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Diagnostic value of induced sputum in interstitial lung disease

Abstract

Introduction: Although induced sputum (IS) has recently been used in studies of interstitial lung disease (ILD), there have been few reports on studies investigating the usefulness of this method in the differential diagnosis of ILD. The aim of our study was to determine the diagnostic value of differential cell counts and CD4+/CD8+ ratio in induced sputum from patients with sarcoidosis and other ILDs.

Material and methods: We enrolled 59 patients in the study (36 with sarcoidosis, 16 with hypersensitivity pneumonitis [HP] and seven with idiopathic pulmonary fibrosis [IPF]). Sputum was induced a minimum of seven days following BAL by inhalation of 5% NaCl solution for five minutes and repeated four times. Differential cell count was determined in Giemsa stained cytopspins by counting 400 mononuclear cells for specific cell types per slide. The analysis of T-cell subtypes was carried out by flow cytometry. The potential for differentiating sarcoidosis from the other ILDs was checked by determining the predictive value for CD4+/CD8+ ratio or by using the object classification method based on the k-nearest neighbour (k-NN) rule. The variables used in the k-NN rule were the following parameters of IS: cell viability, total cell count, percentages of macrophages, lymphocytes, neutrophils, eosinophils, CD4+ lymphocytes and CD8+ lymphocytes, and the CD4+/CD8+ ratio.

Results: Evaluation of IS was possible in 33 patients (15 with sarcoidosis, 11 with HP and seven with IPF). A CD4+/CD8+ ratio in IS exceeding 2.6 had a sensitivity of 100%, and a specificity of 72% with the likelihood of establishing the correct diagnosis while differentiating sarcoidosis from HP and IPF. On the other hand, when we used the k-NN rule, the likelihood of establishing the correct diagnosis was 79% (with an estimated classification error of 21%).

Conclusion: Using the object classification method based on the k-NN rule in the differential diagnosis of sarcoidosis, HP and IPF on the basis of all the IS parameters is not associated with a higher likelihood of establishing the correct diagnosis than the analysis of CD4+/CD8+ ratio alone.

Key words: induced sputum, CD4+/CD8+ ratio, interstitial lung disease, sarcoidosis, hypersensitivity pneumonitis, idiopathic pulmonary fibrosis

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Introduction

Although the examination of sputum in lung diseases can be traced back to the late 19th century, the difficulties associated with obtaining adequate sputum samples in many clinical situations prevented it from being used as a research tool. Solving this problem by introducing the induction of sputum through the inhalation of hypertonic saline solution in patients with asthma more than 18 years ago drew attention to this method and resulted in an impressive expansion of studies investigating its use in respiratory diseases [1]. Being the only entirely non-invasive method of directly observing inflammatory processes occurring in the airways, induced sputum has made it possible to determine the type of inflammation in terms of predominant cell types in various conditions, including asthma [2], chronic obstructive pulmonary disease (COPD) [3] and chronic cough [4]. Between 1992 and 2004, more than 650 papers were published on the use of induced sputum in the diagnosis and treatment of asthma, and more than 200 on its use in COPD and chronic bronchitis [5].

Reports on the use of induced sputum in investigations of interstitial lung disease (ILD) are scarce and most of them concern sarcoidosis. Despite the promising results of attempts to use induced sputum in the diagnosis of ILD, the significance of this method has not yet been established unequivocally. This may stem from the belief that samples obtained by sputum induction and those obtained by bronchoalveolar lavage (BAL) originate from different areas of the lungs. The BAL fluid (BALF) reflects processes occurring in the peripheral air spaces, while induced sputum samples predominantly originate from lung areas characterised by the presence of secretions abundant in neutrophils, and therefore predominantly obtained from large bronchi [6]. Another issue surrounding the analysis of induced sputum is the relatively low number of lymphocytes in this material. However, several studies have favourably assessed sputum induction relative to BALF in various ILDs and have compared induced sputum examination results between patients and healthy volunteers [7–12]. It has been shown that, similarly to BALF, it is possible to detect lymphocytosis in induced sputum from patients with sarcoidosis [8,10–12] and patients with hypersensitivity pneumonitis (HP) [9] and to assess T-cell subpopulations [7, 9, 11–13]. Induced sputum from patients with idiopathic pulmonary fibrosis (IPF), similarly to BALF, was shown to contain higher percentages of eosinophils [7] or neutrophils [14] compared to healthy individuals.

The high percentage of lymphocytes in BALF is not, however, characteristic of sarcoidosis or HP and is also seen in other interstitial, infectious or malignant lung diseases [15]. It is well known that sarcoidosis is characterised by the presence of chronic inflammation in the airways in which a predominant role is played by activated CD4+ lymphocytes and that the increased CD4+/CD8+ ratio, along with a detailed assessment of the clinical picture of the disease, may be of considerable importance in differentiating sarcoidosis from other ILDs [16, 17].

In HP, on the other hand, the repeated inhalation of fine particles of antigens results in the activation and accumulation of T cells in the lower respiratory tract and the pulmonary interstitium. Lymphocyte phenotyping with the use of monoclonal antibodies showed that most of these cells were characterised by the presence of CD8, and only a small subpopulation of cells expressed CD4, leading to the inversion of the CD4+/CD8+ ratio, which normally does not exceed 1 [18]. Some authors believe that determination of CD4+/CD8+ ratio could be useful in differentiating sarcoidosis and other ILDs, including HP [19]. Several studies have shown a strong correlation between the percentages of T cell subtypes (CD4+ and CD8+) and the CD4+/CD8+ ratio in induced sputum and BALF from patients with various ILDs [7, 11–13]. The results show that the analysis of T-cell subtypes in samples of induced sputum may be as useful as the analysis of BALF as a method to identify inflammation in which the predominant role is played by CD4+ lymphocytes and to differentiate sarcoidosis and other ILDs [8].

In our study, we attempted to determine the predictive value of CD4+/CD8+ ratio in induced sputum that would differentiate sarcoidosis from other interstitial lung diseases. We also analysed whether it is possible to establish the diagnosis on the basis of all the parameters of induced sputum in patients with ILD.

Material and methods

We enrolled a total of 59 consecutive patients (33 males and 26 females) admitted to the First Department of Lung Diseases, Institute of Tuberculosis and Lung Diseases, Warsaw, Poland, diagnosed with sarcoidosis, HP or IPF in the course of evaluation.

The diagnosis of sarcoidosis (n = 36; M: 18, F: 18; mean age: 35.3 ± 8.9 years) was established in accordance with the recommendations adopted by an international panel of experts [20]. Sarcoido-

sis was diagnosed on the basis of histopathologic examination of a tissue sample from the affected organ revealing the presence of non-caseating granulomas with a consistent clinical and radiological picture in 25 patients; and on the basis of the typical clinical and radiological picture by conventional methods and by high-resolution computed tomography (HRCT) having ruled out other conditions with a similar clinical and radiological picture in 11 patients. In accordance with the commonly accepted radiological staging classification for sarcoidosis [21], 14 patients were classified as stage I, 20 as stage II and two as stage III disease. There were eight current smokers, four ex-smokers and 24 non-smokers in the group of patients with sarcoidosis.

The diagnosis of HP was made in 16 patients (M: 11, F: 5; mean age: 48.5 ± 13.2 years) according to the criteria published by the American Thoracic Society [22]. Based on the changes detected by HRCT, subacute HP was diagnosed in 11 and chronic HP in five patients [23]. The group of HP patients comprised 12 non-smokers, three ex-smokers and one current smoker.

The diagnosis of IPF in seven patients (M: 4, F: 3; mean age: 65.3 ± 7.6 years) was based on the clinical and radiological criteria published by the American Thoracic Society/European Thoracic Society [24], including the pattern of radiological changes characteristic of IPF discovered by HRCT, and in one female patient the diagnosis was additionally supported by a histopathologic examination of lung tissue samples collected during an open biopsy procedure. The seven patients with IPF included two never-smokers, four ex-smokers and one current smoker.

None of the patients had received, either via inhalation or orally, glucocorticosteroids, immunosuppressant drugs or antibiotics in the three months prior to the study. Each patient read the study protocol and provided written informed consent to participate in the study. The study had been approved by the Ethics Committee of the Institute of Tuberculosis and Lung Diseases, Warsaw, Poland.

Sputum induction

Sputum was induced using the PARI MASTER® inhalation device (PARI GmbH, Germany) with a total output rate of 0.5 ml/min guaranteeing a mass median diameter of $3.6 \mu\text{m}$ adhering to a previously described method [1, 10]. All the patients received nebulisation with a 5% NaCl solution of five minutes' duration repeated four times. After each inhalation, the patients were asked to rinse their mouth and throat with a saline solution

and to expectorate sputum into a sterile plastic container. After each five-minute cycle, spirometry was performed and oxygen saturation measured. If FEV₁ was found to be decreased by at least 20% or manifestations of intolerance were developed (dyspnoea, cough, wheezing, chest tightness) the procedure was stopped.

Sputum processing

Induced sputum was processed using the method proposed by Popov et al. [25]. Sputum was examined directly after induction, never more than two hours after collection. Sputum was transferred to a Petri dish and thick and viscous portions of mucus were selected for further examination. These portions were transferred into a 15-ml conical tube and weighed. A solution of dithiothreitol was then prepared (DDT; Sputolysin® Reagent, Calbiochem, Germany), which was obtained by mixing one part ready-made preparation with nine parts distilled water, and added to sputum in a volume (expressed in μL) exceeding the mass of the selected sputum portions (expressed in mg) by a factor of four.

The sample was stirred mechanically with a pipette by several aspirations and incubated on a rocking platform shaker at 22°C for 15 minutes. The suspension was filtered through a $40 \mu\text{m}$ nylon sieve (Falcon, Becton Dickinson, USA) and the total cell count was determined in a Bürker counting chamber. Cell viability was assessed by mixing 380 μL of trypan blue with 20 μL of the sputum suspension and expressed as a percentage. After centrifuging at 500 g for ten minutes, the supernatant was collected and the cells were suspended in phosphate buffered saline (PBS) to achieve a concentration of 1×10^6 cells/ml. Four cytopreps were prepared by adding 60 μL to each cytopspin, one of them was May-Grünwald-Giemsa stained and the differential cell count was performed on 400 mononuclear cells with the exclusion of squamous epithelial cells. A specimen was considered capable of being evaluated if the percentage of squamous epithelial cells did not exceed 20% and cell viability exceeded 70% [12].

T-cell phenotyping in induced sputum

T-cell subpopulation analysis was carried out by flow cytometry using the FACSCalibur™ system (Becton Dickinson, San Jose, CA, USA) and the TriTEST CD4/CD8/CD3 monoclonal antibodies (Becton Dickinson, San Jose, CA, USA) according to the manufacturer's instructions. The monoclonal antibody panel allowed us to assess the following T-cell subpopulations: CD3 (T cells), CD4 (helper T cells) and CD8 (cytotoxic T cells). The

anti-CD4, anti-CD8 and anti-CD3 antibodies were conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE) and peridinin chlorophyll protein (PerCP), respectively. A suspension of the test cells with a mixture of appropriately labelled mouse immunoglobulins, IgG1 (FITC) and IgG1 (PE) was the control. The analysis of the obtained morphological parameters of cell fluorescence was carried out using CellQuest 3.3. Lymphocyte population was gated according to size and granularity (FSC/SSC). Four hundred lymphocytes detected on the basis of the presence of CD3 was considered the lowest sufficient number of cells for analysis [11]. All the flow cytofluorometric assessments were carried out within four hours of sample collection.

Statistical analysis

In an attempt to find the predictive value of CD4+/CD8+ ratio, we arranged the data from the study in an increasing order, analysed all the possible separation values for CD4+/CD8+ ratio in IS, and determined their respective sensitivity and specificity values and the frequency of correct diagnosis.

We analysed the possibility of establishing the diagnosis on the basis of induced sputum parameters with the object recognition method using the k-nearest neighbours (k-NN) rule [26]. This allows the qualification of an unlabelled object (a case, a patient) into one of the classes (disease entities) represented in the set of already classified objects called the reference set (or the training set). The new unlabelled object is compared to objects from each of the classes in the training set.

To be more precise, in the training set, we select a certain number of objects (k) that are most similar to the unlabelled object. The k value is determined experimentally to minimise the likelihood of mis-classification. The new object is assigned a class which is most abundantly represented among the selected k objects. The operation of this rule for k = 3 and an artificial small two-dimensional training set is illustrated in Figure 1 (the new object is labelled with a star and will be assigned to the class of circles). The similarity of the

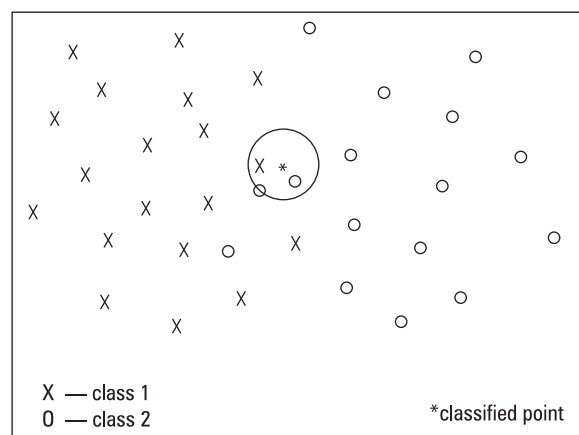


Figure 1. k-NN rule for k = 3 (description in the text)

objects in this rule is measured by the distance, with close objects being considered similar objects, hence the name of the rule.

Results

Induced sputum was able to be evaluated in 33 (56%) out of the 59 patients enrolled the study. Adequate induced sputum was obtained from all the patients with IPF, from 15 (42%) of 36 patients with sarcoidosis, and from 11 (69%) of 16 patients with HP. The analysis of induced sputum was impossible in 26 patients for the following reasons: 16 patients failed to expectorate sputum for examination, induced sputum from one patient contained non-diagnostic cellular debris only, the percentage of squamous epithelial cells in the specimens from seven patients exceeded 20%, no cells were found in a stained cytoprep from one patient, and T-cell subpopulation analysis by flow cytometry was impossible in one patient due to insufficient lymphocyte count in the specimen.

The differential cell counts, the percentages of T-cell subpopulations and CD4+/CD8+ ratios in IS from patients with sarcoidosis, HP and IPF are summarised in Table 1.

Table 1. Differential cell counts, T-lymphocyte subsets and CD4+/CD8+ ratio in induced sputum in the study subgroups (sarcoidosis, HP, IPF)

Group	CLK × 10 ⁶	Macrophages	Neutrophils	Lymphocytes	Eosynophils	CD4	CD8	CD4/CD8
Sarcoidosis	7.4 ± 10.2	45.8 ± 24.8	36.2 ± 28.2	15.7 ± 13.4	2.2 ± 2.4	76.8 ± 14.0	14.1 ± 5.8	6.2 ± 2.6
HP	8.4 ± 7.7	38.5 ± 20.8	38.8 ± 33.1	20.7 ± 15.4	2.0 ± 2.9	46.8 ± 25.3	32.7 ± 17.6	4.9 ± 8.2
IPF	4.2 ± 5.3	51.7 ± 27	30.4 ± 34	15.8 ± 11.5	2.1 ± 3.7	53.7 ± 21.2	33.3 ± 14.1	2.2 ± 1.8

Values are mean ± standard deviation (SD); CLK — total cell count; HP — hypersensitivity pneumonitis; IPF — idiopathic pulmonary fibrosis

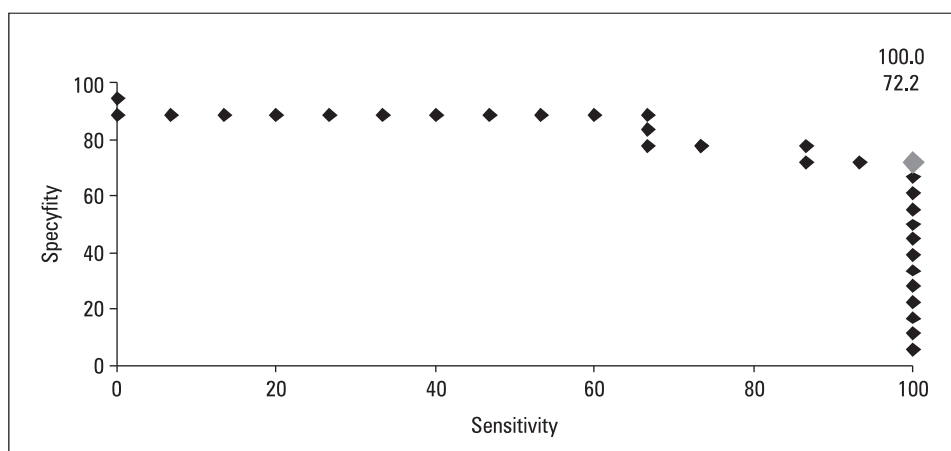


Figure 2. Dependence between specificity and sensitivity for CD4+/CD8+ ratio in induced sputum; grey point on the plot correspond with cutpoint of maximum probability of correct decisions (sensitivity — 100%, specifity — 72,2%)

Table 2. Sensitivity and specificity of different values of the CD4+/CD8+ ratio in induced sputum in the diagnosis of sarcoidosis

CD4+/CD8+ ratio	Sensitivity (%)	Specyfity (%)	Probability of correct diagnosis (%)
1.08	100	44.4	69.7
2.61	100	72.2	84.4
3.55	86.7	77.8	81.8
5.26	66.7	88.9	78.8
7.29	33.3	88.9	63.6
10.23	6.7	88.9	51.5

We arranged the values of CD4+/CD8+ ratio in induced sputum in an increasing order. We then analysed all the possible separation for CD4+/CD8+ ratio values in induced sputum (32 for 33 cases), determining the sensitivity and specificity for each of them and the frequency of correct diagnoses (Fig. 2). Eventually, we selected the value of 2.61 as offering the lowest frequency of errors. For CD4+/CD8+ ratio values exceeding 2.61, 75% of cases were classified as sarcoidosis and 25% as other conditions with a sensitivity of 100% and a specificity of 72.2%. Using a CD4+/CD8+ ratio threshold of 2.61 to establish the diagnosis of sarcoidosis in those who exceeded this value, and to establish the diagnosis of HP or IPF in those who did not exceed this value, the correct diagnosis would be made in 84.4% of cases. Table 2 summarises the sensitivity and specificity values and the estimated likelihood of establishing the correct diagnosis for various CD4+/CD8+ ratio values in induced sputum in the diagnostic evaluation for sarcoidosis.

We analysed the possibility of establishing the diagnosis based on induced sputum parameters with the use of the object recognition method employing the k-NN rule. The set used to create the

classifier (the decision rule) comprised 33 patients and ten features that were the parameters of induced sputum (1 — cell viability in the sputum, 2 — total cell count, 3 — percentage of macrophages, 4 — percentage of lymphocytes, 5 — percentage of neutrophils, 6 — percentage of eosinophils, 7 — percentage of CD3+ lymphocytes, 8 — percentage of CD4+ lymphocytes, 9 — percentage of CD8+ lymphocytes, 10 — CD4+/CD8+ ratio). The training set comprised 15 cases representing the first class (patients with sarcoidosis), 11 cases representing the second class (patients with HP) and seven cases representing the third class (patients with IPF). When we considered the possibility of establishing the diagnosis based on the ten features of induced sputum listed above, we observed a relatively good detectability of sarcoidosis (Fig. 3). It should, however, be pointed out that a considerable percentage of patients with HP and IPF would be mis-classified as sarcoidosis patients. The classifier constructed on the base of the available data would classify all the patients with sarcoidosis correctly, but 45.45% of patients with HP would be mis-classified as sarcoidosis patients. None of the patients with IPF would be classified correctly, as 57.14% would

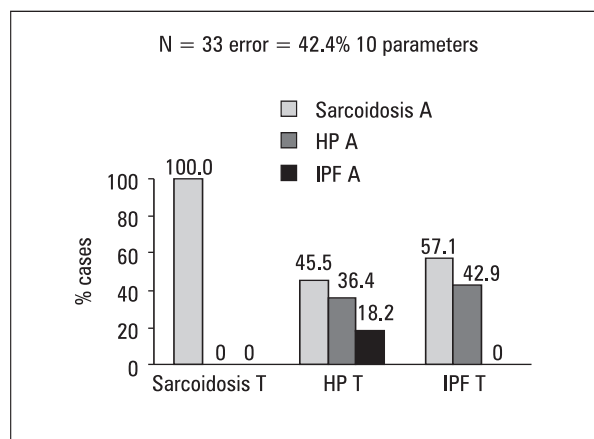


Figure 3. Classification of the patients with interstitial lung diseases on the basis of all the parameters of induced sputum (IS) (3 classes, k-NN rule): percentage of cases from the class T (true class) qualified to the class A (assigned class)

be classified as sarcoidosis patients and 42.86% as HP patients. Based on the k-NN rule taking into consideration ten features, a correct diagnosis would be established in 58% of the cases (which means that 42% of the cases would be misdiagnosed).

If only two classes were included in our model, ie if the training set contained 15 cases from the first class (sarcoidosis patients) and 18 cases from the other class (patients with HP or IPF), the classifier constructed on the basis of all the ten features would correctly classify 11 out of 15 patients with sarcoidosis, with 27% of sarcoidosis patients being mis-classified as representatives of the other class and 17% of patients with HP or IPF being mis-classified as sarcoidosis patients (Fig. 4). The estimated classification error would be $e = 21\%$, meaning that the correct diagnosis would be established in 79% of the cases.

Discussion

In an attempt to determine the significance of CD4+/CD8+ ratio in induced sputum for differentiating sarcoidosis from other interstitial lung diseases, we looked for the best threshold value. We eventually selected the value of 2.6, as it offered the lowest rate of misdiagnosis. Using a CD4+/CD8+ ratio threshold of 2.6 to establish the diagnosis of sarcoidosis in those who exceeded this value, and to establish the diagnosis of HP or IPF in those who did not exceed this value, the correct diagnosis would be made in 84.4% of the cases with a sensitivity of 100% and a specificity of 72%. Fireman et al. [8] compared the cellular composition between induced sputum and BALF from patients with various interstitial lung diseases and

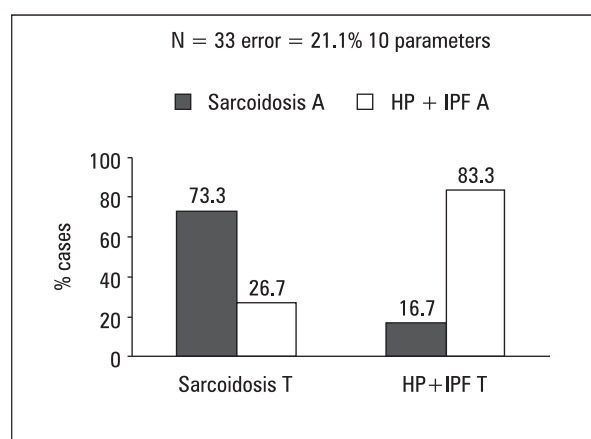


Figure 4. Classification of the patients with sarcoidosis and remaining interstitial lung diseases on the basis of all the parameters of induced sputum (IS) (2 classes, k-NN rule): percentage of cases from the class T (true class) qualified to the class A (assigned class)

found that CD4+/CD8+ ratio above 2.5 in induced sputum had a high predictive value for true positive results (81.2%), a sensitivity of 100% and a specificity of 81% in differentiating sarcoidosis from other non-granulomatous interstitial lung diseases. This was the first study to show that induced sputum was an effective, non-invasive method which might be used to diagnose inflammation associated with the accumulation of CD4+ lymphocytes, which differentiates sarcoidosis from other non-granulomatous interstitial lung diseases.

The results of the studies conducted so far that have evaluated the role of induced sputum in differentiating interstitial lung diseases have made a contribution to the discussion as to the significance of CD4+/CD8+ ratio in the diagnosis of sarcoidosis that has been going on for years now. Our study, similarly to the study by Fireman et al. [8], confirms the usefulness of induced sputum in differentiating sarcoidosis from the other interstitial lung diseases and is consistent with such studies as those by Costabel et al. [27] and Winterbauer et al. [19], which reported specificities of over 90% and sensitivities of 50–60% for high CD4+/CD8+ ratio values. Our study (and these others) however, does contradict those who believe that sarcoidosis patients display a high variability of CD4+/CD8+ ratio [28]. Based on recently published experiences, Fireman et al. [29] considered the possibility of using CD4+/CD8+ ratio in induced sputum in conjunction with pulmonary function parameters to differentiate sarcoidosis from the other interstitial lung diseases. The authors analysed a study that enrolled 120 patients (67 with sarcoidosis and 53 with other interstitial lung diseases), who underwent bronchoscopy with BAL and a transbron-

chial biopsy, sputum induction and HRCT. Two multivariate logistic regression models were employed to determine the likelihood of establishing the diagnosis of sarcoidosis. Model 1 included demographic data and induced sputum parameters, while Model 2 took into account demographic data and a combination of induced sputum parameters and pulmonary function parameters. The area under the curve was 0.899 for induced sputum parameters and 0.914 for the combination of induced sputum parameters and pulmonary function parameters, which proves that this non-invasive procedure may be characterised by a high specificity and sensitivity in the differential diagnosis of sarcoidosis.

Induced sputum as a non-invasive method to assess T-cell subpopulations could be an important diagnostic tool in patients diagnosed with uveitis without an overt involvement of the respiratory system. Neuforfer et al. [30] examined 17 patients diagnosed with uveitis of unknown origin and found, in addition to elevated serum angiotensin-converting enzyme (ACE), high CD4+/CD8+ ratios in induced sputum, which might be suggestive of sarcoidosis. The exchange of lymphocytes between the bronchoalveolar space and the lamina propria of the bronchial mucosa points to the possibility of accumulation of CD4+ lymphocytes in bronchial secretions and therefore to the possibility of obtaining them by sputum induction [31]. In sarcoidosis and HP, lymphocytes show an increased mobility, which obviously could facilitate their accumulation in the respiratory epithelium and lead to the formation of granulomas [32].

When we analysed the possibility of establishing the diagnosis on the basis of the ten features of induced sputum, we discovered a relatively good detectability of sarcoidosis, as all the patients had been classified correctly. However, only 36% of HP patients would have been classified correctly, as 45% of HP patients would have been misclassified as sarcoidosis and 18% as IPF. None of the patients with IPF would have been classified correctly, which should be expected given the fact that the cellular composition of BALF also shows no specific features for this condition.

While BAL does not possess any unequivocal diagnostic value for IPF, it is used to rule out infections and malignancies and those interstitial lung diseases for which BAL is diagnostic (e.g. alveolar proteinosis, Langerhans cell histiocytosis) [24]. Similarly, in the case of induced sputum, it is not to be expected that this method of assessing cellular and non-cellular components that mainly originate from large bronchi could form the basis for

the diagnosis of IPF. On the other hand, in the case of sarcoidosis and HP, the presence of the characteristic differential cell count and abnormalities in the composition of T-cell subpopulations in BALF may be consistent with a specific clinical diagnosis in the absence of a lung biopsy [16, 18].

If we analysed the three groups of patients using the k-NN rule operating on the basis of the ten features of induced sputum, 42% of the patients would be misdiagnosed. A smaller classification error would be made if we divided the patients into two rather than three groups, as did Fireman et al. [8] (i.e. sarcoidosis vs other interstitial lung diseases). The classifier constructed on the basis of all ten features would then result in misdiagnosis of only 21% of the cases.

This is the first study to attempt differential diagnosis of the three commonest interstitial lung diseases on the basis of induced sputum parameters using the statistical method of object recognition based on the k-NN rule. Drent et al. [33] used a different statistical method, multivariate logistic regression, to develop a computer application which enabled them, on the basis of BALF assessment (recovery, total cell count and percentages of macrophages, lymphocytes, neutrophils and eosinophils), to correctly classify 91% of the patients with sarcoidosis, HP or IPF from the training set and 94.5% of the patients from an independent testing set composed of patients from another hospital.

These were undoubtedly better results than ours, as the division of patients into two groups only (patients with sarcoidosis and patients with other interstitial lung diseases) already made it possible to correctly classify 79% of cases on the basis of all the examined features of induced sputum. In this study, the group of patients that made up the training (reference) set consisted of a much larger number of patients than was the case with our study (277 v. 33 patients), which might have affected the quality of the results. The diagnostic evaluation of patients for interstitial lung diseases carried out on the basis of induced sputum parameters in our study and on the basis of BALF parameters in the study by Drent et al. [33] employed different statistical methods. Furthermore, both methods of evaluating the cellular components in the respiratory system investigate material originating from slightly different areas of the lungs. Hence, the assessment of induced sputum probably yielded less favourable results than the analysis of BALF in the diagnostic evaluation of the above interstitial lung diseases. In the case of our patient group, a larger sample size would be required and the results would have to be verified on an independent testing set.

Our study and the study by Drent et al. [33] both evaluated the possibility of differentiating individual disease entities on a set composed of three conditions only: sarcoidosis, HP and IPF. So, the computer application developed on the basis of the results has certain limitations and should be used with caution. How the diagnostic accuracy would be affected by the inclusion of other interstitial lung diseases (e.g. organising pneumonia, eosinophilic pneumonias, acute interstitial pneumonia, interstitial pulmonary changes in the course of connective tissue diseases) is currently unknown and should be investigated.

Conclusions

Our study suggests that the analysis of CD4+/CD8+ ratio alone in differentiating sarcoidosis from HP and IPF is associated with a higher likelihood of establishing the correct diagnosis than using the object classification method based on the k-NN rule using all the parameters of induced sputum. In order to confirm these results, further studies are needed to assess the significance of sputum induction in the diagnostic evaluation of sarcoidosis and the other interstitial lung diseases in larger patient groups than ours.

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