
Effects of oral prednisone on sputum eosinophils and cytokines in patients with severe refractory asthma

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Background: Severe asthma occurs in a heterogeneous group of patients in whom symptoms and airway inflammation persist despite maximal antiasthma treatment.

Objective: To verify whether a short-term course of oral steroids would modify sputum inflammatory cytokine and sputum eosinophil concentrations and whether this effect is related to the presence of sputum eosinophilia.

Methods: In 59 patients with severe refractory asthma, we measured pulmonary function and inflammatory markers in hypertonic saline-induced sputum before and after 2 weeks of treatment with 0.5 mg/kg of oral prednisone ($n = 39$) or placebo ($n = 20$) daily. Selected sputum portions were assayed for total and differential cell counts and supernatant interleukin (IL) 5 and IL-8 concentrations.

Results: At baseline, no statistical differences were found among placebo- and prednisone-treated patients in terms of sputum inflammatory cell percentages and IL-5 and IL-8 concentrations. After treatment, forced expiratory volume in 1 second significantly increased and sputum eosinophil percentages and IL-5 and IL-8 concentrations significantly decreased in the prednisone group, whereas no changes were observed in the placebo group. The positive effect of prednisone treatment was observed only in patients with baseline sputum eosinophilia, whereas in noneosinophilic patients with severe asthma prednisone induced only a significant decrease of sputum IL-8.

Conclusions: Additional high-dose oral corticosteroids improve pulmonary function and reduce not only sputum eosinophil but also sputum proinflammatory cytokine concentrations in patients with severe refractory asthma.

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INTRODUCTION

There is increasing evidence that some patients with bronchial asthma have poor control of the disease despite maximal antiasthma treatment, including high-dose inhaled corticosteroids, long-acting β_2 -agonists, and other drugs.^{1,2} It is known that a persistent eosinophilic airway inflammation accompanies all levels of asthma severity, including mild intermittent asthma.³ Remarkably, persistent eosinophilic inflammation has been shown even in patients with severe asthma and neutrophilic inflammation.^{4,5} The resistance of eosinophilic inflammation to corticosteroid treatment might be related to the lack of inhibition of proeosinophilic cytokines by corticosteroids.

A study from ten Brinke et al⁶ demonstrated that intramuscular triamcinolone leads to reduction in sputum eosinophils and rescue medication and increase in forced expiratory volume in 1 second (FEV₁) in patients with eosinophilic refrac-

tory asthma. All patients recruited for this study had a sputum eosinophil percentage greater than 2%. The authors conclude that the response to triamcinolone is due to the high doses of systemic corticosteroids needed to overcome the high airway eosinophilic inflammation. No measurement of inflammatory cytokines is reported.

In the present study, patients with severe refractory asthma were treated with high-dose oral prednisone, and the effects on sputum eosinophils and interleukin (IL) 5 and IL-8 cytokines were studied. Our hypothesis was that these patients are susceptible to high doses of corticosteroids and that not only pulmonary function but also airway inflammation (both sputum inflammatory cells and cytokines) are responsive to oral corticosteroid treatment.

METHODS

Subjects

From our asthma clinic, we selected 60 outpatients (23 men and 37 women; mean [SD] age, 58.2 [11.9] years) who met the criteria for refractory asthma.² The patients were included if they had been followed up in our clinic for at least 3 years⁷ and were clinically stable in the last 4 weeks before entering the present study. Other eligibility criteria were compliance for functional measurements and treatment, acceptable sputum samples, and no contraindications for the use of systemic

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corticosteroids. All of the patients reported current asthma symptoms and had a history of airway obstruction reversibility (increase in FEV₁ after 400 µg of salbutamol >12% of baseline value). At the time of the first evaluation, 32 patients were atopic (positive skin prick test result to ≥1 common aeroallergen), 18 were ex-smokers, and 4 were current smokers (with a smoking history of <10 packs-years). Only 3 of 60 patients were sensitive to aspirin.

Each patient had been taking 1,600 to 3,200 µg/d of inhaled beclomethasone propionate or equivalent associated with long-acting β₂-agonists in the year preceding the study, with the addition of 1 or more other drugs (oral leukotriene receptor antagonists in 25, inhaled anticholinergics in 16, oral theophylline in 25, and regular low-dose oral corticosteroids in 18 patients).

The study was approved by the university ethics committee, and all patients signed an informed consent for participation in the study.

Study Design

This study had a randomized, parallel-group, double-blind, placebo-controlled design. At baseline, patients underwent pulmonary function tests and sputum induction by means of hypertonic saline inhalation. They recorded daily symptoms and peak expiratory flow (PEF) on a diary card during a run-in period of at least 2 weeks to evaluate PEF variability and symptom score.

Patients were then randomized in a 2:1 ratio to take either oral prednisone (0.5 mg/kg daily) or placebo for 2 weeks, in addition to current regular treatment. Two weeks later, pulmonary function tests and sputum induction were performed again. Antiasthma therapy at study entry was similar in the placebo and prednisone groups except for leukotriene receptor antagonists, which were more frequently used in the placebo than in the prednisone group (14 of 20 patients vs 11 of 39 patients, *P* = .005).

Outcome Measures

Pulmonary function tests were performed by the Biomedin Pulmonary System (Biomedin, Padova, Italy). Skin prick tests were performed using a panel of common inhalant allergens, including house dust mites, Graminaceae mix, *Parietaria officinalis*, dog and cat danders, and molds.

Cumulative daytime and nighttime symptom score (dyspnea, wheezing, cough, chest tightness; maximum cumulative 2-week score, 126) and rescue medication use (total number of occasions during 2 weeks) were obtained from diary cards. The PEF was measured 2 times daily by means of a Mini-Wright PEF meter and expressed according to the following formula:

$$\Delta PEF_{14 \text{ days}} = \frac{[(PEF_{MAX1} + PEF_{MAX2}) - (PEF_{MIN1} + PEF_{MIN2})]}{(PEF_{MAX1} + PEF_{MAX2})} \times 100$$

Sputum was induced according to a validated protocol, and sputum plugs were selected and processed in our laboratory

as previously reported.⁸ Sputum inflammatory cell percentages were counted. Sputum quality was confirmed by high viability index and acceptable percentages of squamous cells.⁸ Sputum eosinophil percentages greater than 2% were considered abnormal.⁹ IL-5 and IL-8 were measured in sputum supernatant by means of the enzyme-linked immunosorbent assay method, as reported in a previous study.⁷

Statistical Analysis

Sputum cell percentages are expressed as median and range. Sputum cytokine concentrations are expressed as geometric mean and range. The other data are expressed as mean and standard deviation.

Sputum data required base 10 log transformation to obtain normal distribution. Values of 0% sputum cell counts were arbitrarily assigned 0.001% before log transformation, and 2-tailed paired and unpaired *t* tests and analysis of variance were performed on these log-transformed data.

A χ² analysis was used to compare categorical data, such as atopy and smoking habit. *P* < .05 was considered statistically significant.

RESULTS

Patient Characteristics

One patient randomized to prednisone withdrew from the study before the end of the treatment period; thus, 20 patients in the placebo group and 39 patients in the prednisone group completed the study. There was no significant difference in age, asthma duration, sex distribution, smoking habit, medication use, atopy and concomitant upper airway diseases, or baseline functional findings between the 2 groups (Table 1). All patients but 2 showed an acceptable baseline sputum sample, whereas all other patients had an acceptable sputum sample at the second examination. There was no difference in the total and differential inflammatory cells and in the cytokine concentrations between the 2 groups (Table 1).

Current and ex-smokers (*n* = 22) and nonsmokers (*n* = 37) showed no significant difference in any clinical, functional, and inflammatory parameters, except for a nonsignificant lower median of the percentage of sputum eosinophils (1.2% vs 6.5%). Also, 28 patients affected by upper airway diseases (rhinitis, sinusitis, and/or nasal polyps) were no different from the remaining patients regarding clinical, functional, and biological findings. All these 28 patients (15 in the prednisone group and 13 in the placebo group) received appropriate treatment for rhinosinusitis (antihistamines and/or low-dose intranasal inhaled corticosteroids).

Effects of Treatments

Prednisone treatment induced a mean (SD) improvement in FEV₁ measured at the second visit, from 1.86 (0.67) L to 2.04 (0.64) L (*P* = .03), whereas placebo treatment induced no effect on FEV₁, from 1.84 (0.57) L to 1.78 (0.55) L.

After prednisone treatment, but not after placebo, sputum eosinophil percentages significantly decreased to values sig-

Table 1. Clinical, Functional, and Biological Characteristics of 2 Groups of Patients With Severe Refractory Asthma at the Time of Randomization to Prednisone or Placebo Treatment^a

Characteristics	Prednisone (n = 39)	Placebo (n = 20)
Age, mean and SD, y	57.2 (13.3)	60.1 (8.7)
Duration of asthma, mean and SD, y	22.2 (14.4)	17.4 (6.8)
Female/male, No.	23/16	14/6
Smoker, yes/ex/no, No.	1/14/24	3/4/13
Upper airway disease, yes/no, No.	15/24	13/7
Atopy, yes/no, No.	23/16	9/11
Regular oral glucocorticosteroid treatment in the last year, yes/no, No.	10/29	8/12
Cycles of oral glucocorticosteroids in the last year, mean (SD)	3.3 (6.9)	3.6 (2.1)
Symptom score at 14 days, mean (SD)	17.8 (16.1)	15.3 (15.6)
ΔPEF at 14 days, mean (SD)	38.0 (19.5)	36.9 (14.8)
FEV ₁ , mean and SD, L	1.86 (0.67)	1.84 (0.57)
FEV ₁ , mean (SD), % predicted	67.6 (16.3)	69.6 (18.1)
Sputum neutrophils, median (range), %	47.2 (2.6–94.5)	50.6 (11.1–91.4)
Sputum eosinophils, median (range), %	6.1 (0.0–68.1)	12.1 (0.0–79.8)
Sputum IL-5, geometric mean (range), ng/mL	82.6 (10.0–417.0)	48.3 (10–113.8)
Sputum IL-8, geometric mean (range), ng/mL	840.2 (164.0–3,641.0)	603.0 (129.0–3,684.0)

Abbreviations: FEV₁, forced expiratory volume in 1 second; IL, interleukin; PEF, peak expiratory flow.

^a *P* values were nonsignificant for all characteristics.

nificantly lower than those after placebo (prednisone: from 6.1% to 0.7%; placebo: from 12.1 to 7.6%; Figure 1). In particular, prednisone decreased sputum eosinophil levels to normal in 11 of 20 patients who had high baseline sputum eosinophil levels, whereas placebo left high sputum eosinophil levels unchanged. Sputum neutrophil percentages did not significantly change after either treatment.

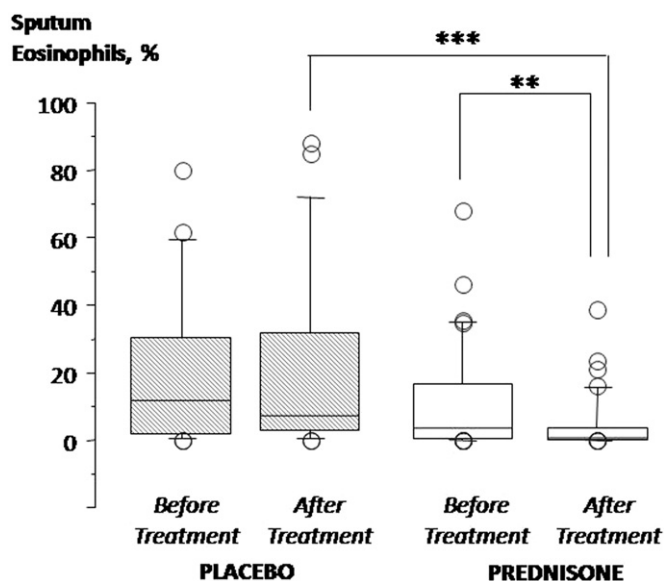


Figure 1. Comparison between baseline and after treatment (placebo or prednisone) for a median of sputum eosinophil percentages. ***P* < .01; ****P* < .001.

The response to prednisone treatment of subgroups of patients, such as current smokers and ex-smokers vs non-smokers or patients with or without upper airway diseases, was similar to that observed in the whole study population (data not shown).

Sputum IL-5 and IL-8 levels were measured before and after treatment in 48 patients (32 taking prednisone and 16 taking placebo). Both IL-5 and IL-8 levels significantly decreased after prednisone but not after placebo treatment (Figure 2A and 2B). In the 32 patients treated with prednisone, sputum IL-5 concentration was higher than 50 pg/mL (value slightly higher than the detection limit with our method) in 29 samples before and in 10 samples after prednisone treatment.

Effect of Prednisone Treatment According to Baseline Sputum Eosinophil Concentrations

At baseline, 35 patients showed sputum eosinophil percentages greater than 2% (eosinophilic group, 20 patients randomized to prednisone), whereas 22 patients had normal sputum eosinophil percentages (noneosinophilic group, 17 patients randomized to prednisone). No significant difference was found in baseline clinical and functional characteristics and in sputum inflammatory markers between the eosinophilic and noneosinophilic patients, except for the number of acute exacerbations requiring oral corticosteroid use, which was significantly lower in eosinophilic patients (Table 2).

However, in the eosinophilic patients prednisone significantly improved FEV₁ and decreased sputum eosinophil percentages and sputum IL-5 but not IL-8 concentrations. In the noneosinophilic patients, prednisone did not affect FEV₁ and sputum eosinophil percentages but significantly decreased the sputum IL-8 concentration; prednisone also decreased the

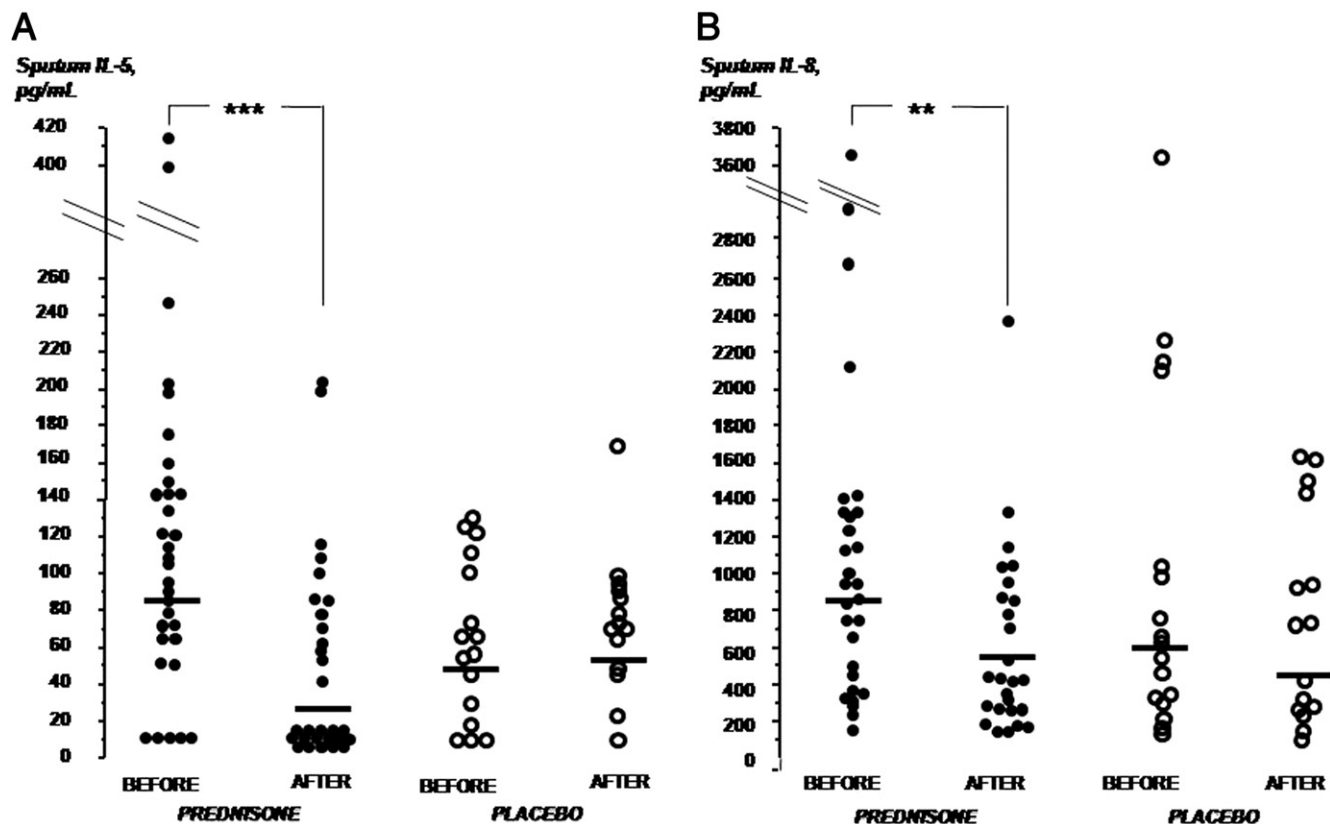


Figure 2. Comparison between concentrations in sputum IL-5 (geometric mean) (A) and sputum IL-8 (geometric mean) (B) from baseline to after treatment in the placebo and prednisone groups. ** $P < .01$; *** $P < .001$.

sputum IL-5 concentrations, but the decrease was not statistically significant ($P = .09$) (Table 3).

DISCUSSION

Our results confirm that a 2-week course of oral prednisone may improve both functional and inflammatory parameters in asthmatic patients with poor asthma control despite maximal pharmacologic treatment (patients with refractory asthmatic or patients with difficult-to-treat asthma). In addition to previous studies, our study shows for the first time, to our knowledge, that not only sputum eosinophils but also sputum IL-5 and IL-8 concentrations are sensitive to a short course of oral prednisone, suggesting that the release of some proinflammatory cytokines may be positively affected by systemic corticosteroid treatment.

These results confirm and extend the conclusions of the study by ten Brinke et al.⁶ We used oral prednisone at a dosage of 0.5 mg/kg daily for 2 weeks instead of intramuscular triamcinolone, with similar effects on pulmonary function and sputum eosinophil concentrations. Moreover, in our study, sputum IL-5 and IL-8 concentrations also decreased after prednisone treatment. IL-5 is a proinflammatory cytokine that increases the recruitment, activation, and survival of eosinophils and is considered of pivotal importance in the

pathophysiology of asthma.¹⁰ IL-5 is highly expressed by T cells, eosinophils, and mast cells in bronchial biopsy specimens from patients with asthma¹¹ and can be measured in sputum.^{7,12,13} IL-8 is a progranulocyte cytokine released by many airway cells that is able to influence migration of both neutrophils and eosinophils in the airways.¹⁴ Our results show that systemic corticosteroids are effective not only on sputum eosinophils but also on the release of sputum proinflammatory cytokines that play a relevant role in perpetuating airway inflammation in patients with refractory asthma. Our data agree with the results of other studies showing that corticosteroids decrease the number of activated T cells expressing IL-4 and IL-5 messenger RNA in bronchoalveolar lavage in both patients with asthma of varying severity¹⁵ and in patients with severe asthma.¹⁶

An important difference from the previous study⁶ is that we also included patients with low sputum eosinophil levels. In the present study, we showed that the positive effect of oral prednisone is mainly attributable to the patients with sputum eosinophilia, in whom prednisone induced an improvement in FEV₁, sputum eosinophil percentages, and IL-5 concentrations. In other words, our results suggest that there is a subgroup of patients with difficult-to-control asthma with low percentages of sputum eosinophils, in whom oral prednisone

Table 2. Baseline Characteristics of Patients According to the Presence of Baseline Sputum Eosinophilia (Sputum Eosinophil Percentage >2%)

Characteristics	Eosinophilic (n = 35)	Noneosinophilic (n = 22)
Age, mean (SD), y	59.9 (9.8)	54.8 (14.7)
Duration of asthma, mean (SD), y	17.7 (10.1)	21.6 (13.3)
Female/male, No.	24/11	12/10
Smoker, yes/ex/no, No.	2/9/24	2/8/12
Atopy, yes/no, No.	17/18	14/8
Upper airway disease, yes/no, No.	19/16	10/12
Regular oral glucocorticosteroid treatment in the last year, yes/no, No.	12/23	6/16
Cycles of oral glucocorticosteroids in the last year, mean (SD) ^a	4.0 (2.8)	2.5 (1.6)
Symptom score at 14 days, mean (SD)	16.8 (14.7)	16.0 (18.2)
ΔPEF at 14 days, mean (SD)	37.6 (16.3)	33.9 (20.7)
FEV ₁ , mean (SD), L	1.85 (0.69)	1.85 (0.58)
FEV ₁ , mean (SD), % predicted	68.4 (17.8)	67.5 (15.8)
Sputum neutrophils, median (range), %	47.2 (2.6, 85.2)	60.7 (11.1, 94.5)
Sputum eosinophils, median (range), % ^b	15.7 (2.3–79.8)	0.6 (0.0–1.8)
Sputum IL-5, geometric mean (range), ng/mL	65.0 (10.0–248.0)	89.0 (10.0–417.0)
Sputum IL-8, geometric mean (range), ng/mL	789.0 (129.0–3,684.0)	782.0 (227.0–2,123.0)

Abbreviations: FEV₁, forced expiratory volume in 1 second; IL, interleukin; PEF, peak expiratory flow.

^a *P* = .02.

^b *P* = .0001.

neither improves respiratory function nor reduces markers of eosinophilic inflammation in sputum. These data are in agreement with other studies of patients with mild-to-moderate asthma,^{17,18} confirming that sputum eosinophilia is a good predictor of response to corticosteroids. The reason for this different short-term effect of corticosteroids, as opposed to the well-known, positive, long-term effect of corticosteroids in most asthmatic patients, is not known. A possible explanation might be that corticosteroid effects on noneosinophilic inflammation may take longer to occur.¹⁹ Our data support the usefulness of sputum eosinophil assessment to predict when patients with severe asthma may benefit from an increase in the dose of corticosteroids.

Patients with or without sputum eosinophilia were no different in regards to sputum IL-5 and IL-8 concentrations before prednisone, although there was a trend for the IL-5 levels to be higher in eosinophilic patients and for IL-8 levels

to be higher in noneosinophilic patients. There is evidence that IL-5 is detectable in induced sputum of asthmatic patients and that the IL-5 level is higher in patients with severe asthma than in those with mild-to-moderate asthma.⁷ The values of IL-5 and IL-8 that we measured in patients with severe asthma were higher than the values measured in a group of untreated patients with asthma and in a group of control subjects studied with the same protocol.⁷ Thus, the number of eosinophils in the airways might not adequately express the level of airway inflammation in patients with refractory asthma treated with high-dose inhaled corticosteroids, whereas sputum IL-5 concentrations might better reflect an increased drive of proinflammatory eosinophilic inflammation in patients with refractory asthma.

Persistent eosinophilic inflammation in the airways despite high-dose inhaled corticosteroids has been demonstrated in several studies,^{4,6,7,20} and it was associated with

Table 3. Effects of Prednisone in Patients With High and Normal Sputum Eosinophil Counts

Characteristics	Noneosinophilic (n = 17)		Eosinophilic (n = 20)	
	Before prednisone	After prednisone	Before prednisone	After prednisone
FEV ₁ , mean (SD), % predicted	66.2 (16.5)	65.6 (11.9)	68.2 (16.9)	80.8 (12.5) ^a
Sputum eosinophils, median and range, %	0.6 (0.0–1.8)	0.4 (0.0–23.9)	15.5 (3.8–66.1)	1.1 (0.1–38.6) ^a
Sputum neutrophils, median and range, %	47.7 (11.1–91.4)	61.0 (9.7–87.5)	51.3 (12.4–85.2)	63.3 (5.7–95.9)
Sputum IL-5, geometric mean (range), ng/mL	88.3 (10.0–417.0)	34.8 (10.0–199.0)	81.3 (53.0–248.0)	25.1 (10.0–203.0) ^b
Sputum IL-8, geometric mean (range), ng/mL	910.0 (227.0–2,123.0)	328.0 (131.0–1046.0) ^a	860.0 (280.0–2,988.0)	587.0 (167.0–2,379.0)

Abbreviations: FEV₁, forced expiratory volume in 1 second; IL, interleukin.

^a *P* < .001 vs before prednisone.

^b *P* < .01 vs before prednisone.

poor prognosis in terms of impaired pulmonary function and severe asthma attacks.²¹ The reason for persistence of eosinophilic inflammation in these patients is unknown. The similarities between our study and previous studies confirm that classifying severe asthma in eosinophilic and noneosinophilic phenotypes may have clinical implications in the choice of the pharmacologic therapy. In addition, patients with severe asthma with sputum eosinophilia despite inhaled corticosteroid therapy may further improve after a short course of systemic corticosteroids, suggesting a dose-dependent sensitivity to corticosteroids in these patients. Possible reasons for this poor corticosteroid responsiveness include high levels of proinflammatory cytokines, which may modify the response of the corticosteroid receptors^{22,23} but may be overcome by increasing the dose of corticosteroids.

We have also evaluated the influence of corticosteroids on sputum neutrophil percentages. In our study, sputum neutrophil percentages were not affected by treatment with high-dose oral prednisone, according to previous studies on neutrophils and their markers in sputum and blood.^{6,24,25} However, the role of neutrophils in determining corticosteroid insensitivity remains an important question to be elucidated. Neutrophils could contribute to the airway inflammation because they can store eosinophil cationic protein produced by activated eosinophils.²⁶ In some patients, prominent neutrophilic inflammation may be demonstrated,²⁷ and these patients appear to be less responsive to corticosteroids.^{17,18}

In our study, the 2 groups of patients with refractory asthma showed a different IL-8 response to corticosteroid treatment. In many studies, an increase in IL-8 in the airways of asthmatic patients was demonstrated, and it was interpreted as a marker of neutrophilic inflammation.^{4,25} Our asthmatic patients had levels of sputum IL-8 higher than normal,⁷ which decreased in both eosinophilic and noneosinophilic patients, although the decrease was significant only in noneosinophilic patients. We can speculate that IL-8 might be produced by a larger spectrum of airway cells than IL-5, and these cells may be sensitive to corticosteroid treatment.²⁷

In conclusion, this study shows that patients with difficult-to-control asthma despite high levels of regular treatment are still able to improve in lung function, sputum eosinophil counts, and proinflammatory cytokine levels after the addition of a course of oral prednisone and confirms the usefulness of sputum cell assessment in selecting patients with refractory asthma who might benefit from systemic steroids. On the other hand, the absence of sputum eosinophilia is a marker of a poor sensitivity to corticosteroid treatment. Measurement of sputum cytokines, particularly IL-5 and IL-8, might provide new information about the characteristics of airway inflammation in different subgroups of patients with difficult-to-control asthma.

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