

Short communication

Detection and treatment of early asthma

Background: We sought answers to two questions:

- 1) how can mild, early asthma best be detected?
- 2) how should it be treated and the treatment effect monitored?

Methods: Eighty adult patients with early, mostly mild asthma and 30 control subjects were examined by lung-function and biochemical tests. Patients were randomly assigned to two treatment groups. One group was treated with an inhaled steroid (budesonide) for 6 weeks, and the other first with an inhaled β_2 -agonist (terbutaline) for 6 weeks and then with an inhaled steroid for 2 weeks.

Results: Treatment with budesonide was effective: symptom scores, PEF, blood eosinophils, and sputum ECP values all improved. Terbutaline was ineffective by these criteria. For the detection of early asthma and for the following treatment effects, sputum and serum ECP assays are useful supplements to lung-function tests.

Conclusions: Treatment of early, mild asthma with an inhaled steroid is effective and worthwhile. Detection of the disease remains a problem, as both lung-function and biochemical tests have low sensitivities.

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It has been claimed that early, mild asthma can effectively be treated with inhaled steroids. Such treatment can stop the progress of the disease at an early stage, reduce or even eliminate present and future symptoms, and possibly improve the long-term outcome (1, 2). The present paper is a further study of the effects of an inhaled steroid, budesonide, as compared with an inhaled β_2 -agonist, terbutaline.

Diagnosis of asthmatic airway inflammation and early asthma is complicated by the lack of laboratory methods of satisfactory sensitivity. The best lung-function test, histamine challenge, has a sensitivity of only 48% when applied to patients with early, mild asthma (3). When supplementary laboratory tests have been sought, sputum – an excretion from the affected mucosa – has appeared to be a promising sample material (3, 4). Inducing sputum secretion and studying the sputum sample is noninvasive, while the results are comparable to those of long-established, invasive bronchoscopic methods (5). We evaluated both sputum and other supplementary tests to see how

well they perform when early asthma has to be detected and the effects of subsequent therapy have to be followed.

Material and methods

Subjects

Eighty consecutive patients with newly detected asthma were recruited in an open, longitudinal study. They had had subjective symptoms (cough, sputum secretion, wheezing, dyspnea, or chest tightness) for less than 1 year. The patients were examined by three lung-function tests. Those admitted to the study had at least one of the following lung-function results outside the reference (normal) range:

- 1) FEV₁ improvement of >15% after an inhaled β_2 -agonist (0.4 mg rimeterol, Pulmadil, 3 M)
- 2) PEF diurnal variation of >15% and PEF increase of >15% after an inhaled β_2 -agonist at least once during a 2-week follow-up period.

In addition, many patients had PD₁₅ of ≤ 1.6 mg in histamine challenge (6). Based on symptom history and lung-function parameters, the responsible clinician classified (without strict criteria) 61 patients (76%) as having mild asthma and 19 patients (24%) as having moderate asthma.

Abbreviations. ECP: eosinophil cationic protein; EPX: eosinophil protein X; FEV₁: forced expiratory volume in 1 s; MPO: myeloperoxidase; PD₁₅: provocative dose of histamine causing 15% reduction in FEV₁; PEF: peak expiratory flow.

Exclusion criteria were treatment with anti-inflammatory medication (corticosteroids, cromones, theophylline), lung diseases other than asthma, and a respiratory tract infection in the previous 4 weeks.

Thirty healthy, nonatopic, nonsmoking control subjects were recruited from the hospital staff. Exclusion criteria were past or present long-term respiratory diseases including asthma, respiratory tract infections in the preceding 4 weeks, and hyperresponsiveness to histamine.

The study groups are characterized in Table 1. The mean ages of the groups did not differ significantly. There were more women than men, especially in the control group. The two patient groups were similar by all clinical parameters examined (Tables 1 and 2).

Study protocol

Patients were randomized into two treatment groups. The budesonide-group patients received 1.2 mg inhaled budesonide per day, divided into two doses (Pulmicort Turbuhaler, Astra-Draco AB, Lund, Sweden). The patients could inhale also bronchodilating terbutaline (0.5 mg Bricanyl Turbuhaler, Astra-Draco AB, Lund, Sweden) when needed. The terbutaline-group patients took only terbutaline when needed. The first treatment period for both groups was 6 weeks. Patients in the terbutaline group were then shifted to budesonide and treated for 2 weeks as described for the budesonide group. No phase of the study was conducted blinded.

Patients visited the hospital three times before treatment and then after 6 weeks of treatment. The terbutaline group made one additional visit after 2 weeks of budesonide treatment. During the first two visits, lung-function and skin prick tests were performed and asthma was diagnosed. On subsequent visits, sputum, blood, and urine samples were obtained. Control persons also gave laboratory samples, and lung-function tests were performed on them. The hospital ethics committee approved the study protocol. All subjects gave their informed consent.

Table 1. Characteristics of control persons and of patients before treatment

Characteristic	Patients treated with budesonide	Patients treated with terbutaline	Control subjects
Number of persons	39	41	30
Mean age (years)	31.0	28.6	39.5
Age range (years)	16–60	16–57	22–59
Female/male	32/7	31/10	28/2
Smokers/nonsmokers	14/25	9/32	0/30
Skin-prick-test-positive/-negative persons	23/16	25/16	0/30
Mild/moderate asthma	31/8	30/11	0/0
Median PD ₁₅ (mg histamine)	0.68	0.58	> 1.6
FEV ₁ (% of predicted), mean ± SD	91.6 ± 10.8	94.5 ± 9.9	97.3 ± 10.9

SD: standard deviation.

Clinical methods

Flow-volume spirometry and histamine challenge tests for bronchial responsiveness were performed by methods previously described (3). All patients measured their morning peak expiratory flow (PEF) (the best of three measurements was recorded) during 2 weeks before inclusion and during treatment weeks 5–6. In addition, terbutaline-treated patients measured their PEF during treatment weeks 7–8. PEF values were expressed as a percentage of the patient's predicted value. The PEF values reported here are the mean morning values for 2-week measuring periods.

At the time of the PEF measurements, patients evaluated the subjective symptoms of asthma which they had experienced during the past 24 h. Symptom scores were graded on a scale from 0 (no symptoms) to 10 (most severe discomfort). Scores are presented as means for 2-week periods.

Skin prick tests were performed with a panel of 11 common inhalant allergens (Soluprick SQ, 10 HEP, ALK, Denmark). A patient was classified as atopic if any allergen caused a wheal with a diameter of 3 mm or more while control solutions (solvent and 10 mg/ml histamine dihydrochloride) gave expected results (7). For control persons, atopy was an exclusion criterion, and all were skin-prick-test negative.

Induction of sputum

Most asthmatics, but few healthy persons, expectorate sputum. We needed sputum samples from patients with mild asthma, patients in remission, and healthy controls. To obtain them, we induced sputum production by the inhalation of nebulized 5% (hypertonic) saline from an ultrasonic nebulizer (Spira Ultra, Spira Ltd, Hengityshoitokeskus, Hämeenlinna, Finland), as previously described (3, 8). No premedication was used. To ensure patients' safety, we made PEF measurements before and after sputum induction. If the PEF decreased more than 10%, the subject was treated with inhaled terbutaline. No one experienced notable adverse effects. Induction was discontinued when a macroscopically adequate sputum sample had been obtained or when 15 min had passed.

Before treatment, an adequate sputum sample was obtained from 73% of the patients and 77% of the controls. After treatment, the success rate for sputum induction was 62% in the budesonide group and 66% in the terbutaline group. Sputum data are given for patients and control persons who produced adequate sputum samples as judged by cytologic examination.

Processing of sputum

The bronchial origin of sputum was confirmed

Table 2. Effect of treatment. Means (arithmetic or geometric) for asthma symptom scores and laboratory variables for control persons and for two patient groups before and after treatment

Variable	Unit	Control persons	Budesonide group		Terbutaline group		
			Before treatment	After 6 weeks of budesonide	Before treatment	After 6 weeks of terbutaline	After 6 weeks of terbutaline plus 2 weeks of budesonide
Asthma symptom score	Arbitrary (0–10)	Not done	2.7	2.0**	2.3	2.0	1.7**
Morning PEF	% of predicted	Not done	89.5	93.3**	92.2	93.2	96.7**
Sputum ECP	µg/l	114	506*	211**	496*	714	283**
Sputum eosinophils	Arbitrary (0–4)	0.6	1.3	1.0	1.1	1.0	1.0
Sputum neutrophils	Arbitrary (0–4)	1.8	2.2	2.1	2.0	1.9	2.2
Blood eosinophils	10 ⁹ /l	0.13	0.20	0.11**	0.16	0.14	0.12**
Serum ECP	µg/l	9.2	13.1	11.1	12.3	14.1	12.1
Serum MPO	mg/l	479	458	496	455	527**	474
Urinary EPX/creatinine	µg/mmol	38.2	33.7	32.3	38.2	38.7	39.6

*Significant ($P < 0.05$) difference between controls and patients.

**Significant ($P < 0.05$) change from baseline data.

cytologically. Samples with no macrophages were assumed to contain mainly saliva and/or inhaled saline and were rejected.

To determine eosinophils and neutrophils, native sputum samples were spread thinly on two microscope slides. The slides were fixed with ethanol and Papanicolaou stained. The same analyst examined all slides. The numbers of eosinophils and neutrophils were determined on a semiquantitative scale graded 0, 0.5, 1, 2, 3, and 4 for each type of cell (9). Drawings of typical fields were used for calibration.

Sputum samples to be used for biochemical analyses were processed within 2 h. The volume was determined. To liquefy the sputum, we added an equal volume of 6.5 mM dithiotreitol solution (Sputolysin, CalbioChem, LaJolla, CA, USA), diluted 10-fold in distilled water. The mixture was vortexed, incubated for 15 min at room temperature, and stored at -20°C until analyzed. Thawed samples were incubated for 1 h with an equal volume of phosphate-buffered saline, pH 7.2, containing 0.4% *N*-cetyl-*N,N,N*-trimethylammonium bromide (to solubilize proteins) and 0.25% human serum albumin. Samples were then centrifuged at 600 *g* for 10 min to remove cell debris and to collect supernatant.

Sputum results were not adjusted to correct for dilution effects, as no biochemical or cellular component suitable for dilution estimates seemed available.

Biochemical measurements

All laboratory analyses were performed blindly with no knowledge of patient data. Clear sputum-sample supernatants were used for ECP assays.

Blood samples were collected by venipuncture with gel tubes (SST tube, Becton-Dickinson, UK), and sera for eosinophil cationic protein (ECP), and myeloperoxidase (MPO) assays were separated under standardized conditions (10). ECP and MPO concentrations

were determined by commercial immunoassay kits (Pharmacia and Upjohn Diagnostics, Uppsala, Sweden). Blood eosinophils were counted with a Technicon H2 instrument (Technicon, USA).

The first urine in the morning was sampled and analyzed for eosinophil protein X (EPX) with a radioimmunoassay research kit (EPX RIA, Pharmacia and Upjohn Diagnostics, Uppsala, Sweden). Samples were diluted 10-fold with the assay dilutor. Urinary creatinine was determined by a modification of the Jaffe method. Results are given as EPX/creatinine (µg/mmol) ratios to correct for the variable amount of water excreted with the urine.

Statistical analyses

As the distribution of results for several assays (blood eosinophils, all ECP, EPX, and MPO assays) was roughly log-normal, logarithmic values were used in statistical evaluations; e.g., geometric means were compared. In other cases, logarithmic transformations were not used. For comparisons between groups, Student's *t*-test and Wilcoxon's rank sum test were used. Correlations between variables and changes were analyzed by Spearman's rank-order test. Two-tailed *P* values below 0.05 were considered significant.

A reference (normal) range for sputum ECP was calculated as geometric mean $\pm$ 2 standard deviations of values obtained for the healthy control group. By definition, this range included 95% of healthy persons.

Results

Test results before treatment

The baseline values for the budesonide and terbutaline groups were not significantly different for any variable examined. Patients with asthma had higher sputum

ECP levels than control persons ($P=0.0002$). No other significant difference in laboratory parameters was observed between patients and controls (Table 2).

Patients with moderate asthma had higher symptom scores ($P=0.0001$), lower PEF levels ($P<0.0001$), higher sputum ECP ($P=0.044$), and higher numbers of sputum eosinophils ($P=0.044$) than patients with mild asthma. No differences between patients with and without atopy (based on skin test results) were detected, nor were there differences between patients who were smokers and nonsmokers.

In all subjects (patients and control persons), serum ECP correlated with blood eosinophils ($r_s=0.56$, $P=0.0001$), and sputum ECP correlated with sputum eosinophils ($r_s=0.37$, $P=0.0012$). Patients' serum ECP and serum MPO correlated as well ($r_s=0.56$, $P=0.0001$). A correlation between sputum ECP and PEF was observed in atopic patients in the budesonide group ($r_s=0.58$, $P=0.02$).

Effect of treatment with budesonide and with terbutaline

In the budesonide group, patients' condition improved significantly after 6 weeks of treatment as judged by symptom scores, morning PEF values, blood eosinophil counts, and sputum ECP concentrations. In the atopic subgroup, serum ECP levels were also reduced. In the terbutaline group, no parameter changed

significantly. When terbutaline patients were shifted for 2 weeks to budesonide medication, their condition improved in a manner similar to that of patients treated with budesonide from the beginning (Table 2 and Fig. 1).

Serum ECP levels tended to decrease in both budesonide and terbutaline groups during treatment with inhaled budesonide, but the changes were not significant. Serum MPO levels increased during 6 weeks of terbutaline treatment. Urinary EPX/creatinine did not change (Table 2).

No correlations were found between changes in sputum or serum ECP and changes in PEF.

Sensitivity and specificity of laboratory tests

When used to detect early and mostly mild asthma, the serum and sputum ECP tests had an identical, relatively low sensitivity of 33%. This is related to the overlap of patient and control result distributions (Fig. 1). Combined, the serum and sputum ECP tests had a sensitivity of 53%. All laboratory tests combined, without lung-function tests, had a sensitivity of 58%. While combining tests increases sensitivity, it reduces specificity. Thus, serum and sputum ECP combined had a specificity of 96%, while all tests combined (without lung-function tests) had a specificity of 83%.

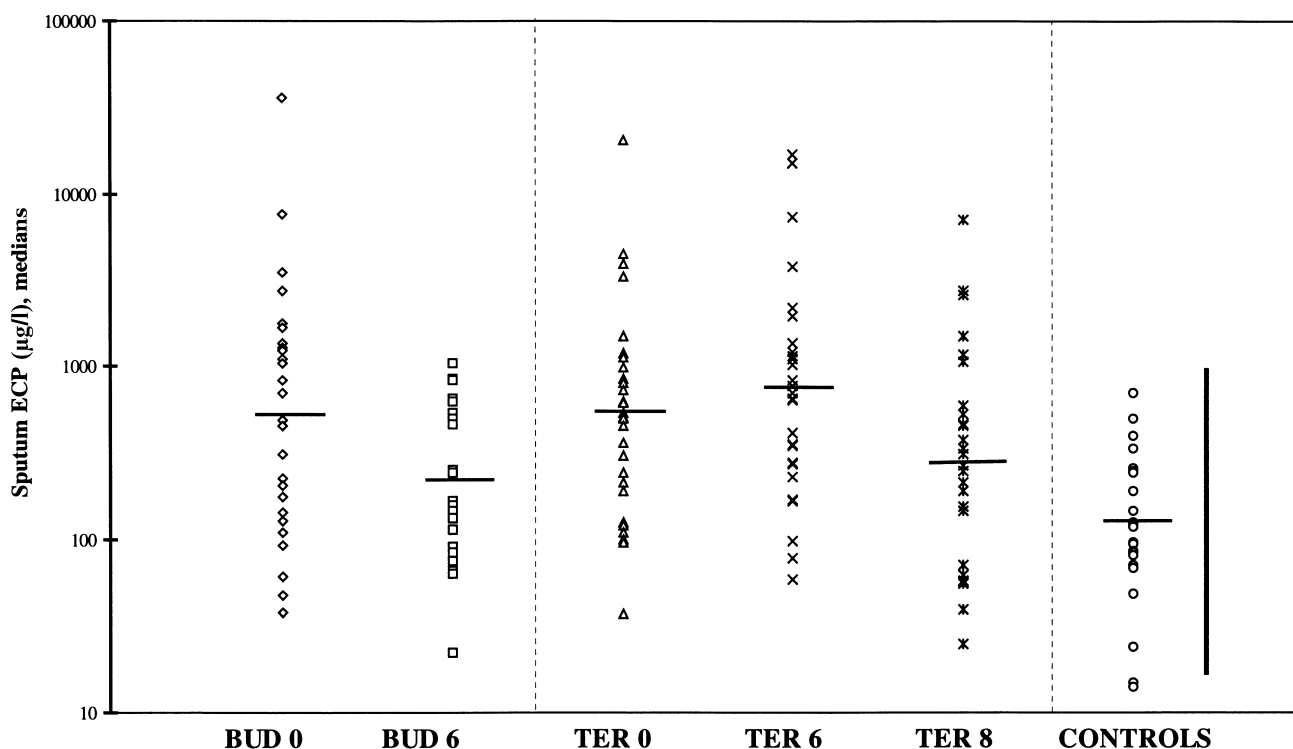


Figure 1. Patients' sputum ECP values before treatment (BUD 0 and TER 0), after 6 weeks of treatment with budesonide (BUD 6) and terbutaline (TER 6), and after treatment with 6 weeks of terbutaline followed by 2 weeks of budesonide (TER 8). Results for control persons are also given. Median value for each group is shown by horizontal bar, and 95% range (reaching 875 $\mu\text{g/l}$) for controls by vertical bar.

Discussion

In 76% of our patients, who had early asthma, the disease remained mild. Detecting mild forms of a disease by laboratory tests is generally more difficult than detecting severe illness. This is illustrated by the mean morning PEF and FEV₁ values in our patient groups; the means were in the range 89.5–94.5% of predicted levels, a finding which suggests normal lung volumes.

Lung-function tests are widely used for the detection of asthma, but even the most sensitive test, the histamine challenge, has a sensitivity of only 48% when applied to patients with early, mild asthma (3). Asthma is caused by an inflammation of the bronchial mucosa accompanied by leukocyte infiltration, particularly by eosinophilic leukocytes. When new laboratory methods have been sought, it has been logical to study the usefulness of inflammation markers, such as ECP, and to use sputum originating in the airways as sample material (3, 8).

Sputum ECP turned out to be the best biochemical test tried for the detection of early asthma and for the monitoring of treatment effects. The sensitivity was a modest 33% when untreated patients were examined, but improved to 53% for the combination sputum ECP plus serum ECP. We found no significant correlation between inflammation marker levels and pulmonary function tests other than between sputum ECP and PEF in atopic patients in the budesonide group. When patients with more severe asthma have been studied, correlations between sputum ECP and pulmonary function have been found (11). Nor did we find a correlation between sputum ECP and sputum eosino-

phils. This may have been due to relatively mild sputum eosinophilia in our patients.

Our choice of control persons is open to some criticism, as only nonatopic nonsmokers were included. Atopic subjects without asthma may have airway eosinophilia (12), and a wider sputum ECP reference range might have been observed if atopics had been included in the control group. However, in our patients, there were no differences in any parameter between skin-prick-test-positive (atopic) and -negative individuals. Nor were there differences between smokers and nonsmokers.

When patients were treated with inhaled budesonide, symptoms were reduced, lung function as measured by PEF improved, and blood eosinophil numbers and sputum ECP came down. The other treatment tried, inhaled terbutaline, was not effective by these criteria – no parameter changed significantly.

We conclude that treatment of early, mild asthma with an inhaled steroid is effective and worthwhile. Detection of the mild disease remains a problem. Few methods, all of them insensitive, are available. The best combination of biochemical tests, sputum plus serum ECP, had a modest sensitivity of 53%. These assays are, however, a useful supplement to conventional lung-function methods, which also suffer from low sensitivity.

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