Eosinophilic airway inflammation is regarded as a typical feature of asthma, while in chronic obstructive pulmonary disease (COPD) neutrophils seem predominant inflammatory airway cells. The aim of the present study was to compare the cellular components of airway inflammation in patients with newly diagnosed mild or moderate COPD and asthma. Seventeen patients with COPD (M/F 10/7, aged 57 ±11 yr) and 22 patients with asthma (M/F 12/10, aged 36 ±14 yr) were enrolled into the study. None of the patients has been treated with steroids for at least 3 months. All patients underwent clinical examination, laboratory examinations, skin-prick tests, pulmonary function tests, methacholine challenge test, and sputum induction with the total and differential cell count assessments. We found increased number of eosinophils in both study groups. However, there were no significant differences in the cellular composition of induced sputum between the asthma and COPD patients. We conclude that eosinophils are important inflammatory cells not only in asthma, but also in COPD.

Key words: asthma, COPD, eosinophilic inflammation, induced sputum

INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are chronic respiratory disorders, characterized by a temporary or permanent reduction of airflow in the lower airways. Chronic airways inflammation is the distinguishing feature of both diseases. However, the pathogenesis and cellular composition of airways inflammatory infiltrate in patients with asthma and COPD are different. The presence of eosinophils is considered typical of asthmatic inflammation,
whereas neutrophils, macrophages, and lymphocytes are the most significant inflammatory cells found in the airways of patients with COPD (1).

The cellular composition of inflammatory infiltrate in the airways may be evaluated through a variety of techniques, including bronchial mucosa biopsy, bronchoalveolar lavage fluid (BALF), and induced sputum. Because sputum induction is relatively non-invasive and a easily repeated procedure, cytological and biochemical evaluation of induced sputum plays an important role in the evaluation and monitoring of the chronic airways inflammation.

Cytologic examinations of induced sputum carried out in COPD patients confirm that neutrophils are the predominant cells, occurring both in the stable period of the disease and during exacerbations (2). The neutrophil count in the airways of COPD patients varies according to the severity of the disease.

The specificity of the cellular composition of inflammatory infiltrate in patients with asthma and COPD is, however, relative. In patients in whom asthma coexists with COPD, a mixed type of inflammatory infiltrates has been observed, with the presence of both eosinophils and neutrophils. This type of inflammation may result in a better response to glucocorticosteroids in this group of patients (3). On the other hand, in asthmatic smokers, the pathological picture of respiratory changes is very similar to that found in COPD (4).

Albeit previous studies have provided quite a broad description of many cellular and biochemical aspects of airway inflammation seen in asthma and COPD, most COPD studies included patients with moderate to severe disease. The small number of studies in patients with mild COPD is explained by the limited and uncharacteristic symptomatology contributing to a delayed diagnosis (2). The aim of the present study was to compare the cellular composition of induced sputum in patients with mild to moderate asthma or COPD.

MATERIAL AND METHODS

The study is part of a research project approved by the Bioethics Committee of the Medical University of Warsaw (No. 172/2003) and each patient gave written informed consent.

The study was conducted in 22 patients with asthma (12 men and 10 women) and 17 patients with COPD (10 men and 7 women). Assignment to treatment groups (asthma or COPD) was based on a specific medical history and the following evaluations: physical examination, chest X-ray, spirometry and flow-volume curve (Lung Test 1000, MES, Poland), bronchial obstruction reversibility test according to the guidelines of the European Respiratory Society (ERS) (5), methacholine bronchial challenge, allergy skin prick tests, and total serum IgE. The severity of asthma and COPD was assessed in accordance with the 2002 guidelines of the Global Initiative for Asthma (GINA) (6) and the 2001 guidelines of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) (7), respectively. Bronchial hyperreactivity was diagnosed on the basis of PC_{20} values below 16 mg/ml in the methacholine challenge test (8).

The study enrolled patients who had not been treated or treated only with bronchodilators during the three-month period prior to study entry. Patients who had received inhaled corticosteroids within three months prior to enrolment were excluded.
A detailed description of the study groups is shown in Table 1. The mean age of asthma patients was 36.1 ±14.5 SD years (range, 18 to 76 years). All patients with asthma had a history of episodic dyspnea and wheezing. The mean duration of symptoms was 16 ±11.2 years (range, 1 to 38 years). Sixteen (73%) patients had signs and symptoms of atopy. The mean FEV1% was 84 ±17%, FVC% was 102 ±14% of predicted values. The mean and FEV1/FVC was 69% ±8%. Twelve patients with asthma (54%) had never smoked, and there were six former smokers (mean number of pack/years, 6.4 ±12.1; minimum time from smoking cessation, 3 years) and four current smokers (mean number of pack/years, 24.5 ±18.4).

The mean age of COPD patients was 56.8 ±11.2 years (range, 31 to 72 years). All COPD patients were current or former smokers (11 and 6 or 65% and 35%, respectively). The mean number of pack years was 38.6 ±13.9. Three (18%) patients were diagnosed with atopy. The mean FEV1% in the COPD patients was 73 ±19%, FVC% was 102 ±23% of predicted values, and FEV1/FVC was 59 ±6%.

The sputum was induced by hypertonic NaCl solutions, in accordance with the ERS standards (9). The detailed protocol for induction has been described previously (10). Induction was discontinued when the patient produced a sufficient volume of sputum (at least 2 ml), or the FEV1 decreased by 20% or more from the baseline (post-bronchodilator FEV1 was taken as the baseline FEV1). The procedure of sputum processing was described in an earlier publication (11). The total cell count was calculated and presented per 1 ml of sputum. The differential cell count was determined in May-Grunwald-Giemsa-stained smears based on the morphology of 300 cells from various fields. The sputum sample was considered adequate if epithelial cells accounted for no more than 50% of total cellularity, and the non-epithelial cell count was at least 200 cells per slide.

All numerical values are presented as means ±SD and ranges. The Shapiro-Wilk test was used to assess the normal distribution of the variables. Comparisons between the groups were performed using the Mann-Whitney and Chi-square tests. Spearman's rank correlation coefficient was used to assess potential correlations between different variables. P<0.05 were considered statistically significant.

### Table 1. Demographic data of the study groups asthma and COPD patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Asthma n = 22</th>
<th>COPD n = 17</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- females</td>
<td>10</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>- men</td>
<td>12</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.1 ±14.5</td>
<td>56.8 ±11.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7 ±3.3</td>
<td>26.4 ±4.2</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking history n (%)</td>
<td>10 (45)</td>
<td>17 (100)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Pack-years (in current and ex-smokers)</td>
<td>6.4 ±12.1</td>
<td>38.6 ±12.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Age at onset of symptoms (years)</td>
<td>18.2 ±19.7</td>
<td>51.1 ±12.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Duration of symptomatic illness (years)</td>
<td>16.0 ±11.2</td>
<td>5.1 ±4.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Atopy n (%)</td>
<td>16 (73)</td>
<td>3 (18)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Chronic rhinitis, n (%)</td>
<td>12 (55)</td>
<td>3 (18)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Chronic sinusitis, n (%)</td>
<td>8 (36)</td>
<td>5 (29)</td>
<td>NS</td>
</tr>
<tr>
<td>Bronchial hyperreactiveness, n (%)</td>
<td>19 (100)</td>
<td>11 (69)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>FEV1 (%predicted)</td>
<td>84 ±17</td>
<td>73 ±19</td>
<td>NS</td>
</tr>
<tr>
<td>FVC (%predicted)</td>
<td>102 ±14</td>
<td>102 ±23</td>
<td>NS</td>
</tr>
<tr>
<td>FEV1%FVC (%)</td>
<td>69 ±8</td>
<td>59 ±6</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Mean values are ±SD.
RESULTS

Thirteen (59%) patients were diagnosed with mild and the remaining nine (41%) with moderate persistent asthma. Mild COPD was present in nine (53%) and moderate COPD in eight (47%) patients. The distribution of disease severity was similar in both groups, although the number of patients with mild disease was slightly higher in the asthma group. All asthma patients had a positive methacholine bronchial challenge test, with a mean PC$_{20}$ of 2.3 ±3.0 mg/ml (range, 0.00-7.99 mg/ml). Methacholine challenge was positive in 11 (69%) patients with COPD and the mean PC$_{20}$ was 9.3 ±8.3 mg/ml (range 0.44-22.80 mg/ml). Bronchial hyperreactivity was significantly more common among asthma patients than in COPD patients (P<0.05). No correlation between PC$_{20}$ and age, BMI, degree of obstruction, or severity of smoking was seen in either group.

No clinical or spirometric features of bronchoconstriction were observed during induction that would necessitate discontinuation of the test (the largest FEV$_1$ reduction did not exceed 20% of the expected value in any of the patients).

Table 2. Comparison of total and differential cell count of induced sputum (IS) in asthma and COPD patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Asthma IS (n = 14)</th>
<th>COPD IS (n = 16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell count (x10$^6$ cells/ml)</td>
<td>3.5 ±2.6</td>
<td>5.1 ±5.8</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>20 ±16</td>
<td>24 ±17</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophils (x10$^6$ cells/ml)</td>
<td>0.8 ±1.0</td>
<td>0.9 ±0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>4 ±2</td>
<td>5 ±7</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocytes (x10$^6$ cells/ml)</td>
<td>0.1 ±0.1</td>
<td>0.2 ±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>22 ±16</td>
<td>30 ±17</td>
<td>NS</td>
</tr>
<tr>
<td>Eosinophils (x10$^6$ cells/ml)</td>
<td>1.0 ±1.4</td>
<td>2.1 ±3.1</td>
<td>NS</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>54 ±22</td>
<td>42 ±21</td>
<td>NS</td>
</tr>
<tr>
<td>Macrophages (x10$^6$ cells/ml)</td>
<td>1.6 ±0.9</td>
<td>1.9 ±2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Epithelial cells (%)</td>
<td>37 ±20</td>
<td>36 ±2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ±SD

Fig. 1. Comparison of cell counts in induced sputum in both groups of patients. There were no significant differences.
Sputum samples (minimum volume of 2 ml) were obtained from 15 (88%) asthma and 17 (100%) COPD patients. Less than 50% of epithelial cells were seen in 77% of all sputum samples, 14 (64%) from asthma and 16 (94%) samples from COPD patients, and, therefore, these were considered appropriate for further analysis.

Cytology of the sputum showed no significant differences between both groups in the total cell count and the absolute and relative count of individual cell types (macrophages, lymphocytes, neutrophils, and eosinophils) (Table 2 and Fig. 1). Increases in eosinophil counts were demonstrated in both COPD and asthma patients; relative to the normal values in healthy individuals based on bibliographical data (12).

COPD patients showed a positive correlation between the absolute and relative sputum eosinophil count and PC$_{20}$ (r=0.6 and r=0.5, respectively; P<0.05) and between the absolute and relative sputum neutrophil count and smoking history in terms of pack-years (r=0.7 and r=0.6, respectively; P<0.05).

In both groups, the correlation between the pulmonary function tests and cellular composition was assessed. In the COPD group, a negative correlation was demonstrated between the FEV$_1$ increase in the bronchial obstruction reversibility test and the sputum eosinophil count (r=-0.65, P<0.05). In the asthma group, a negative correlation was shown between the FEV$_1$ increase in the bronchial obstruction reversibility test and the sputum eosinophil count (r=-0.5, P<0.05). Furthermore, a strong correlation was shown between RV and the relative sputum eosinophil count (r=0.76, P<0.05).

**DISCUSSION**

Macrophages, which account for about 60-70% of all cells, are the predominant sputum cell type in healthy individuals. Neutrophils are the second most common cells (30-40% of all cells), whereas the relative eosinophil count does not exceed 2%. The few studies conducted in healthy, non-smoking volunteers have demonstrated that macrophages and neutrophils are also the most important cellular components of sputum induced by hypertonic saline inhalations. In this material, eosinophils account for an average of 0.4-0.6% of all cells. An increased eosinophil count has been observed in women and atopic patients (12). Previous studies have revealed that more than 50% of asthmatic patients who received no anti-inflammatory treatment have an increased induced sputum eosinophil count (13). Increased eosinophil counts have also been reported in a large proportion of patients with asthma treated with inhaled corticosteroids (14). Evaluation of the cellular composition of induced sputum in asthmatic patients is of considerable practical importance (15). According to Brinke et al (16), the sputum eosinophil count is a good marker of the likelihood of developing persistent airflow obstruction in severe asthma (16), and its absence practically rules out the acute phase of the disease.
In contrast to asthma patients, a pronounced increase in the neutrophil count was demonstrated in COPD patients. Negative correlations between the neutrophil count in induced sputum vs. FEV<sub>1</sub>, and the reduction in annual FEV<sub>1</sub> values (17) suggested a significant effect of neutrophils on pulmonary function and the influence on the severity of the disease. Despite the above-mentioned differences, some publications have reported a similarity between the inflammation seen in severe asthma and that seen in COPD (4, 18).

We found no significant differences between asthma and COPD patients in terms of the cellular composition of induced sputum. We found a surprisingly high neutrophil count in induced sputum in asthma patients and unexpectedly high eosinophil count in induced sputum in COPD patients. This might suggest a similar nature of the airways inflammatory infiltrate in patients with mild to moderate COPD and asthma.

Explaining the high eosinophil count in induced sputum of COPD patients is not an easy task. The first and the simplest hypothesis that could explain our results would be to assume that the individual patients had been clinically misdiagnosed. However, we made every attempt to select the patients in a very thorough manner, to counter any objections as to the correctness of the diagnosis. A substantial number of the patients that were screened for study eligibility were finally excluded from the study due to an equivocal clinical picture. Strict inclusion criteria (with the typical clinical picture that would raise no question as to the diagnosis of asthma versus COPD being the most important) accounted for the long recruitment period (30 months).

Another explanation for the similar cellular composition of induced sputum from patients with asthma and COPD could be the so-called Dutch hypothesis, whereby asthma, chronic bronchitis, and emphysema should be considered as various manifestations of chronic non-specific lung disease (CNSLD) rather than separate disease entities (19). Intrinsic (patient-related) and extrinsic (environmental) factors account for the pathogenesis of this disease. Both inherited predisposition to allergic reactions and bronchial hyperreactivity are considered the main traits determining the development of the disease, and diffuse bronchial obstruction is a common functional abnormality seen in CNSLD.

Sputum eosinophilia is not a new finding in COPD patients. Increased eosinophil counts in the respiratory tract during COPD exacerbation have been discussed in numerous publications (20). Less attention has been paid to the role of eosinophils in patients with stable COPD. Only patients with this form of the disease were analyzed in the present study. If an elevated sputum eosinophil count is defined as more than 3% (21), then sputum eosinophilia was found in as many as 87.5% of COPD patients. Some previous publications have also emphasized the elevated eosinophil count in stable COPD (22), but other authors failed to confirm these findings (15, 17).

Based on the available bibliographical data, it may be assumed that approximately 38-65% of patients with COPD demonstrate eosinophilic
inflammation in the airways (21, 23). The origin, incidence, and clinical relevance of eosinophilic inflammation in patients with COPD are unclear. Increased numbers of eosinophils in the respiratory tract may be predictive of a good response to bronchodilators (21). An overlap of asthma-specific and COPD-specific inflammatory processes in COPD patients is also a possibility (18). Chanez et al (18) demonstrated an improved FEV$_1$ of more than 200 ml and/or 12% over baseline values following oral corticosteroids in nearly half of COPD patients. These patients, compared with patients with a negative bronchial reversibility test, demonstrated specific features of asthma, namely thickening of the basal membrane, elevated eosinophil count, and elevated eosinophil cationic protein (ECP) levels in the BALF.

The phenotype of the disease may be another explanation for the increased eosinophil count in sputum induced from patients with COPD. Enrolment criteria in patients with COPD (in addition to spirometric criteria) included the presence of productive cough and exertional dyspnea. The respiratory eosinophil count seems to be higher in the airways of COPD patients with the phenotype of chronic bronchitis (24).

Exposure to tobacco smoke might be yet another etiologic factor in sputum eosinophilia in patients with COPD. Animal studies have demonstrated increased migration of neutrophils and eosinophils to the respiratory tract following long-term exposure to tobacco smoke (25); these cells were mainly recruited to the lumen of bronchi and bronchioles, rather than the lumen of pulmonary alveoli. However, no correlation was found between the severity of smoking and the composition of induced sputum. Patients with increased sputum eosinophil counts have not been shown to differ from other groups in terms of the incidence of atopy. Moreover, no evidence of peripheral blood eosinophilia was found in this group of patients.

It is impossible to rule out the possibility that, despite the stringent enrolment criteria, no patients prone to allergic respiratory inflammation were included in the COPD group. This might be supported by the fact that COPD patients not only demonstrated increased eosinophil counts in induced sputum (87.5%), but also bronchial hyperreactivity (69%).

Bronchial hyperreactivity is an unspecific feature affecting about 12-14% of adults and 12-18% of children (26). There is no proof to support the hypothesis that bronchial hyperreactivity in childhood might be a risk factor for developing COPD. Studies have shown, however, that bronchial hyperreactivity is an independent predisposing factor for an accelerated decrease in FEV$_1$ and could, therefore, be the cause of COPD later in life (27).

The eosinophilic inflammatory infiltrate in COPD patients might also have been associated with bronchial hyperreactivity, which is a very frequent finding. However, a negative correlation has been demonstrated in these patients between the severity of bronchial reactivity and the sputum induced eosinophil count.
(positive correlation between eosinophil count and $PC_{20}$). Of note, reversibility of bronchial obstruction was not shown in any of the COPD patients.

Although bronchial hyperreactivity is not COPD-specific, it is not an uncommon finding in these patients. According to Bahous et al (28), the incidence of bronchial hyperreactivity in COPD patients is 64%. This observation was confirmed in 70% of patients by Ramsdale et al (29) and in 46% of patients studied by Yan et al (30). A more recent publication, the Lung Health Study, demonstrated the incidence of bronchial hyperreactivity to be 25% in men and 48% in women with moderate COPD (31). On the other hand, if bronchial hyperreactivity were to be defined as an FEV$_1$ decrease of at least 20% with a methacholine concentration of up to 25 mg/ml, the percentage of patients with bronchial hyperreactivity would be 59-63% in male and 85-87% in female patients (31). One of the most important risk factors for the development of hyperreactivity is the reduction in FEV$_1$ and FEV$_1$/FVC (31). Smokers with bronchial hyperreactivity demonstrate particularly high annual loss of FEV$_1$ (32).

Most of our COPD patients were diagnosed with mild disease, with mild symptoms and a relatively short mean duration of symptomatic disease (5 years). Eosinophilia in induced sputum seems to be more common in this patient population. Eosinophils may well play a role in triggering the inflammation in early COPD. This has also been previously suggested by other authors (33). The possibility of an association between COPD and eosinophilic bronchitis or an overlap syndrome between COPD and eosinophilic bronchitis cannot be excluded. Eosinophilic bronchitis is a common cause of chronic cough in middle-aged patients and is characterized by eosinophilia in the sputum (34). Although according to the definition of eosinophilic bronchitis, the disease is not characterized by bronchial obstruction, it may well be envisaged that the latter could be an element of COPD caused by smoking. These patients could, based on the clinical symptoms, be considered COPD patients. This hypothesis could, however, be contradicted by the frequent presence of bronchial hyperreactivity in our COPD patients (which should, by definition, be absent in eosinophilic bronchitis). In conclusion, eosinophils may play a role in the respiratory inflammatory infiltrates not only in asthma patients, but also in subjects diagnosed with COPD.

Conflicts of interest: No conflicts of interest were declared with relation to this work.

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