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The usefulness of induced sputum examination in the diagnostic evaluation of selected chronic inflammatory diseases of the respiratory tract

Abstract

Introduction: Based on the normal values for inflammatory cell counts in induced sputum produced by healthy individuals living in the region of Silesia, Poland, we assessed the usefulness of cytological examination of induced sputum in the diagnostic evaluation of asthma, chronic obstructive pulmonary disease (COPD) and chronic bronchitis.

Material and methods: We analyzed the results of examinations performed in 96 healthy individuals (controls), 42 patients with asthma, 49 with COPD and 30 with chronic bronchitis. We performed spirometry with salbutamol reversibility testing and examination of induced sputum in all the subjects. Those without contraindications underwent methacholine challenge testing.

Results: We found a significantly elevated percentage of eosinophils in all the patient groups compared to the controls ($p < 0.00001$). Median values were 10.3% for asthma, 1.5% for COPD, 1.6% for chronic bronchitis and 0.3% for the controls. We found statistically significant differences in the mean neutrophil percentages in induced sputum between healthy individuals and asthma patients, COPD patients and chronic bronchitis patients ($p < 0.05$). The median values were 45.75%, 38.1%, 77.5% and 58.1%, respectively. The percentage of subjects with positive eosinophil counts (> 2.8%) in the sputum of patients with asthma, COPD, chronic bronchitis and in the controls was 85%, 38%, 20% and 6%, respectively.

Conclusions: 1. Cytological examination of induced sputum is a good test supporting the diagnostic evaluation of chronic inflammatory diseases of the respiratory tract. 2. The percentage of eosinophils in induced sputum exceeding 2.8% is a very good indicator of asthma.

Key words: induced sputum, asthma, COPD, chronic bronchitis

Pneumonol. Alergol. Pol. 2009; 77: 349–356

Introduction

Asthma, chronic obstructive pulmonary disease (COPD) and chronic bronchitis are the most prevalent respiratory diseases. Airway inflammation plays an important role in their pathogenesis and diagnosis is principally on the basis of history, physical examination, pulmonary function testing (spirometry and bronchial obstruction reversibility testing following a short-acting β_2-agonist) and bronchial hyperreactivity testing. Although the clinical pictures of these disease entities overlap, they are distinct conditions in terms of pathogenesis, as evidenced by differing responses to treatment with, for instance, anti-inflammatory drugs. Studies investigating the pathogenesis and pathophysiology of these conditions provide convincing arguments to support the need to identify the type of inflammation before treatment is initiated [1–5].

Numerous studies aimed at analyzing induced sputum from patients suffering from chronic respiratory diseases have shown that the composition of inflammatory cells in the sputum in individual disease entities slightly differs. Sputum from the majority (more than 80%) of patients with

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Received: 8.09.2008

ISSN 0867–7077

uncontrolled asthma, and from more than 50% of patients with well-controlled asthma, is characterized by an increased percentage of eosinophils compared to healthy individuals [3–8], while sputum from COPD patients tends to contain an increased percentage of neutrophils [9–12]. Furthermore, asthma patients and COPD patients include patients with an untypical distribution of cells in the sputum, such as asthma patients with a predominantly neutrophilic profile [13] and COPD patients with an increased percentage of eosinophils [14]. Although numerous studies confirm the importance of eosinophil detection in sputum [6, 15–18] evaluation of these diseases and the monitoring of their treatment based on cytological markers of inflammation are rarely used in clinical practice. This may be because of the labour-intensiveness of the method compared to the determination of eosinophil counts in the peripheral blood. However, inflammatory cells in sputum are a more sensitive and specific marker of pulmonary inflammation than peripheral blood inflammatory cells, because the former directly originate from the inflamed site [19].

The aim of our study was to assess the usefulness of induced sputum cytology in the diagnostic evaluation of chronic inflammatory diseases of the respiratory tract (asthma, COPD, chronic bronchitis) based on the previously established normal values for the composition of inflammatory cells in induced sputum produced by healthy individuals inhabiting the region of Silesia, Poland [20, 21].

Material and methods

The results presented in this paper originate from the samples collected from outpatients managed at the Outpatient Clinic of the Central Teaching Hospital in Katowice-Ligota, Poland, and the participants in health promotion campaigns organized by the hospital. The results were collected over a period of six years at the Laboratory and Pulmonary Function Testing Facility of the Department of Pneumonology.

We analyzed induced sputum from: 42 patients with asthma, 49 patients with COPD, 30 patients with chronic bronchitis, and 96 subjects without respiratory signs or symptoms (controls).

Inclusion criteria

We adopted the following clinical diagnostic criteria:

1. A diagnosis of asthma established by the treating physician; bronchial obstruction reversibility 15 minutes after the inhalation of 200 μg of salbutamol exceeding 12% baseline or confirmation of bronchial hyperreactivity in methacholine challenge testing (PC_{20} < 8 mg/ml); a history of less than 10 pack years of smoking.
2. A diagnosis of COPD established by the treating physician; onset of symptoms after the age of 40; current or former smokers with a history of at least 10 pack years of smoking; no history of atopy; FEV_1 < 85% predicted and FEV_1/VC < 70% predicted on pulmonary function testing' bronchial obstruction reversibility 15 minutes after the inhalation of 200 μg of salbutamol below 10% predicted.
3. A diagnosis of chronic bronchitis established by the treating physician; no history of atopy.
4. Control group: Normal spirometry, including FEV_1 ≥ 85% predicted and FEV_1/VC ≥ 70% predicted. Exclusion criteria included: bronchial hyperreactivity in methacholine challenge testing (PC_{20} < 8 mg/ml) and a history of allergic diseases.

The patients and controls were stable during eligibility testing: meaning that during the testing and within the 30 days preceding testing they had no exacerbation, did not develop any infection, were not hospitalized and underwent no outpatient treatment.

Methods

1. Spirometry was performed on MasterLab (Jaeger) in accordance with the recommendations of the European Respiratory Society and the Polish Society of Pulmonary Diseases [22, 23].
2. Bronchial obstruction reversibility testing 15 minutes after administration of 200 μg of salbutamol.
3. Bronchial hyperreactivity in methacholine challenge testing in subjects without contraindications (e.g. FEV_1 < 70% predicted) was assessed in accordance with the protocol described by Sterk et al. [24].
4. Induced sputum testing was performed in accordance with the protocol based on the method described by Pavord et al. [7] and Popow et al. [25] and in accordance with the recommendations of the European Respiratory Society [26].

   Sputum induction. The induction of sputum was preceded by spirometry before and after administration of 200 μg of salbutamol in order to prevent bronchospasm. Sputum was induced with 3%, 4% and 5% sodium chloride solutions given sequentially in the amount of

7 ml in the form of a spray produced by a Devilbiss ultrasonic nebuliser (flow rate 1 ml/min). Following each dose of sodium chloride inhaled through a mouthpiece, each subject was asked to rinse his/her mouth with water, clean his/her nose and expectorate sputum into a sterile container. Each inhalation was followed by spirometry to check for FEV_1 reduction, if any. If FEV_1 was reduced by 20% of baseline (15 minutes following salbutamol) sputum induction was discontinued. The total duration of inhalation was about 20 minutes.

Processing of the sputum. Sputum was separated from the saliva and weighed. A volume of 0.1% dithiothreitol in HBSS (Hanks balanced salt solution) equal to the quadrupled weight of the sputum expressed in grams was added to the sputum. The resulting suspension was homogenised by aspiration with a pipette and mixed for 20 minutes in a rocking platform shaker. HBSS was added in the amount equal to the volume of the suspension of cells and mixed again for five minutes. The resulting homogenate was centrifuged for 10 minutes at 790 g. The supernatant was frozen at –70°C and the sputum cellular sediment was suspended in a small amount of HBSS. The viability of cells was determined with trypan blue, the total epithelial and non-epithelial cell counts were calculated in Neubauer counting chamber and two cytospin slides were prepared using an MPW-342 centrifuge with a cytoset, centrifuging the sample for six minutes at 600 rpm. Following May-Grunwald-Giemsa staining the percentages of individual cell types were estimated by light microscopy, counting 400 consecutive non-epithelial cells in each of the two slides. Sputum processing began within two hours of it being obtained.

5. Statistical analysis: The results were presented as means, standard deviations (SD), medians, percentile ranges. The distributions of the individual cell types were estimated with the Kolmogorov-Smirnov test. The comparative analysis for the groups was performed with the U Mann-Whitney test and Kruskal-Wallis rank ANOVA. The differences between the groups were considered statistically significant at $p < 0.05$.
6. The diagnostic sensitivity and specificity of induced sputum testing or the ability of the test to detect or rule out the disease were calculated using the following formulae: sensitivity (%) = [TP/(TP + FN)] × 100% and specificity (%) = [TN/(TN + FP)] × 100%, where: TP refers to true positive, FN to false negative, TN to true negative and FP to false positive results [27].

Results

The demographic data in the study groups and pulmonary function test results are summarized in Table 1. The characteristics of the induced sputum for each of the study groups (percentage distributions of inflammatory cells, distributions expressed in absolute terms and the total counts of inflammatory cells per gram of sputum) are summarized in Table 2.

The percentage of eosinophils in induced sputum was significantly increased in all three groups of patients with asthma, COPD and chronic bronchitis ($p < 0.00001$) versus controls, as illustrated by Figure 1. The median values were 10.3%, 1.5%, 1.6% and 0.3%, respectively. Simi-

Table 1. The characteristic of study groups

	Asthma	COPD	CB	Control
Number of subjects	42	49	30	96
Age (years)	46 (20–73)	64 (48–78)	59 (41–75)	44 (17–79)
Females/Males	28/13	8/42	9/21	37/5
Non smokers/Smokers/Ex-smokers	32/3/6	0/29/21	7/13/10	57/27/12
FEV_1 (%)	85.5 (19.3)	54.9 (18.7)	92.3 (16.2)	109.1 (11.1)
FEV_1/VC (%)	72.5 (10.1)	52.6 (9.2)	76.5 (9.9)	86.5 (6.5)
PC_{20} [mg/ml]	0.6 (0.05–3.9)	Not done	9.4 (0.2–25.0)	> 16

COPD — chronic obstructive pulmonary disease; CB — chronic bronchitis; FEV_1 — forced expiratory volume in one second; VC — vital capacity; PC_{20} — provocative concentration causing a 20% fall in FEV_1
Age are expressed as means with ranges; values FEV_1 and FEV_1/VC are expressed as medians and ranges between percentiles, PC_{20} are expressed as means (min.–max.)

Table 2. The characteristic of induced sputum from study subjects

	Asthma	COPD	CB	Control
Total cell count × 10^6/g	2.0 (0.7–9.8)	3.3 (1.1–39.6)	3.2 (0.2–22)	2.0 (0.4–11)
Eosinophils (%)	10.3 (1.3–65.4)	1.5 (0–53)	1.6 (0–48)	0.3 (0–2.8)
Neutrophils (%)	38.1(5.7–74.9)	77.5 (32–92)	58.1 (18.3–94)	45.7 (23–74.6)
Lymphocytes (%)	0.8 (0–5.0)	0.68 (0–4.8)	1.7 (0.5–6.0)	1.0 (0–3.9)
Macrophages (%)	42.3 (11.6–66)	15.4 (6–46)	37.7 (9–65)	51.9 (19.2–78)
Eosinophils × 10^6/g	0.2 (0.04–0.77)	0.09 (0.0–1.29)	0.04 (0.02–0.2)	0.00 (0.0–0.07)
Neutrophils × 10^6/g	0.4 (0.15–1.08)	2.6 (0.97–13.9)	1.8 (0.47–7.4)	0.9 (0.2–3.9)
Lymphocytes × 10^6/g	0.01 (0.0–0.09)	0.02 (0.0–0.33)	0.07 (0.01–0.2)	0.02 (0.0–0.08)
Macrophages × 10^6/g	0.8 (0.6–1.32)	0.8 (0.39–1.48)	1.2 (0.38–2.15)	0.9 (0.5–1.44)

COPD — chronic obstructive pulmonary disease; CB — chronic bronchitis
Values are expressed as medians and ranges between 10 and 90 percentiles

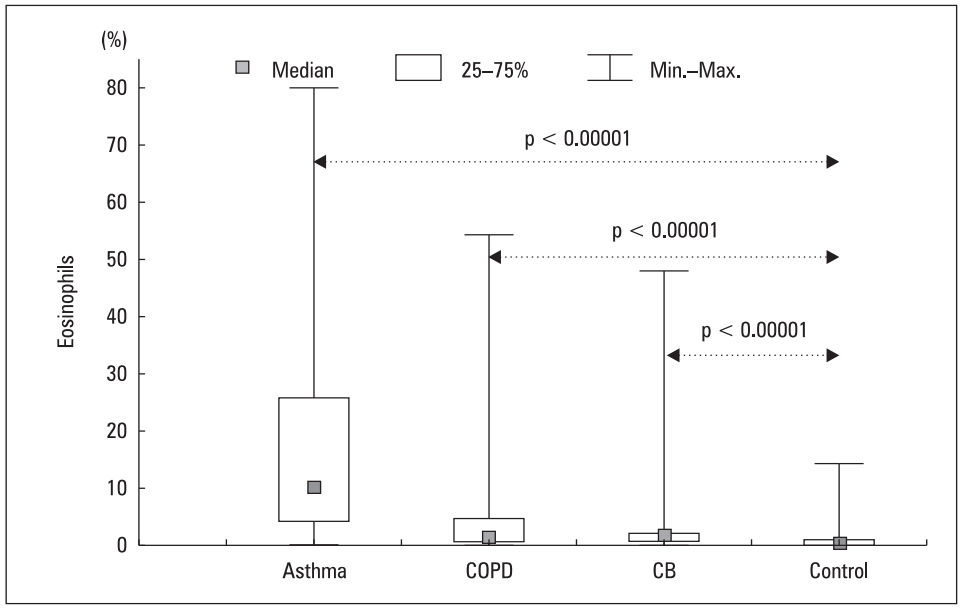

Figure 1. The percentages of eosinophils in induced sputum of asthmatics, COPD and CB patients compared to healthy subjects

lar significant differences were observed when absolute values were analyzed ($p < 0.00001$).

We found statistically significant differences in the mean percentages of neutrophils in induced sputum between healthy individuals and patients with asthma, COPD and chronic bronchitis ($p < 0.005$) with the median values amounting to 45.7%, 38.1%, 77.5% and 58.1%, respectively. Similar significant differences were observed when absolute values were analysed ($p < 0.05$). The differences between the individual groups of subjects are presented in Figure 2.

We found a significantly lower percentage of macrophages in induced sputum from COPD patients compared to patients with chronic bronchitis, asthma and healthy individuals ($p < 0.00001$). The median values were 15.4%, 37.7%, 42.3% and 51.9%, respectively. We found no significant differences in the absolute macrophage counts between the individual study groups.

We found no significant differences in the percentage of lymphocytes in the sputum between the healthy individuals and the patients with asthma or COPD. Significant differences were only present between the healthy individuals and chronic bronchitis patients. The medians were 1.0% and 1.65%, $p = 0.025$. We observed significant differences in the absolute lymphocyte counts between the chronic bronchitis patients and the patients with COPD, asthma and the healthy individuals.

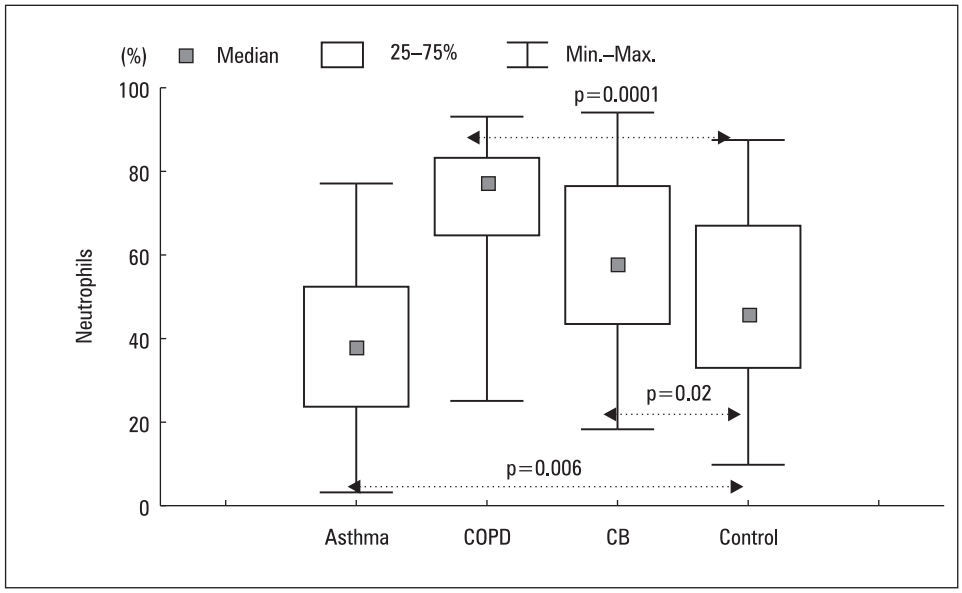

Figure 2. The percentages of neutrophils in induced sputum of asthmatics, COPD and CB patients compared to healthy subjects

We found significant differences in the total cell count per gram of sputum between the healthy individuals and COPD patients (median values: 2.0×10^6/g and 3.3×10^6/g, respectively; $p = 0.0006$) and between COPD and asthma patients (median values: 3.3×10^6/g and 2.0×10^6/g, respectively, $p = 0.0009$).

The sensitivity for detecting eosinophilic inflammation in induced sputum was 83.7% and the specificity for excluding eosinophilic inflammation was 93.9%. The sensitivity for detecting neutrophilic inflammation in induced sputum was 54.5% and the specificity for excluding neutrophilic inflammation was 96.9%.

The mean changes in FEV_1 following hypertonic sodium chloride inhalation in the healthy individuals, asthma patients, COPD patients and the chronic bronchitis patients were –0.61%, –6.5%, –6.7% and –0.78%, respectively. Significant differences were observed between the healthy individuals and COPD patients ($p = 0.0001$).

Discussion

The results of our study were consistent with the findings of other authors [3–8], who have shown an increased percentage of eosinophils in the sputum from most patients with asthma and an increased percentage of neutrophils in the induced sputum from patients with COPD or chronic bronchitis versus healthy controls. In our study, the relative and absolute eosinophil counts in the induced sputum from patients with asthma, COPD or chronic bronchitis were significantly increased compared to controls. On the other hand, the relative and absolute neutrophil counts in induced sputum were significantly increased only in COPD and chronic bronchitis patients, while the asthma patients demonstrated significant reductions.

We compared our results with the normal ranges of inflammatory cell counts in induced sputum reported in our previous publication [20]. Given the very wide range of the reference values for neutrophils, which was calculated at 22.5––74.6% for 96 healthy individuals [20] and at 16.1––77.6% for a larger group [21], it is difficult to establish a threshold value for these cells that is of diagnostic significance. Such a wide range suggests the absence of sharp boundaries between the disease and health in the cytologic picture of induced sputum. It is not uncommon to see disorders manifested by abnormal spirometry and clinical symptoms that are correlated with parameters falling within the reference ranges. In 10% of COPD patients and 27% of chronic bronchitis patients participating in our study, the relative neutrophil counts in the sputum were below the mean value in the control group of 45.7%. On the other hand, in 50% of COPD patients, and 23% of chronic bronchitis patients, the relative neutrophil counts in the sputum exceeded the upper reference value for these cells of 74.6%. The sensitivity for detecting neutrophilic inflammation with the study test was only 54.5%. Nevertheless, it indicates that in some of the patients with suspected

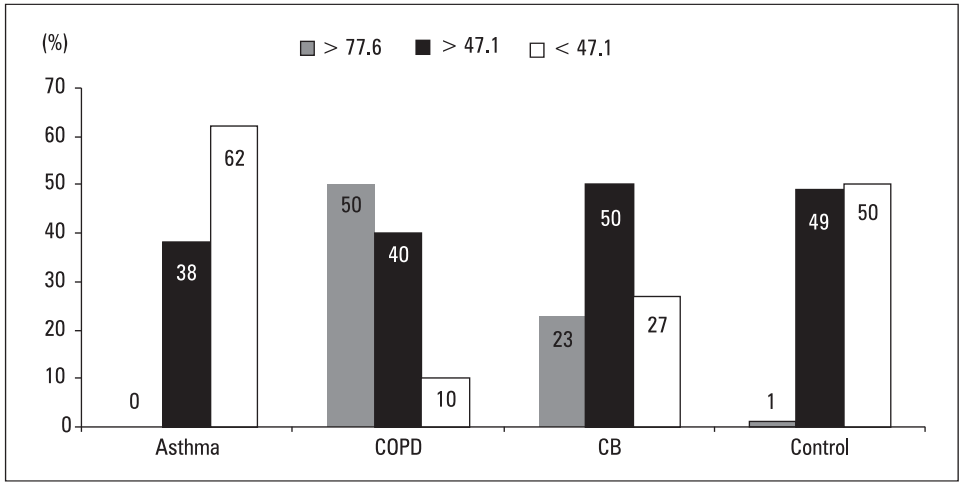

Figure 3. The percentage of subjects with abnormal percentage of neutrophiles in induced sputum (> 77.6%), above the mean values (> 47.1%) and below the mean values (< 47.1%)

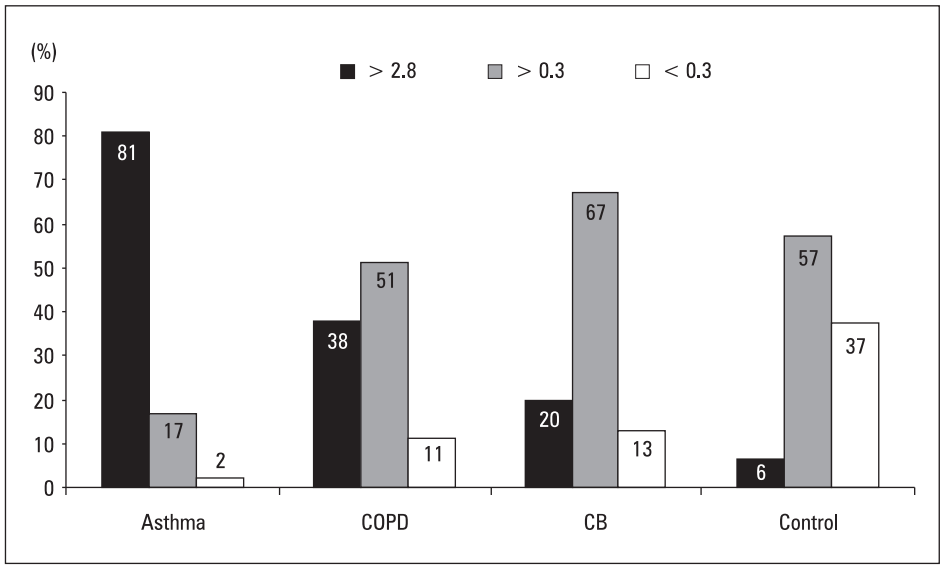

Figure 4. Percentage of subjects with abnormal percentage of eosinophiles in induced sputum (> 2.8%), above the mean values (> 0.3%) and below the mean values (< 0.3%)

COPD or chronic bronchitis a considerably increased relative neutrophil count in induced sputum may be of diagnostic importance (Figure 3).

In clinical practice, the determination of the relative eosinophil count in induced sputum, used in the diagnosis and monitoring of asthma, is of much greater significance. We found that as many as 81% of asthma patients had increased relative eosinophil counts in induced sputum (above 2.8%). The diagnostic sensitivity of induced sputum testing for detecting eosinophilic inflammation was high (83.7%).

In the light of this, **the most important conclusion that may be drawn from our study is that the discovery of increased eosinophil counts in induced sputum confirms, with a very high probability, the diagnosis of asthma.**

In some of the COPD and chronic bronchitis patients (38% and 20%, respectively; Figure 4) the relative eosinophil counts in the sputum were also significantly increased. The identification of patients with increased relative eosinophil counts in induced sputum may be important in the diagnostic evaluation of chronic cough, especially in patients with chronic bronchitis. Early treatment of these patients may prevent the consequences of chronic but untreated respiratory inflammation, such as asthma or the eosinophilic variant of COPD. It is most likely that the COPD patients who have never smoked originate from this group, as pointed out by Birring et al. [28].

The calculation of the absolute and relative lymphocyte counts in the induced sputum from patients attracts less interest among diagnosticians, which may result from the low reproducibility of the measurement [21, 25]. Nevertheless, D'Ippolito et al. and Fireman et al. [29, 30] have demonstrated the usefulness of sputum examination in the diagnostic evaluation of interstitial pulmonary diseases. They showed that relative lymphocyte counts in induced sputum from patients were more than twice as high as those in sputum from healthy individuals. We found that the absolute lymphocyte count in patients with chronic bronchitis versus the other study groups was significantly increased. This finding would, however, require confirmation in further studies.

The value of determination of the relative macrophage count in induced sputum turned out to be low. This is because the relative count of these cells in the sputum reflects changes in neutrophil counts. The low value of determining macrophage counts in the sputum was additionally confirmed by the calculation of the absolute counts of these cells, which did not differ significantly between the study groups. In future, when immunocytochemical methods become available in routine clinical practice, the calculation of relative counts of small and large macrophage populations in sputum may become useful in diagnostic evaluation, such as for the early detection of COPD [31]. However, further studies are required to assess the significance of these cells in the cytodiagnostic evaluation of the inflammatory diseases of the airways.

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