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Low Sputum Eosinophils Predict the Lack of Response to Beclomethasone in Symptomatic Asthmatic Patients*

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Background: **The prognostic role of low sputum eosinophils in steroid-naïve, symptomatic asthmatic patients is controversial.**
Aim: **To verify whether low sputum eosinophils predict poor response to treatment with inhaled corticosteroids.**
Methods: **Sixty-seven symptomatic asthmatic patients with moderate asthma were examined before and after 2 weeks and 4 weeks of treatment with beclomethasone dipropionate, 500 μg bid. None received corticosteroids in the 3 months preceding the study. At each visit, all patients underwent spirometry, methacholine challenge, and sputum induction. The patients recorded symptom scores and peak expiratory flow (PEF) throughout the study.**
Results: **Seventeen patients had low sputum eosinophils despite being symptomatic. Patients with high (> 3%) sputum eosinophils at baseline showed significant improvement in symptoms, pulmonary function, and bronchial hyperresponsiveness after treatment, whereas patients with low sputum eosinophils showed no significant improvement in most clinical and functional outcomes. Among the baseline indexes examined, sputum eosinophils had the highest negative predictive value but low positive predictive value for the response to treatment. Multiple stepwise regression showed that only baseline FEV_1 and sputum eosinophil percentages significantly correlated with changes in FEV_1 after treatment.**
Conclusions: **We suggest that, among the indexes examined, low sputum eosinophils are the best predictor for poor corticosteroid effects in asthma.** ***(CHEST 2006; 129:565–572)***

Key words: asthma; beclomethasone dipropionate; eosinophils; induced sputum

Abbreviations: ECP = eosinophil cationic protein; PD_{20} = cumulative dose of methacholine producing a 20% fall in FEV_1; PEF = peak expiratory flow; ROC = receiver operating characteristic

Inhaled corticosteroids are the treatment of choice for all degrees of persistent asthma, from mild to severe,[1] and are thought to act, at least in part, through a reduction of eosinophilic inflammation.[2] Corticosteroids improve symptoms, pulmonary function, and bronchial hyperresponsiveness, and decrease exacerbation rates and the levels of airway inflammatory markers,[2] although their effects on the different outcomes are dose dependent and time dependent.[2,3]

Bronchial asthma is usually associated with high sputum eosinophil levels.[4] However, some asthmatic patients, although symptomatic, may have normal sputum eosinophil levels.[5] In some cases, this might be due to the effect of previous treatment with oral or inhaled corticosteroids, but patients with low sputum eosinophils despite the presence of active asthma symptoms have been reported.[6] Although

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sputum eosinophilia may be associated with greater severity of the disease in untreated subjects,[7] the clinical significance of normal levels of sputum eosinophils in symptomatic patients is not clear. Some authors[8,9] suggest that sputum eosinophil levels predict the response to treatment with inhaled corticosteroids, whereas others[10] found no difference in the response to inhaled corticosteroid treatment between patients with high and patients with low sputum eosinophils or, at most, found that sputum eosinophil levels added little information to clinical and functional findings, without being the main predictor for response.[11]

In the present study, we wanted to verify whether low sputum eosinophil levels predict a poor clinical and functional response to treatment with inhaled corticosteroids. In this attempt, we selected a group of steroid-naive subjects with mild-to-moderate asthma from our clinic routine, and evaluated their clinical and functional response to 4 weeks of treatment with inhaled corticosteroid according to baseline levels of sputum eosinophilia.

Materials and Methods

Subjects

We screened 72 patients with symptomatic, mild-to-moderate asthma who were recruited from the clinics of the Cardiothoracic Department Respiratory Unit. The diagnosis of asthma was made according to internationally accepted criteria[1] after assessing reversible airway obstruction and/or nonspecific bronchial hyperresponsiveness to methacholine. All but three subjects were defined as having moderate asthma according to at least one of the following criteria: daily symptoms, nighttime symptoms $>$ 1/wk, FEV_1 of 60 to 80% of predicted, or peak expiratory flow (PEF) variability $>$ 30%[1]; the remaining subjects were classified as mild persistent asthmatics. All patients had received antiinflammatory treatment for no $>$ 3 months in the 2 years preceding the study, and none in the last 3 months. Patients with a recent respiratory tract infection or concomitant airway disease were excluded. At the time of the study, all patients had active symptoms and were receiving rescue bronchodilators only. Five patients could not expectorate at baseline evaluation and were thus excluded from the study. The remaining 67 were enrolled and produced adequate sputum samples at all subsequent inductions. The protocol was approved by the local Ethical Committee. Informed written consent was given by all patients.

Study Design

At a screening visit, all patients underwent spirometry, and patients with FEV_1 values $>$ 70% of predicted underwent methacholine challenge (n = 56). Every procedure was always performed in the morning at the same time of the day, ± 1h. All patients recorded daily symptom score, use of rescue β_2-agonists, and morning and evening PEF on a diary card over the whole study period. There was a 2-week run-in period (baseline) to demonstrate the degree of asthma severity. Eligible patients underwent sputum induction with hypertonic saline solution and then started treatment with inhaled beclomethasone, 500 μg bid. Methacholine challenge and sputum induction were repeated on 2 consecutive days, after 2 weeks and 4 weeks of treatment. Blood samples were collected before each sputum induction.

Sputum Induction

Sputum was induced according to the method previously described.[12] Hypertonic saline solution was nebulized with an ultrasonic nebulizer (Sirius; Technomed; Firenze, Italy) with a 2.8 mL/min output, and was inhaled for 5 min periods for up to 30 min. NaCl solution was increased at intervals of 10 min from 3 to 4 to 5%. FEV_1 was measured before and every 5 min during hypertonic saline solution inhalation. Every 5 min after the start of nebulization, subjects were asked to rinse their mouth, discard saliva, and cough sputum into a clean container. The nebulization was stopped after 30 min or when FEV_1 fell by $\geq$ 20% from baseline. At any time during the induction, the inhalation was stopped in the subjects with symptoms of bronchoconstriction, and FEV_1 was then measured.

Sputum Processing

The whole sputum sample was diluted with an equal volume of 0.1% dithiotreithol (Sputasol; Unipath; Basingstoke, UK). Samples were treated as previously reported.[12] Macrophage, lymphocyte, neutrophil, and eosinophil percentages were expressed as percentage of total inflammatory cells, excluding squamous cells. The upper limit of normal range for sputum eosinophils was set at 3% derived from a group of normal subjects.[13]

Blood Processing

Blood samples were examined for total and differential cell counts. The remainder was centrifuged at 1,000*g* for 10 min after 60 ± 10 min of rest at room temperature to allow clotting. The supernatant was again centrifuged to ensure complete cell removal. Serum was then collected and stored at − 80°C for further analysis.

Eosinophil Cationic Protein Measurement

Eosinophil cationic protein (ECP) in sputum supernatant and in serum was measured by means of a radioimmunoassay (Pharmacia RIA; Pharmacia; Uppsala, Sweden) [normal values in serum: ECP, 2.3 to 16 μg/L; lower detection limit, $<$ 2 μg/L]. Since ECP was higher than the upper detection limit of the method in four sputum samples, these samples were diluted 1:10 and the measurements were repeated. Sputum ECP was corrected (multiplied by two) for the processing dilution with dithiothreitol.

Methacholine Challenge Test

Short-acting bronchodilators but not inhaled beclomethasone were withdrawn 8 h before each test. Methacholine (Sigma; St. Louis, MO) was delivered by a jet nebulizer (model 646; Devilbiss; Somerset, PA) using the procedure described elsewhere.[14] The cumulative dose of methacholine producing a 20% fall in FEV_1 (PD_{20}) was computed; a value $<$ 1,000 μg of methacholine was considered as positive for bronchial hyperresponsiveness.

PEF, Symptom Evaluation, Rescue β_2-Agonist Use

Symptoms (wheezing, chest tightness, shortness of breath, cough) were rated on a scale from 0 (none) to 4 (never slept

because of asthma) for nighttime symptoms, and 0 (none) to 5 (could not attend to common daily activities because of asthma) for daytime symptoms, and added up to give the average daily symptom score.[15] Pre-drug morning PEF was expressed as percentage of predicted value. PEF variability, expressed as amplitude percentage mean, was calculated as follows: (highest PEF value − lowest PEF value)/mean daily value × 100; a value > 10% was considered as high. Rescue β_2-agonist use was evaluated by recording the number of times that the patients needed to inhale one or more puffs of short-acting β_2-agonists.

Statistical Analysis

Cell counts and sputum volume are expressed as median (range). Symptom score, morning PEF, daily PEF amplitude percentage mean, percentage of days with abnormal PEF amplitude percentage mean, and short-acting rescue β_2-agonist use were averaged over the last 7 days before each visit and expressed as median (range) except for morning PEF and daily PEF amplitude percentage mean, which are expressed as mean ± SD. FEV_1 is expressed as mean ± SD. PD_{20} is expressed as geometric mean, and was log-transformed for comparisons. Mann-Whitney *U* test and Friedman test were used to compare differential sputum cell counts, ECP, blood eosinophil percentages, and serum ECP between groups and within groups, respectively. Unpaired *t* test and analysis of variance for repeated measures were used to compare FEV_1 and log PD_{20} between groups and within groups, respectively. Based on current knowledge of reproducibility,[16,17] FEV_1 increase ≥ 12% from baseline and PD_{20} doubling dose were considered as a positive response to treatment. The predictive values for each baseline measurement were determined by using 2 × 2 contingency tables, so as to obtain positive and negative predictive values, and sensitivity and specificity.[18] Stepwise regression analysis was performed using treatment outcomes as dependent variables, and some baseline measurements (FEV_1, symptom score, PD_{20}, and sputum eosinophils) as independent variables.

Results

All 67 enrolled patients completed the study. After baseline, all patients produced adequate sputum samples at subsequent evaluations, so that no sputum induction had to be rescheduled. At baseline, 17 patients (26%) had low (≤ 3%) sputum eosinophils. Sputum neutrophils were higher in patients with low sputum eosinophils. Patients with low sputum eosinophils were older and tended to have a slightly longer duration of disease ($p = 0.06$) than patients with high sputum eosinophils (Table 1). There was no difference in sex, smoking habit, atopy prevalence, and asthma severity between high and low sputum eosinophil patients. Baseline FEV_1, morning PEF, PD_{20}, and symptom score were no different between the two groups of patients (Table 2). Baseline daily PEF amplitude percentage mean and percentage of days with abnormal PEF amplitude percentage mean tended to be higher, and baseline rescue β_2-agonist use was higher, in patients with high sputum eosinophils.

After 2 weeks and 4 weeks of treatment, FEV_1, PD_{20}, PEF, and PEF-derived indexes significantly improved in patients with high baseline sputum eosinophils but not in patients with low baseline sputum eosinophils (Table 2). Rescue β_2-agonist use decreased in both groups of patients. Symptom score improved in both groups but reached statistical significance only in patients with high baseline sputum eosinophils. Comparison between groups after 4 weeks of treatment showed that patients with high baseline sputum eosinophils had significantly higher PEF and lower symptoms score than patients with low baseline sputum eosinophils.

Table 1—*Subject Characteristics

Characteristics	High Sputum Eosinophils	Low Sputum Eosinophils
Subjects	50	17
Male/female gender	19/31	3/14
Age, yr	32 ± 11	45 ± 15†
With/without atopy	38/12	12/5
Smoker/nonsmoker/ex-smoker	12/32/6	1/13/3
Disease duration, yr	11 ± 8	16 ± 14‡

*Data are presented as No. or mean ± SD.
†$p < 0.01$.
‡$p = 0.06$.

Baseline blood eosinophil percentages and sputum and blood ECP levels were significantly higher in patients with high sputum eosinophils. After 2 weeks and 4 weeks of beclomethasone treatment, sputum and blood eosinophils and ECP decreased in patients with high baseline sputum eosinophils but not in patients with low sputum eosinophils (Fig 1, Table 3). After treatment, sputum eosinophil percentages decreased to < 3% in 34 of the 50 patients with high sputum eosinophils. However, patients whose sputum eosinophils remained > 3% after treatment did not differ from patients whose sputum eosinophils fell < 3% in terms of functional and clinical indexes except for symptom score, which after treatment was higher in patients whose sputum eosinophil counts remained > 3% (symptom score, 0.3; range, 0 to 3.8; vs 0; range, 0 to 1.8; $p = 0.03$). Among patients with low baseline sputum eosinophils, sputum eosinophils increased > 3% in five patients, three patients after 2 weeks, and two patients after 4 weeks of treatment. This behavior could not be predicted from baseline data, since these five patients did not differ from the remaining patients in terms of clinical and functional data, such as baseline FEV_1, age, smoking habit, or degree of atopy.

Sputum volume, total cell counts, and differential cell counts for lymphocytes and neutrophils did not change in either group of patients, whereas macrophages significantly increased in patients with high baseline sputum eosinophils. Blood neutrophils did not change in either group of patients (Table 3).

Table 2—*Functional and Clinical Indices Measured Before and After Corticosteroid Treatment

Variables	High Sputum Eosinophils			Low Sputum Eosinophils		
	Baseline	After 2 wk	After 4 wk	Baseline	After 2 wk	After 4 wk
FEV_1, % predicted	87.9 ± 15.4	97.3 ± 14.5§	96.9 ± 14.5§	90.2 ± 17.3	91.7 ± 17.7	91.2 ± 20.0
PD_{20}, μg†	135 (2.8)	224 (4.1)§	218 (5.2)§	121 (3.7)	219 (4.3)	185 (5.2)
PEF, % predicted	73.3 ± 16.1	80.9 ± 14.9§	85.0 ± 15.6§	68.4 ± 20.7	70.8 ± 18.4	69.9 ± 20.3
PEF amplitude % mean, %	22 ± 15	11 ± 7§	9 ± 5‡	15 ± 9#	12 ± 7	12 ± 9
Abnormal amplitude % mean, % of days	57 (0–100)	29 (0–86)§	21 (0–92)§	48 (0–77)#	29 (0–92)	33 (0–85)
Symptom score	1.5 (0–3.4)	0 (0–4)§	0 (0–3.8)§	1.2 (0.7–3.6)	0.7 (0.1–2.1)	0.25 (0–2)‖
β_2-Agonist use	1.0 (0–4.4)	0.1 (0–5.3)§	0 (0–3)§	0.4 (0–1.9)¶	0.1 (0–2.9)	0.1 (0–1.4)‡

*Data are expressed as mean ± SD or median (range) unless otherwise indicated.
†Geometric mean (geometric SD).
‡$p < 0.05$ from baseline.
§$p < 0.01$ from baseline.
‖$p = 0.08$ from baseline.
¶$p < 0.05$ between groups.
#$p \geq 0.1$ to < 0.05 between groups.

When a positive response to 1 month of beclomethasone dipropionate treatment was evaluated as a change in $FEV_1 \geq 12\%$ from baseline, or as a doubling of the provocative dose of inhaled methacholine, baseline sputum eosinophilia had the highest negative predictive value for the response to treatment (Table 4). Indeed, of those patients with low sputum eosinophils (≤ 3%), none had an increase in $FEV_1 \geq 12\%$. However, the positive predictive value was low. Similar results were observed when the cutoff value for sputum eosinophils was set at 2% or at 1% (data not shown). The remaining baseline evaluations also had high negative predictive values, but all had low positive predicting values (Table 3). The sensitivity in detecting the increase in FEV_1 after treatment was high for sputum eosinophils > 3% and $PD_{20} < 400$ μg, but low for the other indexes considered. Specificity was high for $FEV_1 < 80\%$ and symptom score ≥ 1 but low for the remaining indexes. Similar results were observed when the outcome considered was PD_{20} doubling dose. With a cutoff value of 50%, high sputum neutrophils showed poor predictive value for the response to inhaled corticosteroids (negative predictive value for neutrophils ≤ 50%, 70%; positive predictive value for neutrophils > 50%, 20%). The positive and negative predictive values for other sputum inflammatory cells (lymphocytes and macrophages) and for blood cells were low (data not shown). Both patients with high (> 5%, upper limit of normal in our laboratory) and low blood eosinophils showed a significant improvement in FEV_1, PEF, PEF amplitude percentage mean, symptom score, and rescue β_2-agonist use after treatment. PD_{20} improved in both groups of patients, but the improvement reached statistical significance only in patients with high blood eosinophils ($p = 0.01$ vs $p = 0.08$, respectively). However, sensitivity, specificity, and positive and negative values of blood eosinophils in predicting PD_{20} improvement were poor (sensitivity, 35%; specificity, 54%; positive predictive value, 54%; negative predictive value, 35%).

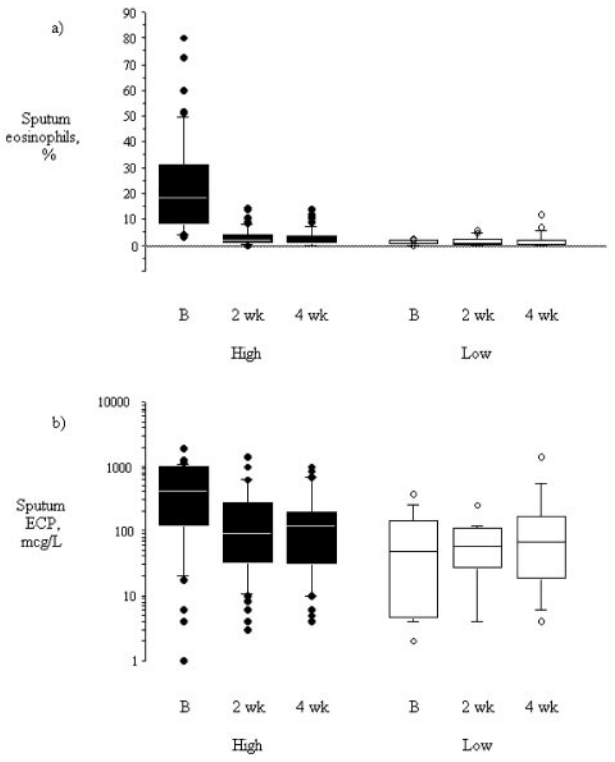

FIGURE 1. Effects of beclomethasone treatment on sputum eosinophils (*top*, *a*) and sputum ECP (*bottom*, *b*) in asthmatic patients with high and low baseline sputum eosinophils. B = baseline.

Receiver operating characteristic (ROC) curves

Table 3—*Induced Sputum and Blood Indices Measured Before and After Treatment

Variables	High Sputum Eosinophils			Low Sputum Eosinophils		
	Baseline	After 2 wk	After 4 wk	Baseline	After 2 wk	After 4 wk
Sputum						
Eosinophils, %	18.2 (3.1–80.1)	1.8 (0–14.5)†	1.4 (0–13.8)†	1 (0–2.7)‡	0.8 (0–5.6)	0.6 (0–11.9)
Neutrophils, %	41 (8.2–86.2)	46.9 (14.8–96.3)	51.5 (5.6–92.2)	60.6 (26.4–94.7)	59.7 (25.9–90.8)	62.5 (0–82.8)
ECP, μg/L	420 (1–1,920)	94 (4–1,370)†	122 (4–1,010)†	24 (2–380)‡	60 (4–130)	68 (4–1,432)
Blood						
Eosinophils, %	6.7 (0.7–22)	3.2 (0.7–13.1)‡	2.6 (0.6–12)‡	2.9 (0.4–7.3)‡	2.5 (0.6–8.0)	1.9 (0.1–7.0)
Neutrophils, %	55.7 (45.7–69.8)	59.1 (53.9–72.8)	62.8 (50.0–75.4)	54.0 (46.5–64.3)	58.0 (48.2–79.1)	57.7 (46.2–73.1)
ECP, μg/L	15 (2–250)	10 (1–60)†	9 (1–115)†	8 (1–48)§	5 (1–30)	6 (1–280)

*Data are presented as median (range).
†$p < 0.01$ from baseline.
‡$p < 0.01$ between groups.
§$p = 0.05$ between groups.

for different cutoff levels of sputum eosinophils, baseline FEV_1, PD_{20}, and symptom score are reported in Figure 2. The area under the ROC curve was low for all predictors but greater for sputum eosinophils in comparison with the other predictors.

Multiple stepwise regression showed that, among the different baseline variables considered, only baseline FEV_1 percentage of predicted value and baseline sputum eosinophil percentage significantly correlated with the change in FEV_1 after treatment. None of the different baseline variables significantly correlated with the change in bronchial hyperresponsiveness as expressed by PD_{20} doubling dose.

Table 4—*Negative and Positive Predictive Values, and Sensitivity and Specificity of Some Baseline Indices in Predicting Two Different Outcomes (FEV_1 Increase ≥ 12% and PD_{20} Doubling Dose) After Corticosteroid Treatment*

Variables	Predictive Value, %		Sensitivity, %	Specificity, %
	Negative	Positive		
FEV_1 increase ≥ 12%				
Sputum eosinophils				
≤ 3%	100		100	
> 3%		34		34
Baseline FEV_1				
≥ 80%	86		59	
< 80%		56		84
PD_{20}				
≥ 400 μg	82		80	
< 400 μg		18		20
Daily symptom score				
<1	87		87	
≥ 1		27		27
PD_{20} doubling dose				
Sputum eosinophils				
≤ 3%	73		84	
> 3%		43		28
Baseline FEV_1				
≥ 80%	59		11	
< 80%		33		86
PD_{20}				
≥ 400 μg	75		89	
< 400 μg		42		21
Daily symptom score				
< 1	54		68	
≥ 1		38		25

Discussion

We found that 26% of symptomatic asthmatic patients enrolled in the present study had low sputum eosinophils. These patients, treated with inhaled corticosteroids for 4 weeks, showed no improvement in respiratory function and bronchial hyperresponsiveness. They only experienced some clinical improvement, as shown by the decrease in rescue β_2-agonist use and in symptom score, which might have been due spontaneous regression to normal or to the idea of being treated. The lack of improvement in FEV_1 might be due to the slightly, although not significantly, higher baseline FEV_1 values in patients with low sputum eosinophils. Conversely, as expected,[19] patients with high sputum eosinophils showed clinical and functional improvement along with a decrease in sputum and blood eosinophils and ECP. The eosinophil cutoff value of 3% yielded a very high negative predictive value and high sensitivity. However, other clinical and functional baseline findings had predictive values similar to those of sputum eosinophilia.

The proportion of patients with low sputum eosinophils in the present study is lower than previously reported.[5,8,10] This might be due to previous or current treatment with inhaled corticosteroids reported in the other studies,[5,18] or to a different level of severity.[5] All our patients were symptomatic, with mild persistent or moderate asthma, and not treated

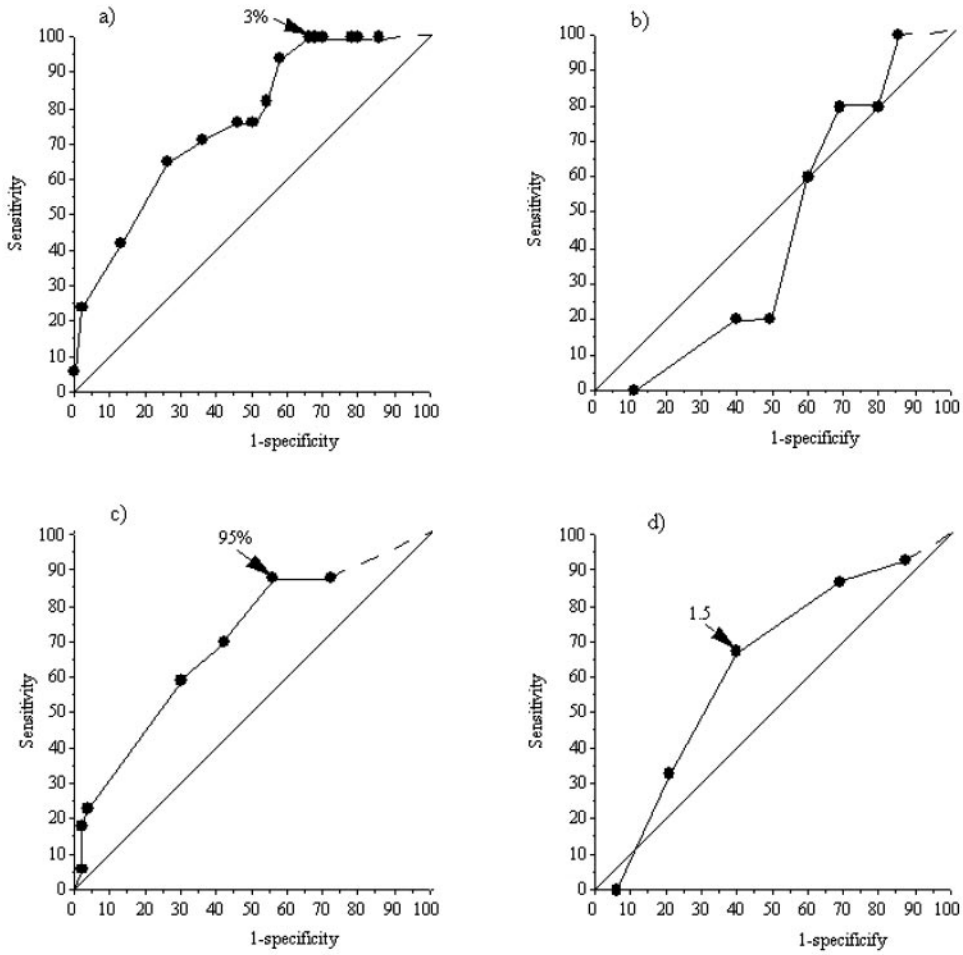

FIGURE 2. ROC curves for sputum eosinophils (*top left*, *a*), PD_{20} (*top right*, *b*), FEV_1 (*bottom left*, *c*), and symptom score (*bottom right*, *d*).

with either inhaled or oral corticosteroids for at least 3 months before the study.

The clinical significance of high levels of sputum eosinophils is not completely clear. While in the study by Anees et al[7] asthmatic patients with high sputum eosinophils were strikingly worse than those with low sputum eosinophils, since they had much poorer lung function and greater bronchial hyperresponsiveness, in other studies[9,20] the differences between high and low sputum eosinophil patients are less marked, and are often due to different selection criteria. The relationship between eosinophil levels and lung function is poor, and the presence of sputum eosinophils cannot predict asthma severity.[21] However, when treatment strategy is aimed at keeping sputum eosinophils low, patients have fewer asthma exacerbations.[22] Thus, high sputum eosinophils may predict higher exacerbation probability and be a marker of less well-controlled asthma, more responsive to inhaled corticosteroid treatment. Indeed, at baseline evaluation, patients with high sputum eosinophils tended to use more rescue medication and to have a greater PEF variability. However, subjects without sputum eosinophilia and prominent neutrophilia may belong to a distinct subgroup of asthmatic patients. As in other studies,[9] noneosinophilic asthmatics were older, suggesting a different specific phenotype of asthma.

After treatment, sputum eosinophils significantly decreased in all patients with high baseline sputum eosinophils but remained above the cutoff limit of 3% in 16 of 50 patients. Compliance to treatment was not specifically assessed, and this might partly explain the lower biological response to treatment in some patients. The persistence of high levels of sputum eosinophils in some patients, however, scarcely affected asthma control, which was almost as good as in patients whose sputum eosinophil levels fell to $< 3\%$. Indeed, most clinical and functional data measured after treatment were no different between the two groups, since only symptom score was significantly higher in patients whose sputum eosinophils remained $> 3\%$.

In the present study, high sputum eosinophils

showed high sensitivity in detecting inhaled corticosteroid responders. However, low sputum eosinophils strongly predict the lack of response to inhaled corticosteroids in symptomatic asthmatic patients. Previous studies[8,10,11] have shown controversial results about the efficacy of corticosteroid treatment in asthmatic patients with low sputum eosinophils. Pavord et al[8] observed that the response to inhaled corticosteroid treatment was poorer in patients with low sputum eosinophils than in patients with high sputum eosinophils. In contrast, Godon et al[10] found that, after inhaled corticosteroid treatment, respiratory symptoms, quality of life, lung function, and bronchial hyperresponsiveness significantly improved in patients with low sputum eosinophils as well as patients with high sputum eosinophils. The characteristics of their patients, however, were more homogeneous, since there was no difference in age between the two groups and atopy prevalence in both groups was higher than that observed in our patients. Meijer et al[11] showed that sputum or blood eosinophils, although providing additional information, were not major predictors of inhaled corticosteroid efficacy. In the latter study, 30% of patients were receiving inhaled corticosteroids at baseline, and this may explain the different results, since our patients had all been steroid free for at least 3 months. Also, in the study by Meijer et al,[11] the duration of treatment was 2 weeks, which may not be enough to outline differences between groups.

In our study, the positive and negative predictive values of baseline $FEV_1 < 80\%$, $PD_{20} < 400$ μg, and daily symptom score ≥ 1 were similar to those of sputum eosinophils $> 3\%$, suggesting that not only sputum eosinophilia but also other indexes can predict the response to inhaled corticosteroids. Indeed, multiple stepwise regression showed that sputum eosinophilia and FEV_1 were independent significant predictors of FEV_1 increase after treatment. No independent predictors were observed for the increase in PD_{20}. Responsiveness to inhaled corticosteroids may markedly vary among patients, and several clinical and functional baseline features, such as age at onset of asthma, smoking habit, total serum IgE, level of pulmonary function and of bronchial hyperresponsiveness, and bronchial reversibility, may affect this response.[23,24] Thus, sputum eosinophilia is not the only determinant of the airway response to inhaled corticosteroids in asthma.

Cigarette smoke has been shown to reduce the efficacy of inhaled corticosteroid treatment.[25] In the present study, however, the number of smokers was low among subjects with low sputum eosinophil levels, suggesting that the lack of sputum eosinophilia in these subjects cannot be ascribed to smoking habit. Furthermore, in our study smokers with sputum eosinophilia responded to corticosteroid treatment as well as nonsmokers with sputum eosinophilia. This fact suggests that eosinophilia, and not smoking habit, may be responsible for the different response to corticosteroid treatment.

Other factors can explain the differences observed in the different studies evaluating the effects of corticosteroids in asthma. It is well known that corticosteroids affect many aspects of inflammation besides eosinophils. Thus, the improvement observed in patients with low sputum eosinophils in some studies may be due to other corticosteroid effects, such as the effects on lymphocytes, on vascular permeability, and on β_2-receptor expression.[2] Another reason might be the spontaneous variations of airway inflammation over time.[26] However, treatment duration was relatively short in all the studies reported,[8,10,11] and spontaneous variations of airway inflammation should therefore be negligible over such short time intervals.[27] We did not measure inflammatory cells in induced sputum before the beginning of the study, and we therefore cannot tell whether sputum eosinophilia is a persistent or a variable marker of airway inflammation in these subjects. However, in a preliminary study, we evaluated a group of 15 steroid-naive symptomatic subjects with mild-to-moderate asthma without sputum eosinophilia. These patients underwent monthly analysis of induced sputum, and sputum eosinophil levels remained low in 13 of 15 subjects over a 3- to 6-month follow-up despite being symptomatic and receiving β_2-agonist treatment only (data not shown).

From our results, one might argue that asthmatic patients with low sputum eosinophils should not be treated with inhaled corticosteroids, since they have no beneficial effects. However, the treatment duration may not have been long enough to induce significant functional improvement through different mechanisms. As mentioned before, inhaled corticosteroids can interfere with markers of airway inflammation other than eosinophils. However, long-acting β_2-agonist monotherapy does not produce better results.[19] However, other antiinflammatory drugs may have better beneficial effects in this group of asthmatic patients.

In the present study, we did not include a placebo group for ethical reasons. Thus, we cannot tell whether the decrease in the use of rescue β_2-agonist and in symptom score observed also in patients with low sputum eosinophils is due to the effect of corticosteroids or to the idea of being taken care of. However, the efficacy of corticosteroid treatment on inflammatory markers in asthma is well known, and therefore the improvement observed in patients with high sputum eosinophils should not simply represent

regression toward the mean. Also, we did not choose a cross-over study design because the duration of the carryover effect of ICS on sputum eosinophils is not completely known, and therefore the washout period for the ICS group might have been too long to get back to baseline sputum eosinophil levels. Furthermore, the number of subjects examined might have been small, but it was still greater than that reported by most previous published studies[8,10,18] on this topic. A larger number of noneosinophilic patients with lower baseline pulmonary function might help appreciate even small changes induced by treatment.

In conclusion, we showed that a fair proportion (26%) of asthmatic patients have low sputum eosinophils despite being symptomatic. After inhaled corticosteroid treatment, these patients show a smaller improvement in lung function and bronchial hyperresponsiveness than patients with high sputum eosinophils. Also, we found that, among different baseline measurements, low sputum eosinophils have the highest negative predictive value for corticosteroid effects on lung function and on bronchial hyperresponsiveness. Further studies are required on the effects of longer corticosteroid treatment duration and of different drugs in these patients.

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