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The inflammatory marker serum eosinophil cationic protein (ECP) compared with PEF as a tool to decide inhaled corticosteroid dose in asthmatic patients

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Abstract The objective of this study was to compare the inflammatory marker eosinophil cationic protein (ECP) with peak expiratory flow (PEF) in determining the therapeutic needs of inhaled corticosteroids in asthma patients assessed as asthma symptoms. A randomized, single-blind study over 6 months was performed at six specialist centres in Europe. In total, 164 adult patients with moderate to severe symptomatic asthma and regular use of inhaled corticosteroids were included. After a run-in period of 2 weeks patients were randomly allocated to the ECP or the PEF monitoring group. The dose of inhaled corticosteroids was adjusted every fourth week based on the current serum ECP value or pre-bronchodilator morning PEF values as surrogate markers of therapeutic needs. At the end of the study there were no statistically significant differences in the mean daily symptom score or the percentage of symptom-free days between the two groups. The mean daily dose of inhaled corticosteroids was similar in the two groups at the start of the study but the algorithms used to adjust the dose of inhaled corticosteroids resulted in an increased use of inhaled corticosteroids in both groups. The mean daily dose of inhaled corticosteroids over the whole study period was significantly lower in the ECP group compared with the PEF group (1246 *vs.* 1667 μg, P=0·026). In the ECP group, forced expiratory volume in 1 sec (FEV_1)% predicted was lower at the end of the study compared with the begining (92% *vs.* 87%, P=0·0009), although there was no significant difference between the two groups. None of the used algorithms for ECP and PEF led to improvement in symptom scores, in spite of increased doses of inhaled corticosteroids. In this respect, both methods were equivalent and insufficient. Recommendations suggesting lung function tests in current guidelines may be difficult to translate into clinical practice, however, a combination of inflammatory markers, lung function and symptoms may still improve asthma control.

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Keywords asthma; corticosteroids; eosinophil cationic protein; inflammation; PEF.

INTRODUCTION

Asthma has been demonstrated to be greatly dependent on inflammatory processes including activated eosinophils (1,2). Various guidelines for asthma management (3, 4) propose that measurements of lung function and specifically peak expiratory flow (PEF) are most helpful in indicating the therapeutic need in moderate to severe asthma. However, lung function variables and PEF relate only indirectly to the underlying inflammatory process in the airways and also reflect the bronchoconstriction related to irritation and hyper-responsiveness (5,6). Thus, it can be argued that, since asthma is an inflammatory disease, there may be a benefit if the inflammatory process could be reflected more directly over time (7).

In active asthma, there is evidence of recruitment of primed eosinophils from the bone marrow into the airways (8). Eosinophil cationic protein (ECP), a cytotoxic granule-derived protein released from activated

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eosinophils, has been found released in the airways of asthmatic patients responsive to corticosteroid therapy (9, 10). Serum ECP is assessed as the *ex vivo* release from primed eosinophil granulocytes incubated at room temperature (11).

Studies have demonstrated ECP release into the blood from activated eosinophils during the late asthmatic reaction (12). Serum ECP levels have been shown to reflect exposure to allergens, deterioration of asthma (9, 13), disease activity (14) and response to corticosteroid treatment (15–18).

The aim of the present study was to compare the clinical outcome when serum ECP, a marker of inflammation, or PEF, a measure of bronchial obstruction, were used to guide the dose of inhaled corticosteroids required to control asthma.

METHODS

Study subjects

One hundred and sixty-four patients with moderate to severe asthma, step 3–4 according to the asthma guideline (3), 16–65 years old with documented symptoms during the last 12 months, $\geq$15% reversibility in PEF or forced expiratory volume in 1 sec (FEV_1) after use of a bronchodilator or in response to a trial of corticosteroid therapy, and regular use of inhaled corticosteroids (400–1600 μg day^{-1} budesonide or 500–2000 μg day^{-1} beclomethasone dipropionate but not fluticasone) were enrolled in a 6-month study. Exclusion criteria were use of oral corticosteroids, anti-histamines, smoking during the last 24 months and/or smoking $\geq$ 5 pack-years during the last 10 years, and any known co-morbidity.

Study design and methods

The study was a parallel group, randomized single-blind study, involving six centres in Gothenburg (Sweden), The Hague (The Netherlands), Antwerp (Belgium) and Veruno, Pavia and Crema (Italy).

At the first visit case history, use of asthma medication, physical examination, spirometry, eosinophil count, serum ECP and specific IgE (Phadiatop®) were assessed. Each patient was given a diary card for daily recording of cough, wheeze or breathlessness during daytime, nighttime and after exercise on an 0–3 scale (maximum total score 21) and to record morning PEF and medication usage during a run-in period of 2 weeks. The personal best post-bronchodilator morning PEF (mini-Wright Peak Flow Meter, Clement Clarke International Ltd, Harlow, U.K.) was established during this period. Treatment allocation to the ECP or PEF group was performed after run-in using a computer-generated randomization schedule prepared for each centre.

After the run-in period, the patients visited the clinic every fourth week $\pm$7 days for clinical assessment. At each visit, the diary card was checked and FEV_1, eosinophil count, serum ECP and pre-bronchodilation PEF were measured at the same time of the day and regardless of group allocation. Patients were instructed not to use short- and long-acting β_2-adrenoceptor agonists 4 and 12 h respectively prior to the FEV_1 measurement. All asthma medications were kept constant during the study period except the as required use of short-acting β_2-adrenoceptor agonists and inhaled corticosteroids. The algorithms used in the present study were based on international consensus (3,4) and clinical data from studies in various countries (12–17). The schedule for dosing inhaled corticosteroids is shown in Fig. 1. The dose steps represent budesonide. For beclomethasone dipropionate, the dose steps were 0–250–500–1000–2000–3000–4000 μg day^{-1}.

Serum ECP (19) was measured with UniCAP® (Pharmacia Diagnostics, Uppsala, Sweden) according to the directions for use. Depending on the PEF values during the preceding period (PEF group) or the current ECP value (ECP group), the patient was instructed within 72 h of each visit to adjust the dose of inhaled corticosteroids based on the algorithm as depicted in Fig. 1.

The primary clinical end point was asthma symptoms, expressed both as symptom score and symptom-free days. Secondary endpoints were use of inhaled short-acting β_2-adrenoceptor agonists, FEV_1 and asthma exacerbations. The criteria for an exacerbation were one or several of the following: morning pre-bronchodilator PEF $<$60% of personal best for $\geq$ 2 days during the last 7 days; nocturnal awakening needing a bronchodilator more than once during 1 night or more than 3 nights in the previous 7 nights; increase of $\geq$ 4 points in symptom

ECP (μg l^{-1})	Dose step	PEF (% of personal best)	Dose steps (μg day^{-1})
			3200
<15[a]	−1	≥85[b]	2400
			1600
≥15 - <20	0	≥85[c]	800
≥20 - <40	+1	70 - 85[c]	400
			200
≥40	+2	<70[c]	0

FIG. 1. Schedule for dosing inhaled corticosteroids. (a) At 2 consecutive visits, (b) during prior 2 months and (c) during last week before visit. The dose steps represent budesonide. For beclomethasone dipropionate, corresponding doses were 0–250–500–1000–2000–3000–4000 μg day^{-1}. In case of exacerbation, the inhaled steroid dose was increased one step after a course of oral steroids.

score for ≥ 2 consecutive days: increase in daily use of short-acting inhaled β_2-adrenoceptor agonists of ≥ 4 doses for ≥ 2 days. Patients who exacerbated were given oral prednisone 30 mg daily for 5–7 days and were kept in the trial on a one step higher dose of inhaled corticosteroids, which was not changed at the next visit.

The study was conducted in accordance with the principles of The Declaration of Helsinki and Good Clinical Practice. Approval from the Ethics Committee at each centre was obtained.

Statistical analyses

The sample size was calculated from an expected risk of having a certain value or higher on the symptom score when ECP was used compared to PEF (odds ratio of 0·4–0·5). The study then required 58–98 patients in each group to have an 80% power of detecting a difference at a two-sided 5% significance level (20). All patients who completed the study, adhered to the protocol conditions and who had not violated a major eligibility criterion were included in the 'per protocol' (PP) analysis. All statistical tests were two-sided with a significance level of 5%. The analyses were based on results from the last seven days preceding each visit. Analysis of variance and analysis of covariance were used for the variable intake of inhaled corticosteroids and FEV_1. Logistic regression (proportional hazards model) was used for the variables symptom score and intake of inhaled short-acting β_2-adrenoceptor agonists. The models included the effects of country, treatment group and values at run-in. Treatment group-by-country interaction terms for the outcome variables were included if the associated statistical test yielded a two-sided P-value of 0·10 or less. Student's t-test (paired) was used to test the within-group changes of FEV_1 and inhaled corticosteroids between visit 2 and visit 8.

Wilcoxon signed ranks test was used for symptom score variables and intake of short-acting β_2-adrenoceptor agonists. Mantel–Haenszel statistic adjusting for country was used for comparison between groups for the proportion of patients with at least one exacerbation during the study period as well as the proportion of patients using long-acting β_2-adrenoceptor agonists.

RESULTS

Of the 164 patients enrolled, 24 did not complete the study (12 for medical reasons and 12 for social reasons) (Table 1), and five left the study after the first or second visit, with no valid data. In total, 135 patients completed the 6-month study. Out of these 135, 118 adhered to all study conditions and were included in the per protocol analysis. Of the randomized patients who were not included in the PP analysis, 17 completed the study but deviated from the intended medication schedule. On the basis of history of allergy and Phadiatop test (screening test for presence of specific IgE), the patients were divided into allergic ($n = 41$), non-allergic ($n = 46$) and mixed asthma ($n = 31$) (Table 2). No difference in baseline characteristics between the two groups was found (Table 2). The concomitant medication is shown in Table 3.

Table 1. Disposition of patients, shown for the whole study population and for the ECP and PEF groups

Number of patients	Total	ECP group	PEF group
Randomized	164	85	79
Completed	135	74	61
Completed and compliant with study conditions (included in PP analysis)	118	65	53
Completed but not compliant with study conditions	17	9	8
Withdrawn during follow-up	24	8	16
Withdrawn with no valid data	5	3	2

Symptoms

The mean daily symptom score was 1·8 at visit 2 and 2·1 at visit 8 in the ECP group, and 1·4 and 1·7 in the PEF group (Table 4). Within-group differences between visits 2 and 8 were not significant, and there was no difference between the groups during the study period. The mean percentage of symptom-free days was 40% at visit 2 and 45% at visit 8 in the ECP group, and 56 and 50% in the PEF group. Within-group differences were not significant, and there was no difference between the groups at the end of the study. There were no significant differences between patients with allergic and non-allergic asthma in any of the groups.

Short-acting β_2-adrenoceptor agonists

The mean percentage of days without intake of short-acting β_2-adrenoceptor agonists at visits 2 and 8 was 73 and 64% in the ECP group, and 76 and 74% in the PEF group, respectively (Table 4). The mean daily intake was not significantly changed in either of the groups and no difference between the groups was found.

Spirometry

Mean FEV_1% predicted in the ECP group at visit 2 and visit 8 were 92 and 87 (P=0·0009), respectively and in the PEF group 90 and 89 (NS). No significant differences were found between the groups, either at the beginning or at the end of the study (Table 4).

Table 2. Clinical baseline characteristics. Mean and 95% confidence intervals

Variable	ECP group	PEF group	ECP *vs.* PEF group
Age (yrs)	42·2 (39·0–45·5)	40·3 (36·4–44·2)	P=0·41
Height (m)	1·69 (1·67–1·72)	1·69 (1·67–1·72)	P =0·93
Weight (kg)	75·9 (72·0–79·8)	72·0 (68·6–75·3)	P =0· 13
Sex (F/M)	36/29	26/27	P =0·48
PEF (% predicted)*	103·4 (97·9–108·8)	103·6 (99·1–108·0)	P =0·92
ECP ($\mu g\,l^{-1}$)	12·0 (10·0–14·4)	11·6 (9·6–14·2)	P =0·87
Eosinophils ($\times 10^9 l^{-1}$)	0·20 (0·17–0·24)	0·22 (0·17–0·27)	P =0·53
Phadiatop (negative/positive)	24/41	16/37	P =0·45
Type of asthma (allergic/mixed/ non-allergic)	23/16/26	18/15/20	P =0·90
Duration of asthma (<2 yrs/2–10 yrs/ >10 yrs)	5/31/29	3/21/29	P =0·29

*The highest of three measurements.

Table 3. Concomitant medication

Type of medication	Used by *n* patients in the ECP group	Used by *n* patients in the PEF group
Long-acting β_2-adrenoceptor agonists	39	30
Xanthin-derivates	5	3
Cromoglycates	1	1
Anti-cholinergics	1	1
Epinephrine	0	1

Exacerbations

During the study 14 patients in the ECP group experienced 18 exacerbations and seven patients in the PEF group experienced eight exacerbations. The difference between the groups did not reach statistical significance (Table 4).

Use of corticosteroids

Based on the treatment algorithm there was a need to change therapy in all patients but one. During the run-in period the mean daily dose of inhaled corticosteroids was 928 μg in the ECP group and 943 μg in the PEF group (NS, Table 4, Fig. 2). In the ECP group and in the PEF group the mean daily dose increased during the study period to 1272 μg (P= 0·03) and 1540 μg (P=0.0004), respectively. Calculated over the whole study period the mean daily dose was significantly higher in the PEF group (1667 μg) compared with the ECP group (1246 μg, P=0·026).

ECP and eosinophil counts

For all patients, the correlation coefficient between ECP and the number of eosinophils at the different visits varied between 0·37 and 0·54. An overall estimate of the correlation coefficient for all eight visits obtained by analysis of covariance was 0·46. Fifty-five per cent of the patients with serum ECP between 20 and 40 $\mu g\,l^{-1}$ had an eosinophil count above the upper reference limit. In patients with ECP $\geq 40\,\mu g\,l^{-1}$, 40% were above this limit.

DISCUSSION

A number of studies have suggested that serum ECP levels may be useful in guiding the dose of inhaled corticosteroids to control asthma (15–18). In the present study an attempt was made to compare this method with a traditional method, using PEF.

The advantages of serum ECP measurement are its objectivity and accuracy. The disadvantages today may be practical problems of blood handling and some delay in results (19). The advantages of PEF are self-use and simplicity. The disadvantages are the need for compliance and the difficulty for some patients, especially small children, to accomplish the measurements properly (5,6).

The clinical decision on how to dose inhaled corticosteroids optimally to maintain asthma control is not always easy. For the individual patient, information including history of symptoms, limitations to daily living, clinical findings and lung function should be evaluated together by the treating physician to best find the optimal dose. It is well known that this optimal situation is not always achieved and suboptimal treatment of asthma is

Table 4. Clinical data. Mean and 95% confidence intervals

Variable	Period	ECP group	PEF group	*P*-value*
Daily symptom score	Run-in	1·8 (1·3–2·3)	1·4 (0·9–2·0)	0·11 †
	End of study	2·1 (1·4–2·8)	1·7 (1·0–2·3)	0·76
Percentage of	Run-in	40 (31–50)	56 (45–68)	0·07‡
symptom-free days	End of study	45 (34–57)	50 (38–62)	0·91
Daily intake of				
short-acting β_2-	Run-in	0·18 (0·09–0·28)	0·13 (0·05–0·21)	0·39†
adrenoceptor agonists (mg day^{-1})	End of study	0·26 (0·14–0·38)	0·17 (0·08–0·26)	0·75
Percentage of days	Run-in	73 (63–82)	76 (66–86)	0·34†
with no intake of	End of study	64 (53–74)	74 (64–84)	0·41
short-acting β_2-adrenoceptor agonists				
FEV_1 in per cent of predicted	Run-in	92·1 (87·2–97·0)	89·8 (85·0–94·6)	0·51‡
	End of study	87·1 (82·6–91·6)	89·1 (84·5–93·6)	0·09
Number of exacerbating patients, *n* (%)	Whole study period	14 (22)	7 (13)	0·20§
Daily intake of inhaled corticosteroids (μg)	Run-in	928 (828–1028)	943 (833–1054)	0·88‡
	End of study	1272 (975–1568)	1540 (1198–1881)	0·25

*Two-sided *P* values for the comparison between ECP group and PEF group. *P*-values at run-in are adjusted for variation due to country. *P*-values at the end of the study are adjusted for values at run-in and variation due to country.
†*P*-values from logistic regression (proportional hazards model).
‡Run-in: *P*-value from analysis of variance. End of study: *P*-value from analysis of covariance.
§*P*-value from Mantel–Haenszel statistic adjusting for country.

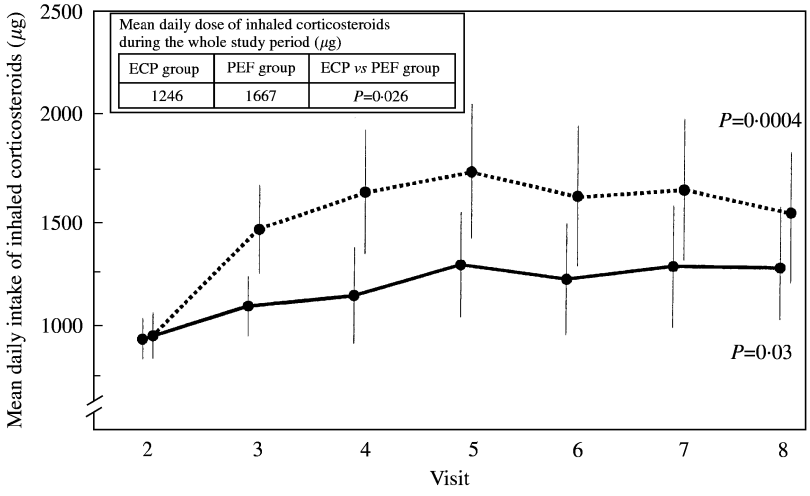

Fig. 2. Mean daily intake of inhaled corticosteroids for the 7 days preceding each visit to the clinic. ECP group (–), *n*=65; PEF group (- - -), *n*=53. Vertical bars show the 95% confidence intervals. *P*-values refer to within-group changes between start and end of the study. The inserted table shows the mean daily dose of inhaled corticosteroids during the whole study period.

common (21–23). To support treating physicians in their therapeutic decisions, guidelines for asthma management have been developed (3,4) but it is not obvious how the recommendations should be translated into clinical practice. The steps of doing the treatment are given, but at what indications should one go from one dose level to another?

Based on the currently available guidelines and clinical experience an attempt was made in this study to construct two algorithms for guiding the inhaled steroid dose, one for ECP as a marker of inflammation, and one for PEF as a lung function test. However, it was shown that there was no difference in symptoms between the two modalities of guiding the dose and, in fact, the primary clinical end points, symptom score and symptom-free days, were not improved. The algorithm for ECP led to a significant fall in FEV_1 despite increase in steroid dose and without change in symptoms. The algorithm for PEF, suggested in published guidelines (3,4), led to a significantly higher dose of inhaled steroids without gain in

symptoms but with a potential risk of more side-effects. Thus, both ways of guiding the steroid dose seem to be insufficient.

It is now known that the dose response curve to inhaled steroids (especially at high doses) is flat, and a mean change in daily dose from 900 to 1200 or 1500 μg, rarely results in much change in lung function or symptoms. In one study, even a four-fold difference (200 *vs*. 800 μg) for one year, in patients of severity similar to that in the present study, did not result in significant differences regarding symptoms or lung function (24). These results were, however, not known when the current study was planned. In a recent study it was suggested that percentage sputum eosinophils, another marker of inflammation, might be useful in guiding the reduction of inhaled steroid doses in asthma (25). As in almost every trial it is not fully known to what extent the patients complied with the given instructions regarding treatment. The reported treatment in diaries was however close to the intended ones, and, in our opinion, there is no reason to believe that poor compliance affected the results considerably.

The study showed that neither ECP nor PEF might be an ideal way for guiding the optimal dose of inhaled steroids. In spite of this, the results are of interest. Firstly, it seems that guidelines have limitations when transferred to clinical practice. Does PEF values below 85% of personal best signal poor asthma control, and is it really correct to increase steroid dose due to reduced PEF? ECP seems to be comparable to PEF in this respect. Secondly, the study seems to support the clinical experience that asthma is a heterogeneous disease. This is indicated by the lack of strong correlation between different parameters of asthma. Increased doses of inhaled steroids, generally considered as potent drugs in asthma, were not followed by an improvement of symptoms. FEV_1 was decreased in the ECP group despite higher doses of steroids, and airway inflammation, expressed as serum-ECP, seems not to have a direct relationship with asthma symptoms. Furthermore, asthma symptoms may also be heterogeneous and all symptoms commonly used in asthma studies, may not reflect reversible bronchial obstruction, e.g. cough may not be induced in the same way as wheezing and breathlessness may not be an 'asthma-specific' symptom (26). Asthma-like disorders, such as sensory hyper-reactivity (27), have also been described where asthma-like symptoms are present but not associated with reversible bronchial obstruction and positive methacholine test (26,27). Although airway symptoms and quality of life are recommended as clinical endpoints, it may be important to further analyse which symptoms are the most 'asthma-specific'.

From a clinical standpoint the conclusion of this controlled study of a marker of inflammation, may be that neither the algorithm for ECP nor PEF lead to improvement of asthma symptoms. However, a reservation must be done for different effects in certain subtypes of asthma and for different airway symptoms. This needs further studies. We also think that a combination of a lung function test and ECP, together with the history of symptoms and physical status, still may be a good way of guiding the steroid dose.

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