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Eosinophilic airway inflammation as an underlying mechanism of undiagnosed prolonged cough in primary healthcare patients

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Abstract Prolonged cough is a common problem in patients seen in general practice. Using a simple method of sputum induction and processing of sputum samples, we determined whether eosinophilic airway inflammation could be a cause of undiagnosed prolonged cough. Eighty-two patients who had had cough for more than 1 month were enrolled into the study, in six primary healthcare centres. Patients with known pulmonary disease, including asthma or chronic obstructive pulmonary disease (COPD), or who were known to have another cause of cough, or to have recently suffered from a respiratory infection, were excluded. Fifty-three healthy individuals served as controls. Sputum was induced by inhalation of 3% saline. Inflammatory cells in smears were studied semi-quantitatively. Concentrations of eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), myeloperoxidase (MPO) and human neutrophilic lipocalin (HNL) were determined. Sputum induction proved safe and adequate samples were obtained from 91%. Sputum eosinophilia (eosinophils accounting for more than 5% of all cells in smears) was present in 14 patients with prolonged cough (19%) but in no healthy individual (P=0·001). Five of the 14 individuals (36%) who exhibited sputum eosinophilia appeared to have asthma, while nine of the 14 (64%) did not. Concentrations of ECP and EPO were higher in patients with prolonged cough than in healthy individuals (P=0·02 for ECP; 0·005 for EPO). We conclude that eosinophilic airway inflammation is a fairly common cause of prolonged cough, even in patients not suffering from asthma or COPD, or in whom no other cause of cough is known to be present. Induced sputum samples obtained in health centres can be studied in a central laboratory. Detection of eosinophilic airway inflammation could aid the decision regarding treatment.

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Keywords eosinophils; induced sputum; prolonged cough; primary care.

INTRODUCTION

Prolonged cough is a common problem in patients seen in primary healthcare. Cough can indicate the existence of or precede the occurrence of asthma. Variable airway obstruction, demonstration of which is the traditional basis for a diagnosis of asthma, is usually associated with chronic eosinophilic inflammation of airway mucosa (1). The existence of such inflammation cannot readily be diagnosed in primary health care because of lack of suitable methods.

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It has been estimated that within the Finnish National Asthma Programme (2), as many as 10% of the population, apart from asthma patients, occasionally exhibits symptoms suggesting that they are suffering from mild asthma, even though results of lung-function tests are normal or close to normal. Many such patients may be exhibiting eosinophilic airway inflammation, the main pathophysiological characteristic of asthma (3–6). Since diagnosis and treatment of asthma is based on demonstration of pulmonary dysfunction, patients of this kind, in whom diagnosis can be ambiguous, may not receive appropriate medication. It has recently been suggested that the term 'asthma-like inflammation' or 'eosinophilic bronchitis' should be employed to describe the disorder from which such patients are suffering (7,8).

The importance of early initiation of anti-inflammatory treatment if a good response to treatment is to be

obtained and asthma is to be prevented from becoming chronic has been emphasized (9,10). Means of confirming the existence of asthma or asthmatic inflammation in cases in which there is no regular pulmonary dysfunction is therefore desirable. Patients with chronic obstructive pulmonary disease (COPD) who exhibit eosinophilic airway inflammation may benefit from anti-inflammatory therapy (11). The same is true of patients with isolated chronic cough and sputum eosinophilia (5,12). A simple method for detection of eosinophilic airway inflammation would therefore be valuable.

Over the past 10 years sputum induction has been found to be a relatively non-invasive way in which the nature and degree of airway inflammation in asthma and other airway diseases can be assessed. Results of sputum analysis may reflect inflammatory processes affecting airway mucosa more accurately than results of blood and serum measurements (13). Methods for sputum induction have developed and have been shown to be reproducible and valid (14,15). They have however been used only in hospitals. We have described a simple method for sputum induction and processing of samples, and have shown this to be valid (16). It can be used in primary health care.

In the study presented here the method was used to assess the nature and degree of eosinophilic airway inflammation in patients with prolonged cough seen in primary health care.

METHODS

Subjects

Eighty-two consecutive patients not known to be suffering from asthma or any other chronic respiratory disease were enrolled into the study, in six health-care centres in the Helsinki area of Finland, between November 1997 February 1998, i.e. outside the pollen season. Each centre serves a population of some 30 000. Patients complaining that they had been coughing daily for more than 1 month were eligible for enrolment into the study. Patients had to be symptomatic at the time of study. They were questioned carefully about respiratory symptoms other than cough. Patients being treated with anti-inflammatory asthma medication (corticosteroids, disodium cromoglycate, nedocromil sodium, theophylline, leukotriene antagonists) or using ACE inhibitors were excluded. Patients who had suffered from a respiratory infection during the preceding 6 weeks or who exhibited symptoms of rhinitis or gastro-oesophageal reflux were also excluded. Patients had to have normal chest and sinus X-rays results, and normal serum C-reactive protein values. Fifty-three healthy control subjects with no history of asthma or other respiratory symptoms were recruited from the staff of the Skin and Allergy Hospital, Helsinki University Central Hospital.

The study had been approved by the Ethics Committees of the Skin and Allergy Hospital, and the health care centres. All subjects gave their informed consent to paticipation in the study.

Clinical methods

Health centre physicians were asked to complete a questionnaire regarding the symptoms and medical history of each patient. Participants were subjected to careful physical examination, including auscultation of their lung sounds. Peak expiratory flow (PEF) values (the highest values of three successful attempts) (Mini Wright Peak-Flow Meter, Clement Clarke International, London, U.K.) were recorded for all patients and controls. If the physician suspected asthma on the basis of the results, the variability of PEF values measured mornings and evenings over a 2-week period was recorded. When feasible, lung volumes were measured, using dynamic spirometry (Vitalograph, Spirotrac III, Buckingham, U.K.) in accordance with standard methods (17). Reversibility in forced expiratory volume in 1 sec (FEV_1) was assessed 15 min after inhalation of 200 μg of salbutamol (Buventol Easyhaler® 100 μg dose^{-1}, Orion Pharma, Espoo, Finland).

Skin prick tests were conducted using 11 common inhalant allergens (Soluprick SQ, 10 HEP, ALK, Denmark), and positive (histamine dihydrochloride, 10 mg ml^{-1}) and negative (solvent) control solutions. A subject was classified as atopic if any allergen caused a weal of 3 mm or more in diameter and control solution gave expected results (18).

Blood samples were taken by venipuncture. Serum was separated under standardized conditions, according to the instructions of the manufacturer (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden), permitting release of leukocyte activation markers.

Sputum induction

Sputum was induced by inhalation of 5 ml of 3% NaCl solution, using an ultrasonic nebulizer (Omron U1, Omron, Germany) for 15 min. Subjects were pre-treated with 200 μg salbutamol (Buventol Easyhaler® 100 μg dose^{-1}), by inhalation. PEF values were measured before and after induction, to ensure safety of the procedure. Subjects were asked to cough during and after inhalation. Sputum samples were collected in empty containers. If PEF fell by more than 15% or troublesome symptoms appeared, patients were treated with 200 μg of inhaled salbutamol.

Sputum sample handling in primary health care

Sputum samples were transferred to Petri dishes and examined against a dark surface. The more viscous parts

were collected, using forceps, and mixed (14). Part of each sample was used to make two smears, which were air-dried (not frozen). The rest of each sample was transferred into a pre-weighed tube and frozen at −20°C.

Sputum analysis in the laboratory

Frozen sputum samples and the air-dried slides were sent to the Skin and Allergy Hospital, where further analyses were undertaken. Samples were processed using a method previously described and validated (16). Briefly, each sample was thawed and treated with a mucolytic agent (Sputolysin™, CalbioChem, La Jolla, U.S.A., diluted 10-fold with distilled water) and a detergent (0.5% cetyl-N,N,N,-trimethylammonium bromide, 0.4% human serum albumin, 100 mM phosphate-buffered saline, pH 7·2). After incubation for 1 h, cells were lysed and markers inside and outside the cells released and solubilized. The sputum supernatant was separated from cell debris by centrifugation. Thawed sputum supernatant concentrations ($\mu g\, l^{-1}$) of two eosinophil- activation markers, eosinophil cationic protein (ECP) and eosinophil peroxidase (EPO), and of two markers of neutrophil activation, myeloperoxidase (MPO) and human neutrophil lipocalin (HNL) were determined. ECP and MPO concentrations were determined using commercially available immunoassay kits (Pharmacia & Upjohn Diagnostics), and EPO and HNL concentrations using prototype kits (Pharmacia CAP System FEIA, Pharmacia & Upjohn Diagnostics) as previously described (19). In serum samples only ECP was measured.

The air-dried slides were stained using eosin and methylene blue. Cell proportions were assessed semi-quantitatively, on a scale from 0 to 4, modified from previously described scales (20). Eosinophilia was graded as 0 (no or occasional eosinophils), 1 (scanty), 2 (moderate), 3 (numerous eosinophils), or 4 (predominance of eosinophils). Approximately, grade 0 corresponded to eosinophils accounting for fewer than 1% of all non-squamous cells, grade 1 to eosinophils accounting for one to 5% of all non-squamous cells, grade 2 to eosinophils accounting for 5–10% of all non-squamous cells, grade 3 to eosinophils accounting for 10–50% of all non-squamous cells and grade 4 to eosinophils accounting for more than 50% of all non-squamous cells. A sample was considered to be adequate, and to originate from the lower airways, if it contained macrophages and fewer than 50% of squamous epithelial cells. All analyses were conducted unaware of the clinical characteristics of the subject.

Data analysis

Data are expressed as means or medians and ranges. Some values for inflammatory markers lay beneath the lowest standard values. Significances of differences between two groups were determined using the Mann–Whitney *U*-test or the χ^2-test, as appropriate. Significances of correlations were determined using Spearman's rank correlation test. A *P*-value below 0·05 in a two-tailed test was considered to indicate significance. The control group was used to establish upper limits of the normal ranges (90th percentiles) for sputum ECP, EPO, MPO and HNL values.

RESULTS

Clinical characteristics

The clinical characteristics of subjects are shown in Table 1. PEF values were normal in all healthy controls. In the patients suffering from prolonged cough, PEF values varied from 47 to 136% of the predicted value (21). Two-week PEF follow-up values were obtained for 41 of the 82 cough patients (50%) and spirometry results for 31 patients (38%). On the basis of PEF values varying by more than 20% between morning and evening over 3 days of the 2-week follow-up period, or a reversibility of more than 15% in FEV_1, 13 of the 82 cough patients (16%) were diagnosed as suffering from asthma (22). None of the 23 cough patients who smoked met the criteria for chronic bronchitis or COPD (22).

Mean duration of cough was 11 months (range 1–96 months); it was 15 months for asthmatics, 10 months for the other patients. Forty cough patients (49%) reported marked sputum production, 38 (46%) wheezing or coughing on exercise, 30 (37%) disturbed sleep, 28 (34%) shortness of breath, and 23 (28%) tightness of the chest with wheezing. Asthmatics experienced sputum production (P=0·03, χ^2-test) and tightness of the chest with wheezing (P=0·04, χ^2-test) more often than patients with prolonged cough not meeting the criteria for asthma.

Sputum induction

Sputum induction was found to be safe. The mean change in PEF during sputum induction was −1·4% (range −13–32%) from baseline (Table 1). No subject required treatment with a bronchodilator following sputum induction. An adequate sputum sample was obtained from 73 patients (89%) and 49 healthy controls (92%) (Table 1).

Eosinophils and inflammatory markers

Results of serum and induced sputum measurements are shown in Table 2. Serum ECP levels were significantly higher in patients with prolonged cough than in healthy controls (P=0·01). Scores for sputum eosinophils were higher in patients with prolonged cough than in controls (P=0·001). No healthy control had a score for sputum

Table 1 Characteristics of subjects

Characteristics	Prolonged cough (n=82)	Healthy controls (n=53)
Age years	46 (16–85)	42 (25–61)
Male/female	25/57	10/43
Atopic*	12 (15%)	9 (17%)
Smokers	23 (28%)	3 (6%)
FEV_1 (% of predicted)†	82 (41–111)	Not determined
PEF (% of predicted)	91 (47–136)	103 (87–120)
PEF-variability (%)‡	13 (0–30)	Not determined
Duration of cough (month)	11 (1–96)	
Change in PEF during induction (%)	−1·1 (−13–32)	−3·0 (−17–19)
Adequate sputum sample§	73 (89%)	49 (92%)

Data are expressed as means and ranges or numbers and percentages of patients.
*Defined as at least one positive result in allergy skin-prick testing (see methods).
†n=30 (21).
‡Peak expiratory flow variability between morning and evening, n=41 (three values showing greatest variability between morning and evening averaged over 2-week follow-up period).
§Defined as a sample containing macrophages and fewer than 50% of squamous epithelial cells.

eosinophils exceeding 1 (5% of eosinophils). A sputum sample was defined as eosinophil-positive if the sputum eosinophil score was 2, 3 or 4. Using this criterion, 14 patients with prolonged cough (19%) had eosinophil-positive sputum samples. Five of these 14 patients (36%) were diagnosed as having asthma, while nine of the 14 patients (64%) were not. On the other hand, eosinophils were found in the sputum of five patients (45%) who were diagnosed as having asthma, and in nine patients with prolonged cough without asthma (15%).

EPO and ECP levels in sputum samples were higher in patients with prolonged cough than in healthy controls (Table 2). Values for the markers of neutrophils in sputum, MPO and HNL, did not differ significantly between patients with prolonged cough and controls. There were no significant differences in inflammatory markers between smokers and non-smokers, or between atopic and non-atopic subjects in any group. When smokers were excluded from analysis differences were similar.

In the healthy controls, the upper reference limit for ECP in serum was 16.5 $\mu g\,l^{-1}$, for ECP in sputum 1987 $\mu g\,l^{-1}$, for EPO in sputum 3899 $\mu g\,l^{-1}$, for MPO in sputum 1012 $\mu g\,l^{-1}$, and for HNL in sputum 14286 $\mu g\,l^{-1}$. Percentages of subjects with values above reference levels are shown in Fig. 1.

DISCUSSION

The importance of measuring airway inflammation in chronic respiratory conditions such as asthma, COPD and prolonged cough has recently been emphasized (23). Airway inflammation can be readily studied using induced sputum samples. Results of such studies have been shown to be reproducible, and similar to those obtained through employment of more invasive bronchoscopic methods (13, 14, 24). However, the studies have been conducted only in hospitals. Sample processing has been laborious. We modified the study method by simplifying sample processing, and showed that results were similar to those obtained using the previously validated reference method (16). In the study reported here, sputum induction took place in 'real-life situations', in office practices where patients are first seen.

The success rate for adequate sputum sample was high, 91%. Sputum induction was supervised by a trained nurse. PEF-values were monitored before and after the procedure. Induction was found to be safe. Since sputum samples for determination of levels of inflammatory markers can be frozen after collection (16), samples can be taken in health centres and studied later, in central laboratories.

We found that 19% of the patients with prolonged cough exhibited sputum eosinophilia (eosinophils accounting for more than 5% of all cells in smears). Approximately two-thirds of those exhibiting sputum eosinophilia were not diagnosed as suffering from asthma. In studies in tertiary consultation clinics, non-asthmatic eosinophilic inflammation in patients with prolonged cough has been found in lower percentages of patients (4, 5). However, these studies included many patients diagnosed as suffering from post-nasal drip or gastro–oesophageal reflux.

Table 2 Induced sputum and serum measurements

	Prolonged cough (n=82)	Healthy persons (n=53)
Sputum sample		
Weight (mg)	290 (10-2980)	110 (3-913)
Eosinophils*		
0-1	59 (81%)	49 (100%)
2	7 (10%)	0 (0%)
3	4 (5%)	0 (0%)
4	3 (4%)	0 (0%)
ECP ($\mu g\,l^{-1}$)†	1015 (44 - 65062)	498 (68 - 2706)
EPO ($\mu g\,l^{-1}$)‡	297 (55 - 33408)	63 (25 - 6830)
MPO ($\mu g\,l^{-1}$)	93 (80 - 34300)	138 (80 - 1524)
HNL ($mg\,l^{-1}$)	7·3 (0.5 - 98)	5·4 (1·1 - 19)
Serum ECP ($\mu g\,l^{-1}$)§	10·5 (2 - 71)	6·7 (2 - 17)

Data are expressed as medians and ranges or numbers and percentages (in relation to eosinophils). Sputum samples were obtained from 73 patients with prolonged cough and 49 healthy persons. Studied semi-quantitatively, on a scale from 0 to 4, P=0·001 (χ^2-test).
†P=0·02.
‡P=0·005.
§P=0·01 Mann–Whitney U-test.

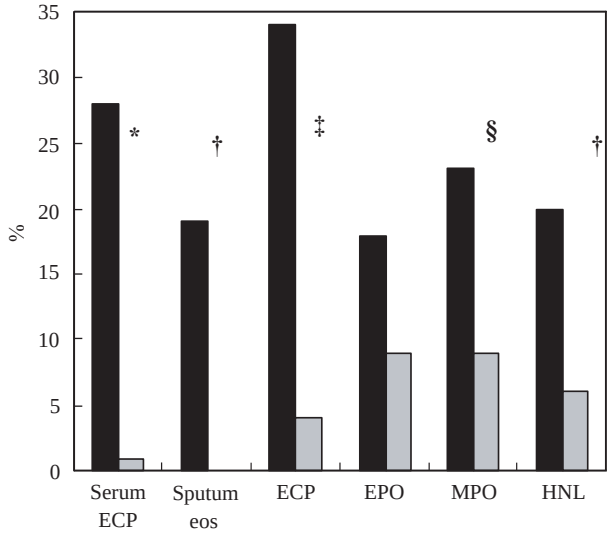

Fig. 1. Percentages of patients and healthy persons with values above reference values in serum or induced sputum analysis. Prolonged cough and healthy persons. *P=0·01, †P=0·001, ‡P<0·0001, §P=0·04 (χ^2-test).

It has been estimated that more than 25% of any population with prolonged cough seeking help from general practitioners will be suffering from asthma (25, 26). In the study reported here, on the basis of results of clinical examinations and lung function tests, 13 of the 82 patients with persistent cough (16%) were diagnosed for the first time as suffering from asthma. However, since PEF follow-up or spirometry were not done in 15 subjects, the possibility cannot be excluded that some of these subjects might have met the criteria for asthma and that the incidence of asthma may therefore have been slightly underestimated. Taken into account the prevalence of asthma in the study population that had lung function testing, we could have missed two to three subjects with asthma. Lung function was however tested in all patients with eosinophilic airway inflammation. As the study was carried out in patients attending for primary care, airway responsiveness could not be measured.

We aimed to exclude common causes of prolonged cough, such as chronic rhinitis, sinusitis and post-nasal drip, gastro–oesophageal reflux and use of ACE inhibitors. Other possible causes of prolonged cough, such as lung parenchymal disease, were excluded on the basis of chest X-ray findings and by careful interview of each patient. Airway eosinophilia can occur in otherwise healthy individuals during and after viral infection (27). We tried to exclude the effects of viral infection by including only patients who had exhibited no signs of respiratory infection during the preceding 6 weeks.

General practitioners treat many patients with prolonged cough, sputum production and occasional wheezing but normal or close-to-normal lung function. The inflammatory process that could be associated with the persistent respiratory symptoms is usually not characterised. Clinical judgements are formed on the basis of indirect information relating to bronchial status, such as results of lung-function or allergy tests. Diagnosis of

early, mild asthma can be difficult, since results of lung-function tests can remain close to normal for long periods, or may be conflicting (28,29). In patients with chronic cough whose lung function is normal it is only by analysis of sputum samples that eosinophilic bronchitis can be diagnosed (4, 5).

The natural courses of untreated or under-treated eosinophilic airway inflammation are unknown (8). Many of the patients concerned may never develop asthma but follow-up studies are needed (6). Eosinophilic inflammation can heal spontaneously, especially if it is a result of exposure to an allergen, which ceases. Therapy with repeated courses of antibiotics, cough mixtures, mucolytic agents or bronchodilators is of little use. Prolonged eosinophilic inflammation requires treatment with an anti-inflammatory agent (5, 12, 30). Effective treatment may have to involve inhaled steroids for 2–3 months (6). COPD patients who exhibit eosinophilia in sputum samples experience relatively rapid declines in lung function (31) and can also benefit from anti-inflammatory therapy (10).

We have shown that eosinophilic airway inflammation is fairly common in primary-care patients with prolonged cough, even in patients not suffering from asthma, COPD or any other known cause of cough. Induced sputum samples can be obtained in health centres and sent for analysis in a central laboratory. Detection of eosinophilic airway inflammation can aid treatment decisions in primary care.

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