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Changes in inflammatory markers following treatment of acute exacerbations of obstructive pulmonary disease

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Abstract The aim of the study was to investigate changes in inflammatory markers following emergency treatment of obstructive pulmonary disease. The study comprised 43 patients. After acute treatment, they were given either 30 mg of prednisolone p.o. or 1600 μg of inhaled budesonide daily for 1 week. Over the following 3 weeks, all the patients were given 1600 μg of inhaled budesonide daily. Blood samples for measurements of eosinophil cationic protein (S-ECP), eosinophil peroxidase (S-EPO), total eosinophil count (B-Eos), myeloperoxidase (S-MPO) and human neutrophil lipocaline (HNL) were taken and spirometry was performed before emergency treatment and after 1 and 4 weeks. There was no difference in the improvement in forced expiratory volume in 1 sec (FEV_1) between patients given prednisolone or budesonide. Patients with an improvement in FEV_1 of $\geq$20% of baseline after 1 and 4 weeks displayed a larger decrease in eosinophil markers. The correlation between ΔFEV_1 and ΔS-ECP was $r = -0{\cdot}37$, $P < 0{\cdot}05$, ΔS-EPO $-0{\cdot}40$, $P < 0{\cdot}01$ and ΔB-Eos $-0{\cdot}44$, $P < 0{\cdot}01$, after 4 weeks. This correlation was highly significant in patients who had smoked ≤ 5 pack-years, while the correlation was not significant in patients with a longer smoking history and chronic airflow limitation (best FEV_1 <80% of predicted). We conclude that the change in eosinophil markers is correlated to the improvement in lung function in non-smokers or short-term smokers following the emergency treatment of obstructive pulmonary disease. This study indicates that following eosinophil markers is more useful in patients with asthma than patients with COPD.

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Keywords asthma; chronic obstructive pulmonary disease; eosinophils; neutrophils; exacerbation.

INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are both characterized by bronchial inflammation. In asthma, one of the most important cells is the eosinophil granulocyte (1), while the neutrophil cell plays an important role in COPD (2, 3). Eosinophil markers such as total eosinophil count and S-ECP have been used to monitor disease activity, especially during treatment with inhaled steroids (4–6). There are, however, not many studies on eosinophil markers after acute exacerbations of obstructive pulmonary disease (7, 8).

We have previously reported that patients with high levels of eosinophil markers before emergency treatment experienced a greater improvement in lung function (9). This investigation was based on common clinical practice at the emergency unit, with a heterogeneous group of patients seeking medical help because of an acute exacerbation of their obstructive pulmonary disease. It comprised both asthma and COPD patients as it is often difficult, especially in the acute phase, to distinguish asthma from COPD and some patients have an overlap syndrome.

The aim of the investigation was to study the changes in eosinophil markers following emergency treatment in patients with exacerbations of obstructive pulmonary disease.

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MATERIAL AND METHODS

Subjects

The study population in this investigation has been previously described (9). It comprised adult patients (>18 years) with acute exacerbations of obstructive pulmonary disease, both asthma and COPD, presenting during the daytime at the emergency room in our department. They were all assessed as being in need of emergency treatment including steroids. The patients were to be free of oral steroids and their daily dose of inhaled steroids was maximized at 1600 μg (800 μg, if fluticasone was used). They were excluded from the study if hospital admission was deemed necessary or if they were unable to perform the lung function tests. All participation was voluntary and the study was approved by the ethics committee at the Medical Faculty at Uppsala University.

Before treatment, all the patients were examined and spirometry (Vitalograph Alfa, Vitalograph Ltd. Buckingham, U.K.) was performed. The predicted value of forced expiratory volume in 1 sec (FEV_1) was calculated for each patient (10). Blood samples were taken before treatment was given. The patients were carefully interviewed about their smoking history and the number of pack-years was calculated. At the follow-ups after 1 week (visit 2) and after 4 weeks (visit 3), spirometry was performed and blood samples were taken.

Treatment

The treatment given in this investigation has been described previously (9). All the patients were given 5–15 mg of nebulized salbutamol (in one to three doses) via Ventstream with a Porta Neb compressor (Medic Aid Ltd, West Sussex, U.K.). The first 28 patients were included as part of a multi-centre study. After randomization, they were treated with 8 mg of nebulized budesonide (2 × 4 mg), 60 mg of prednisolone p.o. or placebo. At discharge and at bedtime on the first day, patients who had received nebulized budesonide or placebo in the acute phase received one dose of 400 μg of budesonide via a dry powder inhaler (Turbohaler®) and the patients who had received prednisolone in the acute phase were given placebo Turbohaler®. The next morning, prednisolone patients started with 30 mg of prednisolone daily for 1 week and the remaining patients continued with 400 μg of budesonide Turbohaler® q.i.d. for 1 week. Up to this point, the study was strictly double-blind. After completing the multi-centre study, a local study was conducted in Uppsala with a further 22 patients in whom the treatment was open and comprised 60 mg of prednisolone p.o. in the acute phase and 30 mg of prednisolone p.o the following week as described earlier (9). Apart from the treatment, the same study protocol was followed as in the multi-centre study. After 1 week, all the patients continued with 800 μg of budesonide Turbohaler® b.i.d. At the follow-up after 1 and 4 weeks, a new spirometry examination was performed.

Serum

Venous blood was collected in SST-Vacutainer tubes and serum was prepared by allowing blood to clot for 60–120 min at 20°C, followed by centrifugation at 1600 g at 4°C for 10 min. The serum samples were kept at −70°C pending analysis.

Inflammatory markers

As eosinophil markers, eosinophil cationic protein (S-ECP), eosinophil peroxidase (S-EPO) and total eosinophil count (B-Eos) were measured, and myeloperoxidase (S-MPO), human neutrophil lipocaline (S-HNL) and total neutrophil count (B-Neutro) were measured as neutrophil markers.

Eosinophil cationic protein (ECP) was measured using the Pharmacia CAP system ECP FEIA (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden), according to the manufacturer's instructions. The inter- and intra-assay variation was less than 8% and the detection limit was 2 μ gl^{-1}.

Eosinophil peroxidase (EPO) was assessed using a prototype immunofluorometric assay utilizing the Pharmacia CAP system. Briefly, EPO, purified with some modifications according to the previously described method (11), was used as a standard ranging from 0.5 to 200 μgl^{-1}. Monoclonal antibodies (MoAbs) were produced using the hybridoma technique. One MoAb was covalently bound to the immunoCAP and the other MoAb was labelled with the enzyme β-galactosidase. The assay procedures were identical to those for ECP. Cross-reactivity with ECP and myeloperoxidase (MPO) in the EPO assay was <0·3 and <0·01%, respectively. The detection limit for the EPO assay was 0·5 μgl^{-1} and the inter- and intra-assay coefficient of variation was less than 10%.

Myeloperoxidase (MPO) was measured using radio immunoassays, according to the manufacturer's instructions (Pharmacia MPO RIA, Pharmacia & Upjohn Diagnostics AB) and human neutrophil lipocaline (HNL) was measured as previously described(12). Inter- and intra-assay variations were less than 10% and the detection limit was 8 μgl^{-1} and 4 μgl^{-1} respectively.

Total neutrophil and total eosinophil counts (B-Neutro, B-Eos) were measured using standard techniques at the hospital's Department of Clinical Chemistry.

Statistical methods

The statistical analyses were performed using the Stat View SE+graphics software from Abacus Concepts Inc. Comparisons between patients were performed using

the Mann–Whitney *U*-test for continuous variables, while the chi-square test was used for comparisons of proportions. Wilcoxon's signed-rank test was used to study changes in inflammatory markers. Correlations between continuous variables on an ordinal scale were performed using Spearman's rank correlation test. A *P*-value of 0·05 or less was regarded as statistically significant.

RESULTS

Fifty patients participated in the study at the first visit, but seven patients subsequently dropped out. This analysis is based on the 43 patients who completed the whole 4-week study period. The mean age was 64 (range 22–87) years and there were 23 (53%) women and 20 men. The mean number of pack-years was 20 (range 0–76) years. Four patients were current smokers and eight patients were lifetime non-smokers. FEV_1 was 49 ± 24% of predicted (mean ± SD).

The levels of inflammatory markers at the emergency visit and at the follow-ups after 1 and 4 weeks are shown in Table 1. All the eosinophil markers had decreased significantly at visit two compared with the emergency visit. By visit three, S-EPO and B-Eos had increased significantly. The neutrophil markers S-MPO and S-HNL showed a slight but significant decrease at visit two compared with visit one. B-Neutro showed an increase from visit one to visit two.

Patients with an improvement in FEV_1 of ≥20% of baseline after 4 weeks displayed a larger decrease in eosinophil markers, which is illustrated in Fig. 1. The correlations between ΔFEV_1 (% of baseline) and ΔS-ECP, ΔS-EPO and ΔB-Eos after 1 week were −0·26, NS; −0·46, $P < 0·01$; and −0·43, $P < 0·05$, respectively. The correlations between ΔFEV_1 (% of baseline) and ΔS-ECP, ΔS-EPO and ΔB-Eos after 4 weeks were −0·37, $P < 0·05$; −0·40, $P < 0·01$; and −0·44, $P < 0·01$, respectively. No correlations were seen between the neutrophil markers and ΔFEV_1.

Twenty-eight patients were given oral steroids during the first week after the emergency treatment. These patients displayed a more pronounced decrease in eosinophil markers at visit two than those treated with inhaled steroids. The effect of oral steroids on the eosinophil markers is illustrated in Fig. 2. B-Neutro only increased significantly at visit two in the group which was given oral steroids for the first week. No differences were seen in the levels of S-MPO and S-HNL between patients who did or did not take steroids orally during the first week. ΔFEV_1 did not differ between patients treated with oral steroids or not.

Nine patients relapsed during the first week and received emergency treatment, including steroids. During the last 3 weeks, another three patients relapsed. These patients did not differ from the others in terms of

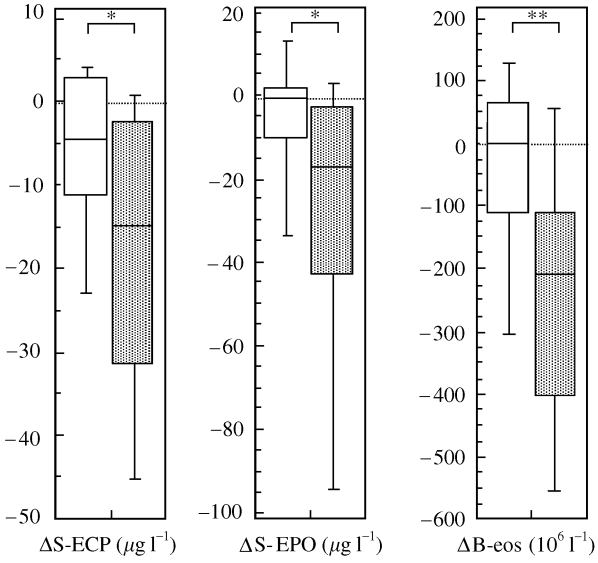

Fig. 1. The change in S-ECP, S-EPO and B-eos after 4 weeks in relation to the change in lung function (ΔFEV_1 % of baseline). The box plots show the values of the median, the 25th and 75th percentiles (box) and the 10th and 90th percentiles. Δ FEV_1 ≤20% (□) and Δ FEV_1 >20% (▨). $P < 0·05$, $^{**}P < 0·01$.

Table 1. Levels of inflammatory markers at visits one, two and three. Mean (± SD).

	Visit 1 (0 h)	Visit 2 (1 week)	Visit 3 (4 weeks)
S-ECP ($\mu g\,l^{-1}$)	30 (22)	17 (13)***	20 (16)***
S-EPO ($\mu g\,l^{-1}$)	38 (40)	14 (20) ***	24 (25)***, †
B-Eos ($10^6\,l^{-1}$)	467 (330)	192 (184) ***	343 (243)*, †††
B-Neutro ($10^9\,l^{-1}$)	4.9 (2.6)	6·4 (2·7) ***	4.4 (1·4)†††
S-MPO ($\mu g\,l^{-1}$)	762 (496)	639 (366)*	636 (354)*
S-HNL ($\mu g\,l^{-1}$)	134 (67)	118 (49)*	110 (47) ***

*$P < 0·05$, ***$P < 0·001$ compared with visit 1.
†$P < 0·05$, †††$P < 0·001$ compared with visit 2.

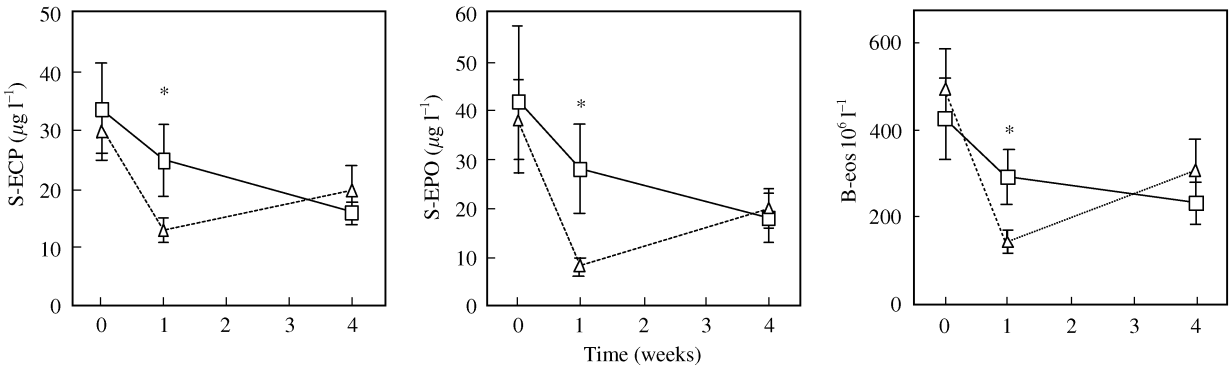

Fig. 2. Eosinophil markers and effect of oral steroids. Patients who relapsed are excluded (mean ± SE). Steroids p.o. (Δ), no steroids p.o. (□). *$P < 0{\cdot}05$.

Table 2. The correlation between ΔFEV_1 (as % of baseline) and Δ eosinophil markers after 1 and 4 weeks in relation to smoking history and best FEV_1 (Spearman's rank correlation test).

	Non- or short-term smokers (≤ 5 pack-years) (n=14)	Long-term smokers with FEV_1 ≥80% of predicted (n=10)	Long-term smokers with FEV_1 <80% of predicted (n=19)
ΔS-ECP 1 week	−0·52	−0·45	−0·09
ΔS-EPO 1 week	−0·74**	−0·38	−0·24
ΔB-Eos 1 week	−0·63*	−0·60	−0·38
ΔS-ECP 4 weeks	−0·59*	−0·52	−0·32
ΔS-EPO 4 weeks	−0·72**	−0·53	−0·13
ΔB-Eos 4 weeks	−0·79**	−0·89*	−0·28

*P < 0·05, **P < 0·01.

inflammatory markers, smoking history or lung function at the start of the study.

Relation to smoking history and lung function

The correlations between the change in lung function and the change in eosinophil markers were also analysed in relation to smoking history and lung function. Patients were divided into three groups. One group comprised patients who had smoked for 5 pack-years or less (non- or short-term smokers) (n=14). The second group comprised patients who had smoked for more than 5 pack-years (long-term smokers) and had an FEV_1 that at least once during the study period was ≥80% of the predicted (n=10). The third group were long-term smokers with chronic airflow obstruction (best FEV_1 < 80% predicted during the study period) (n=19). In the non- or short-term smoking group, the mean number of pack-years was 0·9 ± 1·7 (n=14) and, in the two other group the mean number of pack-years was 30 ± 18 (n=29). In the long-term smoking group without chronic airflow obstruction there was a significant correlation between change B-Eos and FEV_1 after 1 month. Except for this, the change in FEV_1 (Δ) was significantly correlated to the change in eosinophil markers only for non- or short-term smokers. (Table 2).

The levels of the eosinophil and neutrophil inflammatory markers did not differ between short- or long-term smokers with one exception, both groups of long-term smokers had significantly higher serum levels of S-MPO at visit one than the non- or short-term smoking group (Figs 3 and 4). There were no significant differences in change of eosinophil markers between the groups, while there was a significant decrease in S-HNL in both the long-term smoking groups compared to the non- or short-term smoking group 1 week after the emergency visit.

FEV_1 was at all time-points lower in the long-term smokers with chronic airflow obstruction. The reversibility in terms of the change (Δ) in FEV_1 expressed as a percentage of baseline, FEV_1% predicted or FEV_1 (in litres) during the study period did not differ between the three groups, nor was it correlated to pack-years.

DISCUSSION

This study was performed on consecutive patients with an acute exacerbation of obstructive pulmonary disease

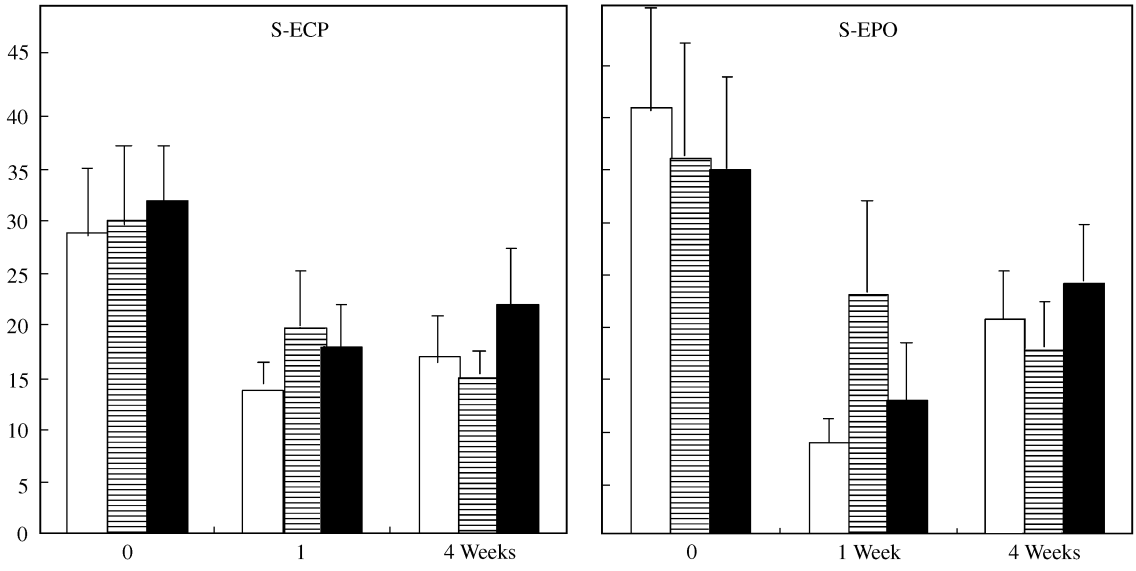

Fig. 3. Eosinophil markers in non- or short-term smokers ($\leqslant$ 5 pack-years) (□) and in long-term smokers ($>$ 5 pack-years) without (▤) and with chronic airflow obstruction (best FEV_1 <80% of predicted) (■).

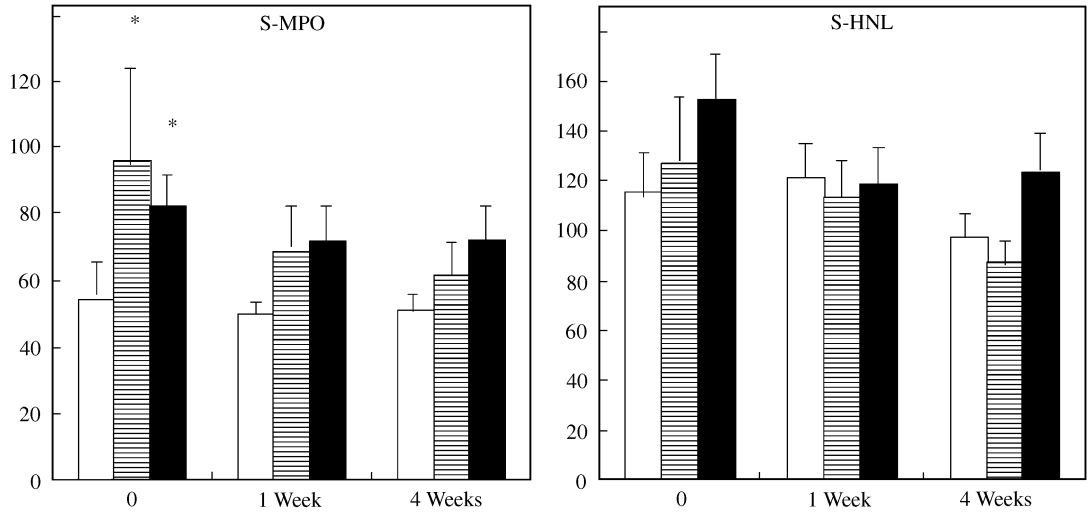

Fig. 4. Neutrophil markers in non- or short-term smokers ($\leqslant$ 5 pack-years) (□) and in long-term smokers ($>$ 5 pack-years) without (▤) and with chronic airflow obstruction (best FEV_1 < 80% of predicted) (■). *$P < 0{\cdot}05$ compared to non- or short-term smokers.

who attended our emergency department. They all had a previous diagnosis of asthma, COPD or both. The main findings of the study are that, for all patients, the decline in eosinophil markers in blood the following month correlated to the improvement in lung function. If patients were grouped according to smoking history, this correlation was, however, only significant in patients with a smoking history of $\leq$ 5 pack-years. The neutrophil markers, S-MPO and S-HNL, decreased after the emergency visit, but the decrease did not correlate to the change in lung function, regardless of smoking history.

In this study we have primarily chosen to study the impact of smoking history and lung function rather than grouping the patients on the basis of diagnosis. We have analysed our data with respect to smoking history, expressed as pack-years, with information that was obtained from the patient in an interview. In our study, we used an arbitrary cut-off point of 5 pack-years to distinguish never-smokers and short-term smokers from long-term smokers. No definite threshold exists when it comes to the magnitude of smoking and the risk of developing COPD. Mensinga *et al.*, however, found that a smoking history of at least 10 pack-years was associated with a significantly lower FEV_1 (13). We therefore chose a cut-off value of 5 pack-years in order to ensure that the patients in the short-term smoking group were purely asthmatics. In the long-term smoking group an FEV_1 lower than 80% of the predicted during the study period

was used to define the group of long-term smokers with chronic airflow obstruction. This cut-off point was chosen on the basis of the British Thoracic Society guidelines (14), in an attempt to separate patients from COPD from asthmatics with a long smoking history. When we compared this classification with the patients clinical diagnoses we found a fairly good agreement. All patients except two who were non-smokers or short-term smokers had a clinical diagnosis of asthma prior to the study, while 15 of the 19 patients who were long term smokers and had chronic airflow limitation had a clinical diagnosis of COPD.

We have shown that an improvement in lung function follows a decline in eosinophil markers in blood after an acute exacerbation. The same result has been demonstrated in studies by Skedinger and Zimmerman (5, 7), but there have also been studies that reveal a lack of correlation (15, 16). In one study, the impact of smoking on the effect of steroid treatment has been studied. Pedersen *et al*. showed that smoking asthmatics did not improve in terms of lung function after treatment with inhaled steroids, nor did the eosinophil markers decrease (17). In our study, ΔFEV_1 did not differ between non- or short-term smokers and long-term smokers, even if the long-term smokers with chronic airway obstruction had a lower FEV_1 during the study. However, only four of our patients were current smokers. Eosinophil markers also decreased after emergency treatment in both the long-term smoking group, but our data show that this decline was very poorly correlated to the improvement in FEV_1, and especially so in the group with chronic airflow obstruction. In contrast, a strong negative correlation was found between the decline in eosinophil markers in blood and ΔFEV_1 in non-or short-term smokers.

Previous studies have found that the eosinophils and eosinophil markers are increased in acute ecxacerbations of both asthma and COPD (8, 18). The increased involvement of eosinophils in the lungs of patients with COPD was previously suggested by high sputum levels of these markers and by biopsy findings of the lung parenchyma (9, 19, 20). It is, however, of interest that the change in eosinophil markers only correlated to change in lung function in the non- or short-term smoking group, which is the group of patients which mainly comprises asthmatics, and not to the lung function in long-term smokers, who are assumed to be predominantly COPD patients. These findings suggest different mechanisms for the activation and attraction of eosinophils in these two disorders and also suggest that eosinophils play different roles in these two conditions.

In recent studies, large numbers of neutrophils were found in the sputum of asthmatic patients with exacerbations (21, 22). Similarly we demonstrated in a previous study that the serum levels of MPO were raised in a group of asthmatics and that these elevated levels normalized upon successful treatment with inhaled corticosteroids (17). HNL is an exclusive marker of neutrophils, whereas MPO also originates from activated monocytes. The fact that S-MPO was further higher in both groups of long-term smoking patients than in non- or short-term smokers could therefore indicate the activation of monocytes as part of the inflammatory process in COPD.

In this study, we have assessed inflammatory markers in blood, which have the disadvantage of being indirect indices of airway inflammation. On the other hand, blood samples are safe, inexpensive and tolerated by most patients except small children.

We conclude that, after the treatment of acute exacerbations of obstructive pulmonary disease, the change in eosinophil markers is correlated to the improvement in lung function. This correlation is, however, only seen in non-smokers and short-term smokers. The neutrophil markers did not display any correlation to lung function regardless of smoking history. Our data imply that it is more valuable to follow eosinophil markers in patients with asthma than in patients with COPD.

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