Vol. **95,** (2001) 670–675 respiratoryMEDICINE

Eosinophil cationic protein (ECP) in saliva: a new marker of disease activity in bronchial asthma

B. SCHMEKEL*, J. AHLNER†, M. MALMSTRÖM‡, AND P. VENGE§

*Department of Clinical Physiology, Institute of Medicine and Care, University Hospital, Linköping; †National Board of Forensic Medicine, Linköping; ‡R&D Department, Primary Care Unit, Mjölby and §Department of Clinical Chemistry, University Hospital, Uppsala, Sweden

Abstract Eosinophil cells play a crucial role in the pathogenesis of asthma, and concentration of eosinophil cationic protein (ECP) in serum has been used to monitor activity of the disease. Our aim was to determine the feasibility and usefulness of measuring ECP in saliva and to use it as a marker of the disease. Thirty-eight patients with asthma and 16 healthy volunteers were included in this study. Repeatability of measurements of ECP in saliva was acceptable [intra-class correlation coefficients (Ri) = 0·74 and coefficients of repeatability (CR) = 0·37 in five healthy subjects]. Levels of ECP in saliva were higher in asthmatics than in volunteers ($P < 0·01$). There was a significant inverse association between a surrogate variable reflecting disease activity (i.e. change over a few weeks in dose of inhaled corticosteroid required by a change in clinical status of asthma) and a change over the same time period in salivary ECP in 19 patients with stable asthma ($r = -0·64$, $P = 0·02$). Our findings indicate that levels of salivary ECP are elevated in patients with asthma and associated with presumed activity of disease as recorded by alteration of taken dose of inhaled corticosteroid.

doi:10.1053/rmed.2001.1123, available online at http://www.idealibrary.com on IDEAL®

Keywords eosinophil cationic protein (ECP); saliva; asthma; bronchial hyper-responsiveness to isocapnic hyperventilation of dry cold air; forced oscillation technique.

INTRODUCTION

Asthma is characterized by variable bronchial constriction and excessive narrowing of the airways in response to antigenic as well as non-antigenic stimuli. Bronchial washing, bronchoalveolar lavage or induced sputum after inhalation of nebulized hypertonic saline is carried out to study inflammatory mediators and cells in the airways. Using induced sputum is more feasible and less invasive than the lavage method, but acute airway constriction may still be evoked by inhaled hypertonic solution, requiring administration of bronchodilators. Furthermore, some healthy persons may fail to produce any sputum at all despite several attemps (1). The method of induced sputum is relatively simple but sputum may be diluted by saliva in the recovered specimens. It has previously been proposed that saliva does not confound results of the analyses, but instead dilutes the fluid phase of sputum (2). Considering the ease at which all subjects, including children, may collect saliva, it would be an advantage if soluble products in saliva, instead of sputum or bronchoalveolar lavage, could be used as markers of asthma in clinical studies.

An important role for eosinophils in the pathogenesis of bronchial asthma has been suggested (3). Eosinophils are the source of pro-inflammatory as well as cytotoxic mediators, among them major basic protein (MBP) and eosinophil cationic protein (ECP). We focused on ECP in saliva and hypothesized that ECP in spontaneously produced saliva would be a useful marker of disease activity in asthma. The aim of the present study was therefore, to evaluate if ECP levels in saliva were higher in asthmatics than in healthy volunteers. We also wished to know if concentrations of ECP in saliva would be related to degree of airway obstruction or change in asthma status presumed to occur after a change of dose of inhaled glucocorticosteroids (ICS). Furthermore, we searched for an association between the concentrations of ECP in saliva and bronchial responsiveness to voluntary isocapnic hyperventilation of cold air (IHCA), which is often used as a model to study bronchial hyper-responsiveness (BHR) (4), a key event in asthma.

Received 10 January 2001 and accepted in revised form 3 May 2001.
Correspondence should be addressed to: Birgitta Schmekel, Department of Clinical Physiology, Institute of Medicine and Care, University Hospital, S-58185 Linköping, Sweden. Fax: +46-013 14 59 49.

MATERIAL AND METHODS

Patients

Patients were included if they had symptoms of asthma as defined by a history of variable airway constriction or wheezing and either bronchial obstruction or proven responsiveness to isocapnic hyperventilation of dry cold air. Subjects with concomitant diseases, exacerbation of asthma or airway infection three weeks prior to inclusion into the study were excluded from participation. Subjects suffering from apparent dental diseases were also not included in this study.

Thirty-eight consecutive patients met the criteria when selected from a pool of 59 patients admitted for breathlessness or asthma-like symptoms. Twenty-five were females and 13 were males, and their mean age was 41 years (range 20–58 years). All but seven patients inhaled a glucocorticosteroid (Budesonid, Astra) in daily doses ranging from 200 μg to 1200 μg (median dose 400 μg), and none used non-steroidal anti-inflammatory drugs. All but 13 patients used short-acting β-agonists on an 'as required basis', and six regularly used a long-acting β-agonist. All but 12 of the patients were allergic to common aeroallergens. Seven of the patients were tobacco smokers and seven had stopped smoking 2–29 years prior to the study.

Sixteen non-allergic and subjectively healthy volunteers (12 females and four males) served as reference persons. Their mean age was 38 years (range 18–54 years). All healthy subjects were non-smokers.

The local Ethics Research Committee approved of the study and informed consent was obtained from all subjects before participation.

Study design

All subjects attended the laboratory for sampling of saliva (after rinsing the mouth with water) and for lung function tests before and after a 4-min hyperventilation challenge with dry cold air. Twenty of the asthmatic patients came to the laboratory a second time for tests. There were no differences in demographic data or lung function tests between patients attending once or twice (data not shown).

Subjectively, perceived symptoms of breathlessness/dyspnoea were scored on a 100-graded visual analogue scale (VAS), and documented before and after the hyperventilation challenge.

Measurement of lung function

Lung function was measured in duplicates at baseline using an MS-IOS Digital instrument (Erich Jaeger AG, Würzburg, Germany) as previously described (5) and performed according to previously published principles (6,7). Resonant frequency (Fres) was recorded at baseline and 4 min after completion of the challenge. Responsiveness to IHCA was defined by an increase of at least 2·4 Hz in resonant frequency (Fres) as recorded by an impulse oscillometer technique (IOS) (5). After the measurements at baseline by means of the IOS technique, forced expiratory volume in 1 s (FEV_1) was measured and expressed as absolute values (l) or as a percentage of the predicted normal value (8,9).

Test technique

Spirometry was measured at baseline, and FEV_1 formed the basis for calculation of the target minute ventilation (V_E) during the test. The provocation was carried out as previously described in detail (5), and target V_E was 70% of the predicted maximum breathing capacity (10). The subjects breathed through a mouthpiece connected to a modified Respiratory Heat Exchange System (RHES, Jaeger AG, Würzburg, Germany) and a Lauda UKT 800 refrigerator (Jaeger AG). The equipment allowed the subjects to breathe dry cold air with an addition of 5% CO_2. The temperature of inhaled air remained at -15°C during the test.

Laboratory methods

After thorough rinsing the oral cavity with tap water, approximately 1 ml of serous saliva was sampled by spontaneous salivation over a few minutes. The admixture of high viscous mucous was avoided, and saliva was collected in a plastic container before any lung function tests. The saliva was immediately transferred to graded tubes and put on ice. The specimens were then diluted 1:1 with 4 M acetic acid, and the degree of dilution was confirmed by weighing the tubes before and after addition of acid. The samples were centrifuged for 10 min at room temperature and the supernatants were kept frozen (-20°C) until analysis. The specimens were diluted 1 : 5 and ECP was analysed using a radioimmuno-assay (Pharmacia ECP RIA, Pharmacia Diagnostics, Uppsala, Sweden) and as previously described (11). The intra- and inter-individual coefficients of variation were less than 8%. The values of ECP in saliva were corrected according to the degree of dilution with acetic acid. Repeatability of ECP concentrations in saliva, as expressed by intra-class correlation coefficient (Ri = variance between subject/variance between subject+variance within subjects) was calculated in five healthy subjects who had stable lung function. Ri was 0·74 and coefficient of repeatability (CR = 2 SD of the mean difference of repeated measurements) was 0·37.

Statistical methods

Mean and standard deviation (SD) or 95% confidence interval (95% CI) was used to describe normally distributed data and median value (lower to upper quartile) was

used to describe non-normally distributed data. Data on ECP concentrations in saliva were not normally distributed and they were logarithmically transformed before statistical evaluations were done. A *t*-test for dependent or independent samples or the Mann–Whitney *U*-test was used for calculating differences and Pearsons correlation coefficient (*r*) or Spearmans correlation coefficient (Rs) was calculated on original or logarithmically transformed data. A *P*-value of less than 0·05 was set to indicate statistical significance.

RESULTS

Asthmatics *vs*. volunteers

Distribution of age, height, weight or sex were similar in the groups of subjects with asthma and healthy volunteers ($P > 0{\cdot}05$, all comparisons). The value of ECP in saliva was significantly higher in specimens obtained from the asthmatics than from the healthy volunteers [486–845 $\mu g\,l^{-1}$ *vs*. 246–467 $\mu g\,l^{-1}$ (95% CI of log-transformed values), $P < 0{\cdot}01$, Fig. 1]. Lung function tests differed significantly between asthmatics and volunteers, suggesting increased airflow obstruction among the asthmatics (Table 1, $P < 0{\cdot}05$–$0{\cdot}01$). Also, subjectively-perceived dyspnoea was rated higher among the asthmatics than among the volunteer persons [VAS: 16(5–30) *vs.* 1(0–3), median value (lower – upper quartile), $P < 0{\cdot}01$ (Mann–Whitney *U*-test)].

ECP and airways obstruction

Levels of ECP in saliva obtained before the lung function tests were inversely related to baseline values of FEV_1 (but not values of F_{res} recorded by impulse oscillometry) among the asthmatics ($r = -0{\cdot}36$, $P = 0{\cdot}03$). There were no associations between concentrations of ECP in saliva and subjective scoring of dyspnoea or doses of pre-test treatment with ICS in the asthma group ($P > 0{\cdot}05$, both comparisons).

Repeated measurements of ECP and lung function

Twenty patients arrived twice at the laboratory and the visits were separated by time periods ranging from 6 to 20 weeks. Seventeen of the patients had improved their lung function in the meantime (range of decreases in $F_{res} = -12{\cdot}7$ Hz to $-0{\cdot}3$ Hz) while three of the patients had decreased lung function (range of increases of $F_{res} = 4{\cdot}5$–$18{\cdot}3$ Hz). Ten of the patients kept their doses of ICS unchanged and 10 had individually titrated changes of their doses between the two visits (five increases and five decreases), due to changes in clinical status. There was an inverse relationship between change of dose of

TABLE 1. Lung function tests recorded in 16 healthy volunteers and 38 patients with asthma, involving FEV_1 expressed as percentage of predicted normal values (percentage predicted) and absolute values (l). Values of resonant frequency (F_{res}) recorded by impulse oscillometry (see text for explanation) at baseline and 4 min after completion of voluntary isocapnic hyperventilation with cold air (IHCA) performed in 35 eligible asthma patients and 16 volunteers are given.

	Volunteers (*n*=16)	Asthma Patients (*n*=38)
Lung function at baseline		
Forced expiration:		
FEV_1 (% pred)	111 ± 17	92 ± 18***
FEV_1 (l)	3·6 ± 1·0	3·0 ± 0·9*
Impulse oscillometry:		
F_{res} (Hz)	10·2 ± 3·7	15·5 ± 6·7**
Lung function 4 min after IHCA		
	Volunteers (*n*=16)	Asthma Patients (*n*=35)
Impulse oscillometry:		
F_{res} (Hz)	11·2 ± 3·8	21·7 ± 7·8***

Significant differences between asthma patients and volunteers are indicated by *=$P < 0{\cdot}05$; **=$P < 0{\cdot}01$; ***=$P < 0{\cdot}001$. Mean (SD) is given.

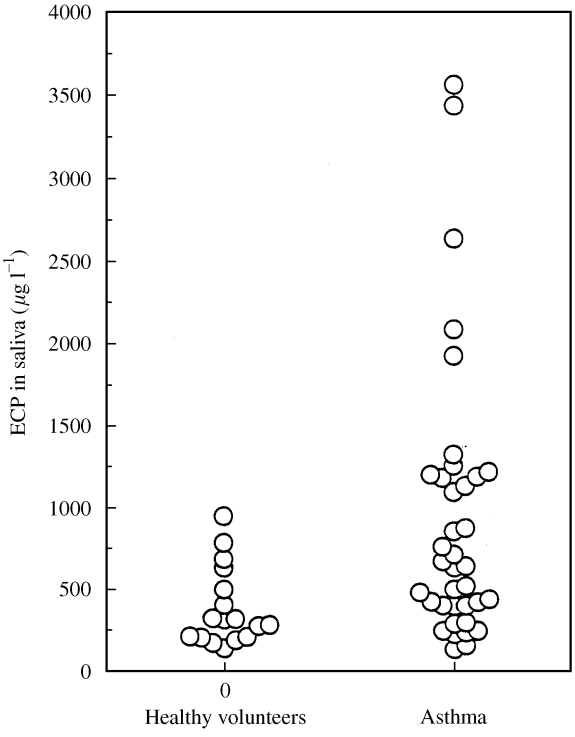

FIG 1. Volume-corrected concentrations of eosinophil cationic protein (ECP) measured in saliva obtained from 16 healthy volunteers and 38 patients with asthma.

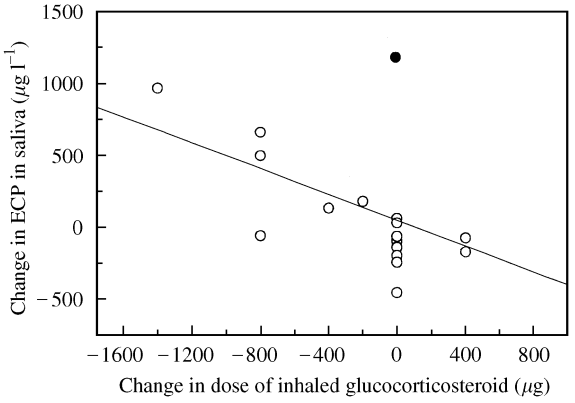

Fig 2. Change in salivary concentrations of eosinophil cationic protein (ECP) *vs.* change in dose of inhaled glucocorticosteroids in 20 patients with asthma, as recorded at two separate visits (Rs = −0·52, *P* = 0·07). Three sets of data are superimposed in the figure, and the filled circle (●) indicates data obtained from one patient who had an exacerbation of asthma during the second visit; when excluding this individual from the statistical evaluation, data were significantly related to each other (Rs = −0·65, *P* = 0·003)

ICS and change of ECP levels in saliva in these 20 individuals ($RS = -0{\cdot}52$, $P = 0{\cdot}07$). However, when excluding data obtained from one patient who had an exacerbation of asthma on his second visit, as judged from the recorded large increase in F_{res} (i.e. increase of 18·3 Hz), a more homogenous group was created (Fig. 2). In this group of patients with stable asthma, a significant association was recorded between change in ICS dose and change in ECP levels ($r = -0{\cdot}65$, $P = 0{\cdot}003$, $n = 19$).

ECP and bronchial hyper responsiveness

Thirty-five of the patients underwent IHCA; the remaining three asthmatics had too low values of FEV_1 at baseline (less than 60% of predicted normal value) to allow a safe bronchial challenge. Significantly higher bronchial responses to challenge, as recorded by increases of F_{res} after challenge, were documented in asthmatics than in healthy volunteers ($P < 0{\cdot}001$, Table 1). Furthermore, the increase of subjective scoring of dyspnoea was higher in the asthmatics than in the volunteers ($P < 0.001$ Mann–Whitney *U*-test, data not shown). There were no associations between levels of ECP in saliva obtained before the challenge and the outcome of the IHCA tests.

DISCUSSION

This study showed higher level of ECP in saliva obtained from patients with asthma than healthy volunteers. Eosinophils, the source of ECP, are often found in the lower airway mucosa of asthmatic patients (12–14), and in nasal mucosa of patients with allergic rhinitis (15). The fact that circulating eosinophils may be found at high levels in peripheral blood drawn from patients with asthma suggests a generalized character of asthmatic inflammation. This is also suggested by findings of increases of inflammatory progenitors in bone marrow in asthma patients (16). Our present data of increased levels of ECP levels in saliva may reflect a pathophysiological consequence of eosinophils present in the whole extent of the airways, also including the oral mucosa. Supporting this notion, infiltration of cells typical for the asthmatic inflammation has been demonstrated in minor salivary glands biopsied from patients with asthma (17). Furthermore, the oral mucosa per se may have become leaky by noxious activities of eosinophils, allowing for circulating ECP to be more rapidly transferred from vascular spaces to saliva. We did not biopsy oral mucosa in our patients, nor did we measure concentrations of ECP in serum. Levels of ECP in serum obtained from 23 patients with unstable severe asthma ($< 100\ \mu g\ l^{-1}$, geometric mean +SD) or 98 healthy volunteers ($< 20\ \mu g\ l^{-1}$) (18) were several times lower than the majority of values of ECP recorded in saliva of our present patients. On the other hand, levels of ECP in saliva, which are similar to our own, were recorded in a study on sputum obtained from asthmatics (19). The reason for such a discrepancy of levels in blood and airway secretion is unclear and may involve a number of contributing, so far unknown, factors. Immuno-cytochemical staining of EG2, an indicator of activated eosinophils, in oral mucosa and salivary glands in an adequate number of asthmatics seems highly warranted to enable firm conclusions on the location of activated eosinophils in various organs of asthmatics.

A relationship between ECP levels in sputum or bronchoalveolar lavage fluid and clinical expression of asthma has previously been shown by several authors (20, 21). We found merely weak or no significant association between levels of ECP in saliva and FEV_1 or bronchial hyper-responsiveness to cold air hyperpnoea. Neither did we find any association between ECP and given dose of ICS in the group of asthmatics, data which are in agreement with previous findings (22). High inter-individual variability in intensity of the asthmatic inflammation at the time of sampling of saliva may have interfered with data recorded in our group of patients. ICS is known to reduce the intensity of inflammation in asthma and to decrease levels of ECP in blood (23). A change in dose of ICS required by a change in clinical status of asthma was judged to be an acceptable surrogate variable, reflecting disease activity in the present study. Our finding of a significant association between this surrogate variable and the response in salivary ECP concentrations suggest that ECP concentrations in saliva obtained from asthmatics mirror a relevant clinical event. A relevant endpoint in studies of disease activity of asthma may be symptom score or number of exacerbations during a certain peri-

od before or after the test situation. We did not measure any of those endpoints, instead we recorded scoring of subjectively perceived breathlessness, and lung function measured before and after a bronchial provocation test. Despite low or moderate activity of asthma, judged from baseline lung function and subjective scoring of breathlessness, we confirmed peripheral airway constriction elicited by IHCA in our asthmatics as previously observed (24). Our asthma patients also perceived more symptoms of breathlessness after the IHCA tests than the volunteers. We conclude that BHR was evident in our patients, and that there was no association between the degree of responsiveness to cold air and the salivary levels of ECP. Disagreement between levels of ECP or eosinophils and BHR have previously been shown in blood, bronchoalveolar lavage and sputum (25–27). Our present data tend to agree with the discrepancy between the expression of bronchial hyper-responsiveness and levels of eosinophil cell products. It is however, not likely that there should be a close relationship between the ease at which bronchial constriction is elicited by cold air hyperventilation and the level of ECP. Although several hypotheses have been suggested for mechanism underlying airway responses to IHCA, none of the hypotheses have included eosinophil involvement (28,29).

Taking into account the values of Ri, repeatability of ECP salivary tests seems to be adequate and the data to be reliable (30). We recorded levels of ECP in saliva at similar levels as previously found in saliva obtained from asthmatics (2) as well as in sputum (22,31), but higher than those found by others (32). In contrast to those data, our data on ECP reflect a total view of the state of activity, due to lysis of cells after addition of acetic acid to the test samples of saliva. Our data on ECP in saliva thus represents the presence of cells, as well as their intra- and extra- cellular mediator content. The kinetical retrieval of ECP in bronchoalveolar lavage fluid is dependent on a mechanism that does not appear to be identical to that of albumin or urea (33,34). These latter compounds have previously been utilized as denominators for dilution of bronchoalveolar lavage fluid. Dissimilarities of transfer rates across the mucosal membranes, however, may invalidate the use of albumin, urea or possibly also other compounds, as reliable denominators. Similar problems with dilution arise when determining the quantity of proteins in saliva. We, therefore, refrained from correcting the levels of ECP in saliva and expressed the levels per volume unit.

In conclusion, we found elevated levels of ECP in saliva, suggesting primed eosinophils to be located in the oral cavity and/or salivary glands of patients with asthma. Alternatively elevated levels of ECP in saliva may reflect increased permeability of mucous membranes and/or increased circulating levels of ECP. Based on our results, we suggest that analyses of ECP in saliva may be adequate to enable clinically relevant assumptions on the status of the airways in patients with asthma.

Acknowledgements

The Swedish Medical Research Council B96-04X-11553-01A, The Heart and Lung Foundation, The Asthma and Allergy Foundation, the Vårdal Foundation and Östergötland County Council supported this study.

REFERENCES

1. Pin I, Gibson PG, Kolendowicz R, *et al.* Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax* 1992; **47:** 25–29.
2. Fahy JV, Liu J, Wong H, Boushey HA. Cellular and biochemical analysis of induced sputum from asthmatic and from healthy subjects. *Am Rev Respir Dis* 1993; **147:** 1126–1131.
3. Thomas LH, Warner JA. The eosinophil and its role in asthma. *Gen.-Pharm.* 1996; **27:** 593–597.
4. Eliasson AH, Phillips YY, Rajagopal KR, Howard RS. Sensitivity and specificity of bronchial provocation testing. An evaluation of four techniques in exercise-induced bronchospasm. *Chest* 1992; **102:** 347–355.
5. Schmekel B, Smith HJ. The diagnostic capacity of forced oscillation- and forced expiration techniques: isocapnic hyperventilation of cold air. *Eur Respir J* 1997; **10:** 2243–2249.
6. Vogel J, U Smidt. *Impulse Oscillometry: Analysis of Lung Mechanics in General Practice and the Clinical, Epidemiological and Experimental research.* Frankfurt am Main, Germany: PMI Verlagsgruppe GmbH, 1994.
7. Bisgaard H, Klug B. Lung function measurement in awake young children. *Eur Respir J* 1995; **8:** 2067–2075.
8. Fridriksson HV, Malmberg P, Hedenström H, Hillerdal G. Reference values for respiratory function tests in males: prediction formulas with tobacco smoking parameters. *Clin Physiol* 1981; **1:** 349–364.
9. Hedenström H, Malmberg P, Agarwal K. Reference values for lung function tests in females. Regression equations with smoking variables. *Bull Eur Physiopathol Respir* 1985; **21:** 551–557.
10. Gandevia B, Hugh Jones P. Terminology for measurements of ventilatory capacity. *Thorax* 1957; **12:** 290–293.
11. Peterson CGB, Enander I, Nystrand J, Anderson AS, Nilsson L, Venge P. Radioimmunoassay of human eosinophil cationic protein (ECP) by an improved method. Establishment of normal levels in serum and turnover in vivo. *Clin Exp Allergy* 1990; **21:** 561–567.
12. Bradley BL, Azzawi M, Jacobson M, *et al*. Eosinophils, T-lymphocytes, mastcells, neutrophils, and macrophages in bronchial biopsy specimens from asthmatic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. *J Allergy Clin Immunol* 1991; **88:** 661–674.
13. Moqbel R, Barkans J, Bradley BL, Durham SR, Kay AB. Application of monoclonal antibodies against major basic protein (BMK-13) and eosinophil cationic protein (EGI and EG2) for quantifying eosinophils in bronchial biopsies from atopic asthma. *Clin Exp Allergy* 1992; **22:** 265–273.
14. Azzawi M, Bradley B, Jeffrey PK, Kay B. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am Rev Respir Dis* 1990; **142:** 1407–1413
15. Erjefält JS, Greiff L, Andersson M, *et al*. Allergen-induced eosinophil cytolysis is a primary mechanism for granule protein release in human upper airways. *Am J Respir Crit Care Med* 1999; **160:** 304–312.

16. Wood JJ, Inman MD, Watson RM, Foley R, Denburg JA, O'Byrne PM. Changes in bone marrow inflammatory cell progenitors after inhaled allergen in asthmatic subjects. *Am J Respir Crit Care Med* 1998; **157:** 99–105.
17. Tonnel AB, Janin A, Copin MC, Gosset P, Gosselin B, Wallaert B. Airway-like inflammation of minor salivary glands in bronchial asthma. *Am J Respir Crit Care Med* 1994; **150:** 802–809.
18. Griffin E, Håkansson L, Formgren, J, Peterson C, Venge P. Blood eosinophil number and activity in relation to lung function in patients with asthma and with eosinophilia. *J Allergy Clin Immunol* 1991; **87:** 548–557.
19. Bacci E, Cianchetti S, Ruocco L, *et al*. Comparison between eosinophilic markers in induced sputum and blood in asthmatic patients. *Clin Exp Allergy* 1998; **28:** 1237–1243.
20. Ronchi MC, Piragino C, Rosi E, Amendola M, Duranti R, Scano G. Role of sputum differential cell count in detecting airway inflammation in patients with chronic bronchial asthma or COPD. *Thorax* 1996;**51:** 1000–1004.
21. Ädelroth E, Rosenhall L, Johansson S, Linden M, Venge P. Inflammatory cells and eosinophilic activity in asthmatics investigated by bronchoalveolar lavage. The effects of antiasthmatic treatment with budesonide or terbutaline. *Am Rev Respir Dis* 1990;**142:** 21–99.
22. Fujimoto K, Kubo K, Matsuzawa Y, Sekiguchi M. Eosinophil cationic protein levels in induced sputum correlate with the severity of bronchial asthma. *Chest* 1997; **112:** 1241–1247.
23. Wempe JB, Postma DS, Breederveld N, Alting-Hebing D, Van Der Mark TW, Koeter GH. Separate and combined effects of corticosteroids and bronchodilators on airflow obstruction and airway hyperresponsiveness in asthma. *J Allergy Clin Immunol* 1992; **89:** 679–687.
24. Wesseling GJ, Vanderhoven-Augustin IML, Wouters EFM. Forced oscillation technique and spirometry in cold air provocation tests. *Thorax* 1993; **48:** 254–259.
25. Ferdousi HA, Dreborg S. Asthma, bronchial hyperreactivity and mediator release in children with birch pollinosis. ECP and EPX levels are not related to bronchial hyperreactivity. *Clin Exp Allergy* 1997; **27:** 530–539.
26. Hoshino M, Nakamura Y. Relationship between activated eosinophils of the bronchial mucosa and serum eosinophil cationic protein in atopic asthma. *Int Arch Allergy Immunol* 1997; **112:** 59–64.
27. Crimi E, Spanevello A, Neri M, Ind PW, Rossi GA, Brusasco V. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. *Am J Respir Crit Care Med* 1998; **157:** 4–9.
28. Desjardin A, De Luca S, Cartier A, L'Archeveque J, Ghezzo H, Malo J-L. Nonspecific bronchial hyperresponsiveness to inhaled histamine and hyperventilation of cold dry air in subjects with respiratory symptoms of uncertain etiology. *Am Rev Respir Dis* 1988; **137:** 1020–1025.
29. Wilson NM, Barnes PJ, Vickers H, Silverman M. Hyperventilation-induced asthma: evidence for two mechanisms. *Thorax* 1982; **37:** 657–662.
30. Chinn S. Repeatability and method comparison. *Thorax* 1991; **46:** 454–456.
31. Keatings VM, Barnes PJ. Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. *Am J Respir Crit Care Med* 1997; 155: 449–453.
32. Fahy JV, Wong H, Liu J, Boushey HA. Comparison of samples collected by sputum induction and bronchoscopy from asthmatic and healthy subjects. *Am J Respir Crit Care Med* 1995; **152:** 53–58.
33. Schmekel B, Hörnblad Y, Hvatum M, Norlund A-L, Venge P. Kinetic retrieval of eosinophil cationic protein, hyaluronan, secretory IgA, albumin, and urea during BAL in healthy subjects. *Chest* 1995; **108:** 62–67.
34. Ward C, Fenwick J, Booth H, Walters EH. Albumin is not suitable as a marker of bronchoalveolar lavage dilution in interstitial lung disease. *Eur Resp J* 1997; **10:** 2029–2033.