Eosinophil Cationic Protein Levels in Induced Sputum Correlate With the Severity of Bronchial Asthma*

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 activated eosinophils play an important role in the pathogenesis of bronchial asthma. In this study, we analyzed the inflammatory leukocyte population and the concentrations of eosinophil cationic protein (ECP) and albumin in induced sputum from patients with mild to severe asthma (n=36), and assessed the findings in relation to the severity of their asthma. Both the eosinophil numbers and the concentrations of ECP in the induced sputum were significantly increased in the patients with asthma compared with those in healthy subjects (n=9). There were significant positive correlations between the ECP levels and both the eosinophil counts (r=0.45) and the albumin concentrations (r=0.53). When the asthmatics were classified as having mild (n=12), moderate (n=14), or severe (n=10) asthma as evaluated by their symptoms and peak expiratory flow rate (PEFR), the ECP levels showed significant increases in accordance with the severity of asthma. The eosinophil counts in the patients with severe asthma were significantly higher than those in the patients with mild and moderate asthma; there was no significant difference between those with mild and moderate asthma. The eosinophil counts and ECP levels were also significantly positively correlated with the mean weekly total symptom scores (r=0.52 and r=0.48, respectively) and negatively with the mean percent PEFR on waking (r=−0.50 and r=−0.65, respectively) recorded for 2 weeks prior to the sputum collection. These findings suggest that the eosinophil activation in the airway is closely linked to the symptoms and airflow obstruction of asthma, and that the ECP concentration in induced sputum could serve as useful marker for evaluating the severity of asthma and monitoring airway inflammation to achieve the optimal control of asthma.

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Key words: albumin; asthma symptom score; bronchial asthma; eosinophil; eosinophil cationic protein (ECP); induced sputum; peak expiratory flow rate (PEFR)

Abbreviations: BDP=beclomethasone dipropionate; ECP=eosinophil cationic protein; PEFR=peak expiratory flow rate

Airway inflammation characterized by eosinophil infiltration into the airway underlies the pathogenesis of bronchial asthma.1,2 Eosinophil infiltration into the airway is commonly observed in both extrinsic and intrinsic bronchial asthma and even in mild asthma.3 Eosinophils can produce and/or release various chemical mediators and cytotoxic proteins that cause bronchoconstriction, mucus hypersecretion, bronchial edema, and epithelial damage, and eosinophils have been suggested to contribute to the development of airway hyperreactivity.4 Allergen inhalation has produced an increase in eosinophils in airway secretion and bronchial biopsy specimens, and the increase in eosinophils correlated with the severity of the late asthmatic response and airway hyperreactivity in patients with asthma.5,6 In addition, the number of eosinophils in bronchial biopsy specimens5,7 and in BAL fluid,8-11 and eosinophil cationic protein (ECP) degranulated from activated eosinophils in BAL fluid8,9 and sputum12-14 have been shown to be increased and correlated with the severity of asthma, airway obstruction, and bronchial hyperreactivity. Therefore, eosinophil infiltration and activation in the airway, as well as symptoms or monitoring of peak expiratory flow rate (PEFR), are very important indexes not only for diagnosis, but also for the evaluation of the severity of asthma. However, the currently used invasive techniques for the assessment of airway inflammation, such as bronchial biopsy and BAL, are limited in their
applicability by the discomfort, inconvenience, and risk of exacerbation caused, especially in patients with severe asthma; these techniques also cannot easily be applied repeatedly. Sputum induction was recently proposed as a noninvasive method for evaluating airway secretions\(^5\),\(^13\)\(^-\)\(^18\) and provided a valid index of airway inflammation in agreement with those from BAL and biopsy specimens in bronchial asthma patients\(^{19,20}\). Sputum induction also yields samples more concentrated and richer in airway secretions than those obtained by bronchoscopy, and various chemical substances have been detected in the supernatant of sputum. It was reported that the number of eosinophils and eosinophil granule proteins in spontaneously expectorated sputum or induced sputum from patients with relatively stable or mild asthma was increased and correlated with the severity, airway obstruction, and airway hyperreactivity.\(^{12,14}\) However, there is little information available concerning sputum examinations of a wide range of subjects with mild to severe asthma, or the relationships between cellular or biochemical components in sputum and the severity of asthma evaluated by symptoms and PEFR. In this study, we analyzed the inflammatory leukocyte population and concentrations of ECP and albumin in induced sputum from patients with mild to severe asthma, and assessed these findings in relation to the severity of asthma. We also determined the correlation between the asthma symptom scores and PEFR, monitored daily on waking and before the evening meal.

**Materials and Methods**

**Subjects**

The 36 patients, with mild to severe bronchial asthma, were recruited from our outpatient clinic. Asthma was defined as a clinical history of intermittent wheeze, cough, chest tightness, or dyspnea, documented reversible airflow limitation either spontaneously or with treatment, and bronchial hyperresponsiveness to methacholine.\(^21\) Seventeen patients were men, and nineteen were women. The ages of the patients ranged from 17 to 74 years, with a mean of 45 years. The numbers of patients with atopic and nonatopic types of asthma (atopic type was defined as a positive reaction to one or more inhalant allergens in a radioallergosorbent or skin-prick test) were 26 and 10, respectively. Nine patients with asthma were current or former smokers. All asthmatic patients were taking an inhaled \(\beta_2\)-agonist when needed; 31 inhaled beclomethasone dipropionate (BDP) with a spacer (8 in a dose <500 \(\mu\)g and 13 in a dose 800 to 1,600 \(\mu\)g); 5 took oral prednisolone (5 to 20 mg/d); and 25 took oral theophylline and/or \(\beta_2\)-agonist. All patients were free from airflow obstructions for 1 month prior to sputum collection. The nine normal healthy volunteers included seven men and two women, ranging in age from 24 to 50 years, with a mean age of 33 years; all were nonsmokers, nonatopic, had no symptoms or history of eczema, rhinitis, or spontaneous wheeze, and were taking no medication. All subjects gave informed consent to the study, which had the approval of the hospital’s ethical committee.

**Study Design**

Prior to the sputum collection, the clinical symptoms and PEFR were measured on waking and before the evening meal, and before \(\beta_2\)-agonist inhalation, using a Wright mini-peak flowmeter (Clement Clarke; Harlow, UK). The symptoms and PEFR values were recorded on a diary card. The two sets of PEFR values (on waking and before the evening meal) were averaged weekly from the diary card records. The severity of each asthma symptom was classified according to the criteria of the Japanese Society of Allergology: wheeze or chest tightness without dyspnea, mild attack defined as “patient is experiencing dyspnea, but is able to walk and to assume a supine position,” moderate attack defined as “patient has difficulty walking and prefers a sitting position due to dyspnea,” and severe attack defined as “patient has orthopnea and difficulty in speaking and is unable to walk due to dyspnea.” The severity of asthma was determined based on the recorded clinical symptoms and PEFR.\(^2\) The “mild” asthmatic patients were those with the clinical features of intermittent wheeze or chest tightness without dyspnea \(\leq 4\) times a week, a brief mild attack \(\leq 2\) times a week, nocturnal asthma attacks with sleep disturbance \(< 2\) times a month, and weekly mean PEFR values both on waking and before the evening meal \(> 80\%\) of the predicted value. “Moderate” asthma patients were those with the clinical features of wheeze or chest tightness without dyspnea \(\geq 5\) times a week, an asthma attack \(\geq 3\) times a week, or weekly mean PEFR values either on waking or before the evening meal 60 to 80% of the predicted value. “Severe” asthma patients were those with the clinical features of moderate attack \(\geq 5\) times a week, a severe attack \(\geq 1\) to 2 times a week, nocturnal asthma attacks almost daily, or weekly mean PEFR values either on waking or before the evening meal \(< 60\%\) of the predicted value. All 36 patients were classified into one of three groups (mild, moderate, and severe) according to the above criteria, and the inflammatory cell population and biochemical substances in induced sputum were compared among these three groups. In addition, symptoms were scored as recommended by the Japanese Society of Allergology; symptoms were recorded four times a day by patients, and 1, 3, 6, and 9 points were assigned to each episode of wheeze or chest tightness without dyspnea, each mild attack, each moderate attack, and each severe attack. The weekly total symptom score was determined by summing up the points each week. The correlations between the airway inflammation and the mean weekly total symptom scores and between the airway inflammation and each mean percent PEFR for 2 weeks prior to the sputum collection were determined.

**Sputum Collection and Analysis**

Sputum was induced by having the subject inhale hypertonic saline solution as previously described.\(^13\),\(^17\) Briefly, prior to the induction of sputum, all subjects inhaled a \(\beta_2\)-agonist to avoid hypertonic saline solution-induced bronchoconstriction. The hypertonic saline solution was nebulized with an ultrasonic nebulizer (NE-V10B; Omron; Tokyo, Japan) at maximum output for 5-min periods, up to 20 min. The concentration of saline solution was increased at 10-min intervals, from 3.5 to 4.5%. If the FEV\(_1\) fell by \(> 10\%\) from the postbronchodilator value, the concentration of saline solution was not increased. If the FEV\(_1\) fell by \(> 20\%\) or if troublesome symptoms occurred, the nebulization was discontinued. Every 5 min, the subjects were asked to rinse their mouths and throats, and then to try to cough sputum into a sterile plastic container. The nebulization was continued for at least 10 min and stopped after 20 min or earlier if a \(\geq 2\) mL sputum sample of good quality was obtained. The volume of the induced sputum samples was determined. A small aliquot of the
sample containing an adequate mucus plug for cell counting was overlaid with an equal volume of Hanks’ balanced salt solution containing 1 mM dithiothreitol (Sigma Chemicals; Poole, UK) and mixed gently by vortex mixer, and then incubated at 37°C for 15 min. The incubated suspension was washed with Hanks’ balanced salt solution two times. After the residual mucus was removed by filtering the suspension with gauze, the eluent was provided for total and differential cell counts. The total cell count except for squamous cells was determined using a standard hemocytometer, and normalized for weight and expressed as cells \( \times 10^6/g \) wet weight sputum. Cell smears were prepared with a centrifuge (Autosmear; Sakura; Tokyo, Japan). The slides were fixed in methanol and stained with May-Grunwald-Giemsa stain for the differential cell count, which was carried out by an observer blind to the clinical characteristics of the subjects. The slides were coded and 500 cells were counted for the differential leukocyte count. A sample was considered adequate when the percentage of squamous cells was <20%. The results of the differential leukocyte counts are expressed as a percentage of nucleated cells excluding squamous and epithelial cells. To the remaining sputum samples was immediately added an equal volume of normal saline solution, and the samples were then mixed by vortex mixer and centrifuged at 2,000 g for 20 min. The supernatant was aspirated and frozen at \(-80^\circ C\) until the ECP and albumin concentrations were measured. The ECP concentration was measured in duplicate using \(^{125}\)I-ECP radioimmunoassay kits (Pharmacia Diagnostics; Uppsala, Sweden). The albumin concentration was measured by laser nephelometry.

Data Analysis

The values shown in the text, tables, and figures are expressed as mean±SEM. The data distribution of the variables in each group was first assessed using Bartlett’s test. When the data for the variables showed normal distribution, the comparison was performed with a one-way analysis of variance, and then multiple comparisons were performed by the Tukey-Kramer method. When the data for the variables did not show normal distribution, the variables were compared using the Kruskal-Wallis test, and then multiple comparisons among groups were performed by the nonparametric Tukey-Kramer method. The correlation between variables was examined by calculating Pearson’s product correlation coefficient. A p value of <0.05 was considered significant for all statistical tests.

RESULTS

Forty-two of the 45 subjects were able to expectorate at 10 to 15 min of nebulization. Only two asthmatic subjects developed a wheeze, and the drop in FEV\(_1\) in these patients exceeded 20% after 10 min nebulization; however, the wheezing and drop in FEV\(_1\) were quickly reversed by the inhalation of a \( \beta_2 \)-agonist. Adequate sputum samples were obtained at the first or second attempt in all subjects.

Comparison of Total and Differential Cell Counts and Chemical Substances Between Healthy Subjects and Asthmatic Subjects

The results of the total cell and differential cell counts and the concentrations of albumin and ECP in the healthy and asthmatic subjects are shown in Table 1. The sputum from the patients with asthma contained a greater median total cell count compared with the sputum from the healthy subjects, but a significant difference was not observed. In the differential cell counts, the median percentage of macrophages was significantly lower and the percentage of eosinophils was significantly higher in the sputum from the asthmatic subjects compared with those in the sputum from the healthy subjects. The concentrations of albumin in the sputum from the asthmatic subjects were increased compared with those in the healthy subjects, but a significant difference was not obtained. However, the median concentration of ECP in the sputum from the asthmatic subjects was significantly increased, about 30 times greater compared with that from the healthy subjects. The sputum eosinophil counts in the asthmatics were positively correlated with the concentrations of ECP (r=0.45, p<0.01; Fig. 1). Their ECP levels were also positively correlated with the concentrations of albumin in sputum (r=0.53, p<0.01).

Comparison of Total and Differential Cell Counts and Chemical Substances Among the Three Groups Classified According to Severity of Asthma

Of the 36 asthmatics, 12, 14, and 10 patients were classified as having mild, moderate, and severe asthma, respectively. The mean weekly total symptom score, mean percent PEFR of predicted value, and total and differential cell counts in each asthmatic subgroup are summarized in Table 2. The mean weekly total symptom score and mean percent PEFR both on waking and before the evening meal were significantly increased in accordance with the severity of asthma. The median total cell count also increased in accordance with the severity of asthma, but significant differences among the three subgroups were not obtained. In the differential cell count, eosinophil counts in each subgroup were

| Table 1—Comparison of Cellular and Biochemical Components in Induced Sputum Between Patients With Bronchial Asthma and Healthy Subjects* |
|-----------------|-----------------|------------------|
|                 | Healthy Subjects (n=9) | Bronchial Asthma (n=36) |
| Total cell counts, \( \times 10^6/g \) | 5.18±1.49 | 13.41±3.95 |
| Macrophage, % | 62.3±9.6 | 34.1±4.1* |
| Lymphocyte, % | 6.7±1.5 | 6.2±0.5 |
| Neutrophil, % | 30.8±8.3 | 41.4±4.3 |
| Eosinophil, % | 0.04±0.02 | 18.2±3.6* |
| Albumin, mg/L | 143.9±40.7 | 427.8±98.3 |
| ECP, \( \mu g/L \) | 18.4±2.2 | 526.1±128.2* |

*Results are expressed as mean±SEM.

p<0.05 vs healthy subjects.
significantly increased compared with those in the healthy subjects. Although there was no significant difference in eosinophil counts between the mild and moderate asthmatic groups, the eosinophil counts in the severe asthmatic group were significantly greater than those in both the mild and moderate asthmatic groups. The concentrations of albumin in the sputum were increased in accordance with the severity of asthma; however, only in the severely asthmatic group were significant differences from the values in the healthy subjects and mildly asthmatic patients obtained. The ECP concentrations in the sputum from each asthmatic group were significantly increased compared with those in the healthy subjects. The concentrations of ECP in both the moderate and severe asthma groups were significantly greater than those in the mild asthma group, and those in the severe asthma group were also significantly greater than those in the moderate asthma group (Fig 2). The ECP concentrations in sputum were increased in accordance with the clinical severity of asthma, with no relationship with the dose of inhaled BDP or oral prednisolone received as therapy prior to the sputum collection (Fig 3). The eosinophil counts in sputum were positively correlated with the total symptom score \((r=0.52, p<0.01)\) and inversely correlated with the mean percent PEFR on waking \((r=-0.50, p<0.01)\). The ECP levels in sputum were also positively correlated with the total symptom score \((r=0.48, p<0.01)\) and inversely correlated with the percent PEFR on waking \((r=-0.65, p<0.01)\) (Fig 4). The albumin levels were also inversely correlated with the percent PEFR on waking \((r=-0.48, p<0.01)\).

**Discussion**

Several examinations of sputum induced by hypertonic saline solution nebulization with a pretreatment of inhaled \( \beta_{2} \)-agonist have been reported, and the repeatability,\(^{13,14} \) responsiveness,\(^{6,16,18} \) and validity,\(^{13-15,17} \) of this method have been demonstrated. Sputum induction has thus been accepted as a noninvasive and useful method of evaluating airway inflammation in bronchial asthma. However, contamination with saliva or dilution by inhaled hypertonic saline solution is to some extent inevitable and becomes a problem in the evaluation of soluble components, whereas the relative differential leukocyte counts excluding squamous cells are not affected.\(^{14,15} \) Pizzichini et al\(^{14} \) suggested the selection of the sputum portion that appears free of salivary contamination to minimize dilution by saliva. In the present study, adequate sputum samples were obtained with 10 to 15 min nebulization in 42 of the 45 subjects, and there were no differences in the duration of nebulization among the four groups. To

![Figure 1. Correlation between relative eosinophil counts and concentrations of ECP in induced sputum from patients with bronchial asthma classified into mild (triangles, \( n=12 \)), moderate (open circles, \( n=14 \)), and severe (closed circles, \( n=10 \)) asthma. A significant correlation was observed.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21754/)

**Table 2—Comparison of Weekly Total Symptom Score, Percent PEFR, and Total and Differential Cell Counts Among the Three Groups of Asthmatics Classified in Accordance With the Severity of Asthma**

<table>
<thead>
<tr>
<th></th>
<th>Mild (( n=12 ))</th>
<th>Moderate (( n=14 ))</th>
<th>Severe (( n=10 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weekly total symptom score</td>
<td>0.4±0.2</td>
<td>17.9±2.2(^{1} )</td>
<td>38.1±6.7(^{11} )</td>
</tr>
<tr>
<td>%PEFR on waking</td>
<td>89.0±3.1</td>
<td>74.3±2.9(^{1} )</td>
<td>48.9±3.5(^{11} )</td>
</tr>
<tr>
<td>%PEFR before evening meal</td>
<td>92.5±3.6</td>
<td>80.6±2.9(^{1} )</td>
<td>55.8±5.4(^{11} )</td>
</tr>
<tr>
<td>Total cell counts, ( \times 10^{9}/g )</td>
<td>8.53±2.55</td>
<td>13.27±7.85(^{1} )</td>
<td>19.45±8.74</td>
</tr>
<tr>
<td>Macrophage, %</td>
<td>39.2±8.6</td>
<td>40.5±5.8(^{1} )</td>
<td>19.1±4.9(^{1} )</td>
</tr>
<tr>
<td>Lymphocyte, %</td>
<td>5.8±0.9</td>
<td>6.5±0.8(^{1} )</td>
<td>6.2±1.2(^{1} )</td>
</tr>
<tr>
<td>Neutrophil, %</td>
<td>45.5±8.4</td>
<td>42.2±6.7(^{1} )</td>
<td>35.5±7.4(^{1} )</td>
</tr>
<tr>
<td>Eosinophil, %</td>
<td>9.2±3.1</td>
<td>10.8±4.0(^{1} )</td>
<td>39.4±8.1(^{11} )</td>
</tr>
</tbody>
</table>

\(^{1} p<0.05 \) vs patients with mild asthma.

\(^{11} p<0.05 \) vs patients with moderate asthma.
Eosinophil counts in BAL fluid or samples from bronchial biopsies have been reported to correlate with the severity of asthma assessed by the clinical symptom score and bronchial hyperresponsiveness.\textsuperscript{3,7-11} The ECP levels in BAL fluid and sputum are closely correlated with the severity of asthma.\textsuperscript{9,12-14} Thus, it has been suggested that activated eosinophils are the most important effector cells in bronchial asthma. The purpose of the present study was to determine the correlation between the eosinophil activation in the airway evaluated using the induced sputum method and the severity of asthma including severe asthma, and whether this noninvasive measurement of eosinophil activation could serve as a useful marker for monitoring airway inflammation. As previously reported,\textsuperscript{14} the eosinophil counts and ECP levels in induced sputum were significantly increased in asthmatics, and a significant correlation between eosinophil counts and ECP

minimize the contamination of saliva, a sample was considered adequate when the percentage of squamous cells was lower than 20%; there were also no differences in the squamous cell counts among the groups. Therefore, the data were considered to be reliable for evaluation.

Eosinophils have commonly been found in increased numbers in the airway in patients with both extrinsic and intrinsic asthma, even in those with mild asthma.\textsuperscript{1-3} It is now accepted that eosinophilic inflammation is a hallmark of bronchial asthma.

![Figure 2](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21754/)

**Figure 2.** Comparison of albumin (open bar) and ECP (closed bar) levels in induced sputum among healthy subjects (n=9) and patients with mild (n=12), moderate (n=14), and severe (n=10) asthma. Asterisk: p<0.05 vs healthy subjects; plus sign: p<0.05 vs patients with mild asthma; two plus signs: p<0.05 vs patients with moderate asthma. The ECP levels in the sputum showed significant increases in accordance with the severity of asthma.

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21754/)

**Figure 3.** Comparison of ECP levels in induced sputum between asthmatic patients receiving therapy without steroid, with inhaled BDP in a dose <800 \(\mu\)g/d, and with BDP in a dose of 800 to 1,600 \(\mu\)g/d, and/or oral prednisolone in a dose of 5 to 20 mg/d prior to the sputum collection. Triangles, open circles, and closed circles represent patients with mild, moderate, and severe asthma, respectively. The ECP levels showed increases in accordance with the severity of asthma, and no relationship with the received dose of inhaled or oral steroid therapy.

![Figure 4](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21754/)

**Figure 4.** Correlations between the ECP levels in induced sputum and the mean weekly total symptom scores (top) and the mean PEFR percentage of predicted value on waking (bottom) for 2 weeks prior to the sputum collection. Significant correlations between the ECP levels and symptom score and between the ECP levels and percent PEFR on waking were observed.
levels was observed. In the present study, when the asthmatics were examined in the three groups in accordance with the severity of asthma, the ECP levels of each asthmatic group showed significant increases in accordance with the severity. The eosinophil counts in those with severe asthma were significantly higher than those with mild and moderate asthma, but there was no difference between the patients with mild and moderate asthma. In addition, the ECP levels were more closely correlated with the percent PEFR as a parameter of airflow obstruction than were the eosinophil counts. It has been established that the ECP levels in sputum and BAL fluid are significantly correlated with the parameters of airflow obstruction such as FEV1, FEV1/vital capacity, and forced expiratory flow rate between 25% and 75% FVC. However, these parameters of airflow obstruction were measured only at the time when the sputum or BAL fluid was collected. The monitoring of daily PEFR, which serves as a good parameter of changes in airflow obstruction, is more reliable for evaluating the longitudinal severity of airflow obstruction. Thus, the infiltrated eosinophil number in the airway by itself does not always serve as a good marker for evaluating the severity of asthma. The ECP level, which is a marker of degranulation from activated eosinophils, may serve as a better marker for evaluating the severity.

In patients with asthma, plasma-protein leakage in the airway appears to correlate with indirect indexes of airway inflammation. We also found in the present study that the albumin levels in sputum were increased in accordance with the severity of asthma, and significant correlations between the albumin levels and both the ECP levels in sputum and percent PEFR were obtained. Pizzichini et al. demonstrated that symptomatic asthmatics had a significantly higher proportion of eosinophils and concentrations of eosinophil granule proteins and albumin in induced sputum than did those with few or no symptoms among patients with stable asthma; the eosinophil counts, eosinophil granule proteins, and albumin levels were inversely correlated with symptom score and airflow obstruction or airway hyperreactivity. These findings suggest that the infiltrated eosinophils are activated in accordance with the severity of asthma and release cytotoxic granule proteins (which may induce the injury of the bronchial epithelial wall) and bronchoconstrictive substances, resulting in plasma-protein leakage and airflow obstruction. The successful treatment of asthmatic patients with inhaled or oral steroids is accompanied by a decline in the eosinophil number and reduction of eosinophil activation in the airway or peripheral blood, which correlates with the improvement of symptoms and airway obstruction. However, in some patients relatively resistant to steroid therapy and with poor improvement of symptoms, the high eosinophil counts and ECP levels in induced sputum persist. In the present study, although a high dose of inhaled steroid or oral prednisolone was received by some patients, their sputum eosinophil counts and ECP levels were increased in accordance with the severity of their clinical features of asthma. Therefore, concerning the management of asthma, it is very important to monitor the airway inflammation as well as the severity of symptoms and changes in PEFR. Our findings indicate that the noninvasive measurement of airway inflammation by the induced sputum method serves as a useful marker for evaluating the severity of asthma and monitoring airway inflammation to achieve the optimal control of asthma.

**References**

4. Leff AR. Inflammatory mediators of airway hyperreactivity by peripheral blood granulocytes: the case for the eosinophil. Chest 1994; 106:1202-05
validity of cell and fluid-phase measurements. Am J Respir Crit Care Med 1996; 154:308-17