the various inflammatory phenotypes, eosinophilic asthma is associated with a good response to inhaled corticosteroids and a benefit from new specific biologics such as omalizumab or IL-5 antagonists. In addition, high eosinophilia is associated with an increased risk of exacerbations [1]. Eosinophilic asthma is usually defined as that presenting eosinophil levels greater than 3% in sputum samples. However, the several well-known limitations of sputum analysis mean that the technique is only available in highly specialized centers [2].

In a previous study of 124 nonsmokers with mild or moderate persistent asthma, we showed that the fraction of exhaled nitric oxide (FeNO), an easily obtained inflammatory marker, was highly sensitive for detecting eosinophilic asthma in patients who did not receive treatment with corticosteroids [3]. In the same cohort, we assessed the predictive capacity of another inflammatory marker that is also accessible in routine clinical practice, namely, eosinophil cationic protein (ECP), a marker of activation of eosinophils, alone or combined with FeNO measurements. We also assessed the influence of corticosteroid treatment on this predictive capacity. The methodology used has been described in detail elsewhere [3], namely, a cross-sectional study with data collected at a single visit. Induced sputum was analyzed according to the methodology of Fahy et al [4].

Peripheral blood eosinophil counts were obtained from standard complete blood counts, ECP was measured using the standard kit ImmunoCAP ECP (Thermo Fisher Scientific), and FeNO was assessed using an online device at a constant flow of 50 mL/s (Niox Mino; Aerocrine AB). Receiver operating curves (ROC) were constructed to assess the discriminative ability of FeNO, ECP, and blood eosinophils to classify eosinophilic asthma (eosinophils in sputum ≥3%). The statistical comparison of the AUC/ROC curves was conducted using the de Long test. Sensitivity, specificity, and positive and negative predictive values were estimated for different cut-off points for each marker and their combinations in the whole sample and stratified according to the use of inhaled corticosteroids.

The results of the AUC/ROC curve analysis are presented in the Table, A. Determination of blood eosinophils followed by FeNO had the highest AUROC (best predictive capacity of eosinophils in sputum), although the differences were not significantly different. When both parameters were associated, the predictive capacity improved discretely, but again not significantly. On the contrary, ECP had a very poor predictive capacity. The multivariate logistic regression model showed that eosinophils in blood were significantly associated with sputum eosinophilia ≥3% (OR, 1.01; *P*=.022) and, albeit marginally, with FeNO (OR, 1.04; *P*=.052). A cut-off point for eosinophils in blood ≥200 mm^3 had high sensitivity, but low sensitivity, for both treated and untreated patients (Table, B). When combined with determination of FeNO, blood eosinophilia significantly improves specificity, especially in patients not treated with corticosteroids. Treatment with inhaled corticosteroids worsened the predictive capacity, especially of FeNO. Blood eosinophils combined with FeNO showed a negative predictive value of 67%. Our data allow us to conclude that 40% of patients would be misclassified, if blood eosinophils alone or in combination with FeNO are used

The relationship between eosinophils in blood and sputum is controversial, with contradictory data in children [5] and adults [6], especially in populations with more severe asthma than the present one. However, Zahng et al [7] reported that blood eosinophils were highly predictive of eosinophilic asthma, at a blood eosinophil cut-off of 260/mm^3. In a population with mild or moderate asthma whose results were replicated in an external validation cohort of severe asthmatics, Wagener et al [8] reported similar results and suggested, as do we, that FeNO did not demonstrate a clear added value to the blood eosinophil count. Finally, in a recent survey, Demarche et al [9] were able to correctly classify asthma as eosinophilic or noneosinophilic in only 58% of patients from a cohort of 869 patients using a combination of FeNO, blood eosinophils, and serum total IgE as a surrogate marker. To our knowledge, no previous studies have directly assessed the influence of inhaled corticosteroids, because almost all the populations studied had large numbers of patients treated with them. This therapy rapidly decreases FeNO levels and to a greater extent than sputum or blood eosinophils. Blood eosinophils transmigrate quickly into lung tissue and the airways, with the result that the association between them and airway inflammation might be transient. Fully activated eosinophils are located outside the bloodstream, and the real significance of serum ECP is not well known, thus possibly explaining why it fails as a good surrogate marker of eosinophilia in sputum. Furthermore, local eosinophilopoiesis, which could eliminate the generally closer relationship between blood and sputum eosinophils, is present in cases of severe eosinophilic asthma, although it may also be observed in milder forms of the disease [10]. Our study is limited by the fact that we did not replicate our findings in an external validation cohort. Nevertheless, splitting our

Table. Results

A. ROC Curve Analysis

Predictor	AUC (95% CI) Total Sample	AUC Non–ICS-Treated	AUC ICS-Treated
Surrogate marker			
FeNO	0.82 (0.73-0.91)	0.85 (0.74-0.97)	0.73 (0.55-0.91)
Blood eosinophils	0.84 (0.73-0.94)	0.92 (0.84-0.99)	0.70 (0.47-0.93)
Serum ECP	0.66 (0.52-0.80)	0.75 (0.59-0.91)	0.51 (0.27-0.76)
Combined markers			
FeNO+Blood eosinophils+ECP	0.87 (0.77-0.97)	0.94 (0.87-1.00)	0.87 (0.72-1.00)
FeNO+ Blood eosinophils	0.87 (0.78-0.96)	0.92 (0.84-0.99)	0.82 (0.65-0.99)

B. Sensitivity, specificity, PPV, and NPV of different surrogate markers, and their combination using alternative cut-off points to diagnose eosinophilic airway inflammation (less than, more than, or equal to 3% sputum eosinophils), stratified by treatmenta

	Total sample				ICS-Treated				Non–ICS-Treated			
	Se	Sp	PPV	NPV	Se	Sp	PPV	NPV	Se	Sp	PPV	NPV
FeNO ppb												
FeNO ≥17	97	10	77	50	96	0	76	0	98	14	78	67
FeNO ≥21	91	48	85	62	87	14	77	25	93	64	89	75
FeNO ≥26	82	67	89	54	78	57	86	44	84	71	90	59
FeNO ≥31	73	71	89	45	65	57	83	33	77	79	92	52
Blood eosinophils												
Be ≥150	97	28	81	71	95	29	78	67	97	27	83	75
Be ≥200	97	33	82	75	95	29	78	67	97	36	84	80
Be ≥250	79	78	92	54	68	57	81	40	85	91	97	62
Be ≥300	79	78	92	54	68	57	81	40	85	91	97	62
Be ≥350	50	94	97	37	42	86	89	35	54	100	100	38
FeNO ppb + Blood eosinophils												
FeNO ≥21 & eosinophils≥250	75	76	91	48	61	50	79	30	82	91	97	59
FeNO ≥21 & eosinophils≥200	88	65	89	61	83	50	83	50	90	73	92	67

Abbreviations: AUC, area under the curve; FeNO, fraction of exhaled nitric oxide; ICS, inhaled corticosteroid; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; Se, sensitivity; Sp, specificity.
[a]Bold letters mark the best cut-off levels.

sample would have precluded studying the effect of inhaled corticosteroids on these surrogate markers.

In conclusion, blood eosinophilia has good sensitivity but lower specificity for detection of eosinophilia in sputum, even in patients treated with inhaled corticosteroids, in contrast with FeNO, whose sensitivity decreases in patients treated with corticosteroids. Serum ECP seems useless as a surrogate marker of sputum eosinophilia. In treated patients, the balance between specificity and sensitivity improves only discretely when blood eosinophil counts are associated with FeNO measurements. Although these surrogate markers have clinical value, they cannot substitute induced sputum cytology.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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