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Cellular composition of induced sputum in sarcoidosis

Ocena składu komórkowego plwociny indukowanej w sarkoidozie

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Abstract

Introduction: The aim of this study was to evaluate the cellular composition of induced sputum (IS) in sarcoidosis and its role in assessing the disease activity. The safety of the procedure was also determined.
Material and methods: Sputum induction by inhalation of hypertonic saline was performed. Twenty-one samples from the healthy controls, 32 from patients with active disease, and 33 from subjects with inactive disease were analysed.
Results: The percentage of lymphocytes in IS was significantly higher in active sarcoidosis than in inactive disease and the control group (9.7% vs. 3.1% vs. 2.9%), and was the highest in the patients with parenchymal changes and active disease (13.3%). The percentage of macrophages was significantly lower in active sarcoidosis than in normal subjects (76.8% vs. 83.4%). It was also significantly lower in IS in active disease and stages II and III than in both subgroups with active and inactive stage I of sarcoidosis. There were no significant differences in the IS cell percentages between the whole sarcoidosis group and the controls. Sputum induction was well tolerated and stopped only four times, two of them because of dyspnoea or the decrease of PEF. The symptoms were well reversible after administering salbutamol.
Conclusions: Sputum induction by inhalation of hypertonic saline is safe, but the evaluation of IS differential cell counts is not useful in sarcoidosis diagnosing. However, it could be used in assessing the activity of the disease, especially in patients with interstitial lung changes.

Key words: sarcoidosis, induced sputum, safety

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Streszczenie

Wstęp: Celem pracy była analiza składu komórkowego plwociny indukowanej (PI) u chorych na sarkoidozę i ocena, czy różnice we wzorze odsetkowym komórek pozwalają określić aktywność choroby. Oceniano także bezpieczeństwo procedury.
Materiał i metody: Indukcję plwociny przeprowadzano przez inhalację hipertonicznego roztworu chlorku sodu. Do analiz włączono 21 materiałów uzyskanych od osób zdrowych, 32 od chorych z aktywną i 33 od osób z nieaktywną sarkoidozą.
Wyniki: Odsetek limfocytów był istotnie wyższy w PI w aktywnej sarkoidozie niż w chorobie nieaktywnej i u osób zdrowych (9,7 *v.* 3,1 i 2,9%). Najwyższy był on w materiale od chorych z aktywną chorobą i zmianami miąższowymi w płucach (13,3%). Odsetek makrofagów był istotnie niższy w aktywnej chorobie niż u osób zdrowych (76,8 *v.* 83,4%). W PI od osób z aktywną chorobą w fazach II i III był on także niższy, niż w fazie I sarkoidozy, niezależnie od aktywności choroby. Nie było natomiast różnicy we wzorze odsetkowym komórek w PI pomiędzy całą grupą chorych na sarkoidozę a grupą kontrolną.
We wszystkich badanych grupach procedura indukcji plwociny była dobrze tolerowana, przerwano ją tylko 4 razy, w tym dwukrotnie z powodu duszności lub spadku PEF. Objawy te szybko ustąpiły po podaniu dodatkowej dawki salbutamolu.
Wnioski: Indukcja plwociny przez inhalację hipertonicznego roztworu chlorku sodu jest procedurą bezpieczną, ocena jej składu komórkowego nie przydaje się jednak w diagnostyce sarkoidozy. Może ona natomiast służyć ocenie aktywności choroby, zwłaszcza u osób ze zmianami w miąższu płuc.

Słowa kluczowe: sarkoidoza, plwocina indukowana, bezpieczeństwo

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Introduction

Inhalation of hypertonic saline (NaCl) aerosol as a safe method of provoking sputum production and expectoration was first described in 1958 by H. Bickerman, who applied it in patients with lung cancer in order to obtain material from the lower respiratory tract for cytological analysis [1]. The method was also used for diagnostics of pulmonary tuberculosis and pneumocystodosis [2, 3]. In 1992, Isabelle Pin suggested using the induced sputum technique (IS) for the assessment of airway inflammation in patients with asthma [4]. Since then, the method has been developing and is now widely applied in other diseases of the respiratory tract [5–8].

Sarcoidosis is a multisystem granulomatous disease of young and middle-aged adults, which predominantly affects the lungs and intra-thoracic lymph nodes [9]. The granuloma formation in lung parenchyma is preceded by the accumulation of inflammatory cells with macrophages and lymphocytes predominance [10, 11]. The type of cells engaged in lower respiratory tract inflammation could be assessed by bronchoalveolar lavage (BAL) fluid analysis, which is a commonly used diagnostic method in sarcoidosis [9, 12]. It has been demonstrated that cellular pattern of BAL fluid reflects the cellular composition of inflammatory infiltrates in lung parenchyma [13, 14]. Non-invasive sputum induction method was recently suggested for this purpose [5, 15–17]. The potential role of this investigation in interstitial lung diseases has not yet been established.

The aim of the study was to analyse the cell composition of IS from patients with sarcoidosis affecting thoracic organs as well as to assess if varying cell differential counts can reflect the activity of the disease.

The safety of the procedure was also assessed in the studied population.

Material and methods

The study group included 87 patients with sarcoidosis affecting thoracic organs, who were not treated with glucocorticosteroids or immunosuppressants within at least three months preceding induction. The study subjects were recruited from patients of the Pneumonology Department of the Medical University of Gdańsk (MUG) and of the outpatient clinic for lung diseases.

The control group included 32 healthy persons, comprising medical students and friends and personnel of the Pneumonology Department of MUG.

Persons included in the study were non-smokers and had no symptoms of respiratory tract infection during the four weeks preceding the trial. All participants gave written informed consent, the formulary of which had been approved by the Independent Bioethical Committee of the MUG.

Anamnesis was taken, and physical examination was performed in all subjects, followed by spirometry (spirometer Pneumo-Record, abcMed, Poland). The results of the spirometry testing were interpreted according to the generally accepted criteria [18]. Chest X-ray was taken in patients with sarcoidosis and then used for assessment of disease stage [9, 19]. All patients in the control group had normal chest X-rays, taken as part of periodic health assessment at their workplace or school within 12 months preceding the current study.

Other investigations were performed in patients with sarcoidosis when needed, as part of routine diagnostic work-up and depending on particular indications. These included bronchofiberoscopy with BAL (in 37 patients), high resolution computed tomography (HRCT) scanning of the chest (in 56 patients), and microscopic evaluation of tissue or cytological samples. Diagnosis of sarcoidosis was made according to recommendations of the international expert panel of the American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Diseases (ATS/ERS/WASOG) [9].

Sarcoidosis was classified as active (group A) in patients having signs of Löfgren syndrome or presenting two or more of the following signs: lymphocytosis in BAL fluid exceeding upper normal limit, i.e. 15% [20], CD4+/CD8+ ratio in BAL fluid > 3.5; ground-glass attenuation on chest HRCT scans as well as clinical symptoms (cough, dyspnoea, chest discomfort) observed for the first time or progressing during the previous three months; worsening of spirometry results; or disease progression confirmed radiologically in chest scans [9, 21]. In all other cases, sarcoidosis was classified as non-active (group B).

Sputum induction was performed 15 minutes after inhalation of 200 μg salbutamol, during four cycles of 5 minutes each, according to the previously described protocol [22, 23], modified through the usage of 5% saline solution (NaCl). An ultrasound nebuliser Thomex MB (MEDBRYT, Warsaw, Poland) was used for aerosol production. Each participant rinsed his or her mouth with water after each inhalation cycle, and, if coughing began, spat out the entire expectorate into a container. Before and after each inhalation cycle three measurements of peak expiratory flow (PEF) were

performed and the maximal obtained reading was recorded. In cases of PEF decrease ≥ 20% from baseline value, the procedure was interrupted, an additional dose of salbutamol was administered, and PEF was controlled again. In several subjects who did not produce IS during the first attempt but agreed to have another one, induction was performed again after one week.

Induction procedures were carried out early in the day, following BAL, not earlier than three days (average 5.9 days) after bronchofiberoscopy.

The volume of expectorate was measured, and samples smaller than 1 ml were excluded from the study [24]. Samples of adequate volume were examined within 2 hours. The whole expectorate of sputum plus saliva was processed according to procedures described by other authors and to recommendations of the ERS working group [25–27].

In order to homogenise the obtained samples, 0.1% dithiothreitol (DTT) solution was added in the same amount as the expectorate and then put in a shaking water bath at 37°C for 15 minutes. Subsequently, phosphate buffer saline solution (PBS) was added at an equal amount to the sample containing IS and DTT.

Total cell count per μl of the processed sample was then assessed by flow cytometry (Cell Dyn 3200, Abbott, USA), and two cytospin slides were prepared using 300 ml of the solution for each of them (Cytospin 3, Shandon Southern Instruments, USA). These were stained with May-Grünwald--Giemsa and analysed by an experienced cytologist, who received no information about the patients. Differential cell count was performed incorporating all the cells contained in the cytospin preparations, including at least 400 non-squamous cells. Differential count of macrophages (M), eosinophils (E), lymphocytes (L), neutrophils (S), and bronchial epithelial cells (N), excluding squamous epithelial cells, was then reported [28, 29]. The percentage of squamous epithelial cells was reported separately as a marker of sample contamination by cells from the oral cavity (saliva). Only adequate samples were included in the final analysis, these being of more than 1 ml in volume [24] and containing <80% squamous epithelial cells [29–31]. Total cell count (cytosis) was calculated for each obtained induced sputum sample, including the total amount of inflammatory cells in the sample (M, E, L, S and N), and excluding squamous epithelial cells originating from the nasopharynx.

Sputum induction was performed 94 times in 87 patients with diagnosed or suspected sarcoidosis. These procedures yielded adequate material in 68 cases, of which three were excluded because diagnosis of sarcoidosis was not confirmed in these subjects. Among the remaining 65 persons, active disease was diagnosed in 32 subjects (group A) and non-active sarcoidosis in 33 patients (group B).

Group A included 32 patients with sarcoidosis diagnosed for the first time ever (20 subjects) or presenting relapse or recurrence of the previously diagnosed disease (12 persons). Diagnosis was confirmed histopathologically in 24 patients (75%). Group A included 15 patients with stage I, 13 subjects with stage II, and four persons with stage III disease.

Group B consisted of 33 patients, of whom 18 had stable disease, 12 had radiological signs of regression, and three were currently diagnosed with sarcoidosis (for the first time ever) but had non-active disease. Histopathological confirmation was obtained in 28 cases (84.8%). The group included 12 patients with stage I, 10 with stage II, and 10 with stage III disease. In one patient the disease stage was classified as II/IV.

Sputum induction was performed 35 times in 32 persons in the control group, obtaining diagnostically adequate samples in 22 cases. Of those, one sample was excluded from final analysis as being clearly aberrant; this was a sample from a woman in her thirties, working in microbiology laboratory, which disclosed a high level of lymphocytosis (13%). The remaining samples from 21 patients were included in the analysis, and are referred to as group K (control group).

Patient characteristics are presented in Table 1.

Statistical analysis

Normal distribution of the obtained differential counts in IS was verified using Shapiro-Wilk test. Differences in percentages of respective cell types and in total cell count between the groups were analysed using Mann-Whitney *U* test. Statistical significance of changes in PEF value during induction was assessed using Wilcoxon test for matched pairs. The level of statistical significance was adopted for $p < 0.05$.

Results

The volume of the obtained expectorate did not differ significantly between the groups. The respective mean volumes were: 3.8 ml in group A (range 2–12.5), 4.2 ml in group B (2–9.8), and 4.2 ml in group K (1.6–9.8). The percentage of squamous epithelial cells was significantly lower in samples from healthy subjects as compared to patients with sarcoidosis, with mean values of 39.1% in group A (range 4–79; $p = 0.042$), 39.9% in group B (2–79; $p = 0.027$), and 26.6% in group K (2–79).

Table 1. The characteristics of examined groups — a number of cases with each abnormality

	The number of cases	The mean age (range)	FEV_1 % pred.	VC % pred.	Abnormal spirometric test	Symptoms from respiratory tract	Löfgren syndrome	Lymphocytosis in BAL > 15 %	CD4/CD8 in BAL > 3,5	„Groung glass opacity" in HRCT
Group A	32 (14K,18M)	39.1 (24–68)	84.44	85.14	17	21	13	6	20	9
Group B	33 (15K,18M)	42.6 * (23–64)	89.79	90.22	12	7	0	0	1	0
Group K	21 (11K,10M)	35.2 (23–69)	104.15	104.11	0	0	0	–	–	–

* the difference is statistically significant versus group K ($p < 0.05$)

The distribution of total cell count per sample ml and distribution of lymphocyte percentage in IS were normal in group K, the others were inconsistent with normal distribution.

Patients with active sarcoidosis (group A) had significantly higher lymphocyte percentage in IS as compared to groups B and K. These patients also had significantly fewer macrophages than persons in group K. There were no differences in differential cell counts in IS between patients with non-active sarcoidosis and healthy subjects. The percentage of bronchial epithelial cells in group A was higher than it was in group K, with a nearly significant difference ($p = 0.05$). Detailed results are presented in Table 2.

For the purpose of further analysis, patients with sarcoidosis were assigned to subgroups of different disease stage. Two subgroups were thus identified in the group of patients with active disease as well as in the group with non-active sarcoidosis. The respective subgroups consisted of cases with parenchymal lung changes (combined stage II and III disease), and subjects with stage I disease. The highest lymphocyte percentages were found in IS from patients with active stage II and III sarcoidosis, and were significantly higher as compared to samples from patients with non-active disease, both stage I ($p = 0.048$), combined stage II and III ($p = 0.0048$), and compared to the control group ($p = 0.006$). Lymphocyte percentages were also higher compared to samples from patients with stage I active disease, but the difference was non-significant ($p = 0.22$).

The percentage of macrophage in IS in stage II and III active sarcoidosis was significantly lower compared to stage I active disease ($p = 0.024$), stage I non-active disease ($p = 0.0016$), and to the control group ($p = 0.007$). In patients with stage II and III non-active sarcoidosis, macrophage percentages in IS were significantly lower than in stage I non-active disease ($p = 0.018$). Differential cell counts in IS from patients in the respective subgroups and control group are presented in Table 3.

Analysis of all patients with sarcoidosis (groups A and B combined) showed no significant differences in differential cell count in IS as compared to the control group. There was a trend for higher lymphocyte percentage in patients with sarcoidosis as compared to the control group (6.3% vs. 2.9%) but the difference was not statistically significant ($p = 0.26$). Detailed results are presented in Table 2.

The procedure of sputum induction was well tolerated by the healthy subjects and the patients with sarcoidosis. Peak expiratory flow (PEF) values were measured before and after each induction cycle, for safety reasons. A small but statistically significant decrease in PEF value from baseline was observed after each induction cycle. In the entire analysed population, mean PEF decrease from baseline was 18.68 l/min (4.1%) after the first cycle, 16.25 l/min (3.5%) after second, 20.43 l/min (4.4%) after the third, and 20.31 l/min (4.4%) after the fourth induction cycle. Figure 1 demonstrates the decrease in PEF value during sputum induction in patients with sarcoidosis and in the control group.

Sputum induction was interrupted four times during all 129 procedures. A female patient in her fifties, with stage I sarcoidosis and normal baseline spirometry, experienced a PEF decrease of 33.9% (from 530 l/min to 350 l/min) after the first induction cycle but reported no dyspnoea. An additional dose of salbutamol was administered, and 30 minutes later PEF increased again to 500 l/min. A week later sputum induction was performed again in the patient, and maximal observed PEF decrease was of 9.8% from baseline, measured

Table 2. The absolute cell counts and differential cell counts of IS in examined groups. The values are mean ± SD

%	Group A+B (n = 65)	Group A (n = 32)	Group B (n = 33)	Group K (n = 21)
Macrophages	80.1 ± 16.4	76.8 ± 19.1	83.2 ± 12.7	83.4 ± 18.5*
Lymphocytes	6.3 ± 9.1	9.7 ± 11.6	3.1 ± 3.6*	2.9 ± 2.2*
Eosinophiles	0.6 ± 1.9	0.9 ± 2.4	0.3 ± 1.3	0.7 ± 1.5
Neutrophiles	10.6 ± 11.8	10.0 ± 11.6	11.1 ± 12.1	11.7 ± 18.4
Bronchial epithelial cells	2.4 ± 3.3	2.5 ± 2.9	2.3 ± 3.7	1.3 ± 1.6
Absolute cell count	0.997 ± 1.061#	1.174 ± 1.166	0.825 ± 0.933*	1.445 ± 1.005

The absolute cell count presents a numer of cells x $10^3/\mu l$ material
*the difference is statistically significant versus group A ($p < 0.05$)
#the difference is statistically significant versus group K ($p < 0.05$)

Table 3. The differential cell counts of IS according to stage and activity of the disease. The values are mean ± SD

%	Stage I A	Stage II/III A	Stage I B	Stage II/III B	Group K
Macrophages	85.3 ± 9.0*	69.3 ± 22.6#	89.9 ± 6.5*	79.5 ± 13.9#	83.4 ± 18.5*
Lymphocytes	5.6 ± 5.4	13.3 ± 14.3#	3.9 ± 4.4*	2.6 ± 3.0*	2.9 ± 2.2*
Eosinophiles	1.1 ± 2.2	0.8 ± 2.6	0 ± 0	0.5 ± 1.6	0.7 ± 1.5
Neutophiles	6.2 ± 7.8	13.4 ± 13.5	5.1 ± 5.0	14.5 ± 13.7	11.7 ± 18.4
Bronchial epithelial cells	1.7 ± 1.2	3.2 ± 3.8	1.2 ± 1.5	3.0 ± 4.4	1.3 ± 1.6
The numer of cases in groups	15	17	12	21	21

Stage IA — active sarcoidosis in stage I; stage IB — inactive sarcoidosis in stage I; stage II/III A — active sarcoidosis in stage II and III; stage II/IIIB — inactive sarcoidosis in stage II and III; group K — control group
*values significantly different versus stage II/III A ($p < 0.05$)
#values significantly different versus stage IB ($p < 0.05$)

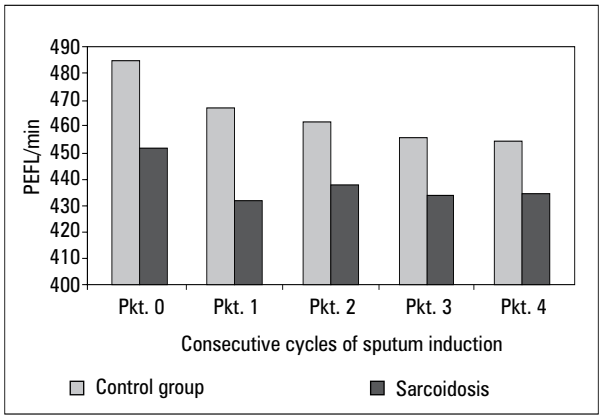

Figure 1. PEF values before and after each inhalation cycle in healthy group and patients with sarcoidosis

after the fourth induction cycle. In another female patient, aged 52, presenting stage III/IV sarcoidosis and spirometric signs of obturation (FEV_1 of 76.2% of the expected value), the procedure was interrupted after the fourth inhalation cycle due to a feeling of dyspnoea; the PEF value decreased by 10% from baseline at that time (from 300 l/min to 270 l/min). Dyspnoea regressed five minutes after administration of an additional salbutamol dose, and the PEF value returned to baseline after 15 minutes. All patients reported a salty taste in their mouth during the procedure, but this was the reason for early test interruption only in a single subject (induction interrupted after two minutes of the fourth cycle). Three persons reported nausea during induction but only a single female patient interrupted the procedure because of that, after the second nebulisation course.

Discussion

The procedure of sputum induction is a promising technique, which can contribute information concerning cellular constituents but also chemical composition of the bronchial fluid

liquid phase. Changes in sputum cell composition may reflect bronchial inflammation in patients with asthma and chronic obstructive pulmonary disease [4, 28, 32], and the obtained results are comparable to data from analysis of BAL samples and biopsies of bronchial mucosa [24, 26, 31, 33]. Sputum induction in diagnostics of tract diseases is proposed as an alternative to more invasive procedures [7, 8, 16].

Bronchoalveolar lavage (BAL) is a widely accepted diagnostic method in interstitial lung diseases, including sarcoidosis [9, 12]. Cytological data from samples obtained through this procedure reflect the composition of inflammatory infiltrate in lung parenchyma [13, 14]. Induced sputum is obtained from more central parts of the bronchial tree. However, during a 20-minute-long procedure, cell material can be obtained also from distal parts of the respiratory tract and lung alveoli [30]. Moreover, in patients with sarcoidosis, lymphocytic infiltration was found not only in lung interstitium but also in bronchial epithelium [34, 35]. These findings suggest that not only BAL but also induced sputum can have significantly different cell composition in patients with sarcoidosis and in healthy subjects.

Many authors have compared cell composition of induced sputum from patients with sarcoidosis and from subjects with other interstitial lung diseases, suggesting the applicability of this method for differential diagnostics [5, 17, 36, 37]. A higher percentage of lymphocytes was found in IS from patients with sarcoidosis as compared to healthy subjects [38–40]. No such difference was observed in the presented study when all patients with sarcoidosis were analysed as one population (group A+B). The percentage of lymphocytes was clearly higher in patients with sarcoidosis than in the control group, but the difference was not statistically significant. This discrepancy can be explained by the composition of the patient group. In the above-cited studies, included patients had newly diagnosed, active sarcoidosis, in which numerous lymphocytes can be found at the sites of inflammation [10, 41]. In the presented study, patients with ongoing as well as subjects with clinically inactive sarcoidosis were included, which resulted in less prominent differences in lymphocyte count as compared to healthy subjects. When patients with non-active sarcoidosis were excluded to a separate group in analysis, no differences in lymphocyte percentage were observed in IS as compared to healthy controls. These findings can suggest that analysis of cell composition in IS may not yield diagnostically important information in the entire population of patients with sarcoidosis or in persons with non-active disease.

When patients with active sarcoidosis were analysed as a separate subgroup, a significantly higher percentage of lymphocytes and lower macrophage counts were found in IS in these subjects as compared to healthy controls, similarly to reports from other authors. The observed lymphocyte percentage in IS in sarcoidosis is similar to values reported by other authors, who also analysed the entire amount of the obtained expectorate [37, 38]. There were no significant differences in eosinophil or neutrophil percentages between the studied groups in the presented study, which is in accordance with data from literature [38–40]. D'Ippolito also assessed the amount of bronchial epithelial cells in IS, and found a higher bronchial epithelial cell content in patients with sarcoidosis as compared to healthy persons [38]. In the presented study, the percentage of bronchial epithelial cells was also higher in subjects with sarcoidosis, but the difference was not significant, although nearly significant when comparing healthy persons to patients with active sarcoidosis. Bronchial epithelial cell content was not analysed in many studies analysing samples from patients with sarcoidosis and healthy persons [15, 36, 39, 40].

In the presented study, the cell composition of IS was evaluated in various stages of sarcoidosis. Particularly high lymphocyte percentage was observed in samples from stage II and III active sarcoidosis, clearly exceeding values found in patients with non-active disease or in healthy subjects. The difference in lymphocyte percentage was not significant between stage I active sarcoidosis and other groups. Many of these patients had relatively few lymphocytes in IS; however, in three subjects lymphocyte percentage exceeded 10%. The highest value was observed in a male patient in whom conventional chest X-ray yielded diagnosis of stage I sarcoidosis but HRCT scans revealed ground glass attenuation, pointing to an active interstitial disease. Higher lymphocyte percentage in IS from patients with stage II and III sarcoidosis may possibly be explained by the admixture of cells originating from the most peripherally located parts of the respiratory system or by a higher intensity of inflammation in the central parts of the bronchial tree in patients with interstitial changes. The above-mentioned studies investigated IS from patients with stage II sarcoidosis only [37] or from patients with different stages of disease analysed as one group [5, 17, 36, 38, 39].

In the presented study, a relatively low percentage of neutrophils was observed in IS from

patients in all of the studied subgroups. Many authors, investigating IS from patients with sarcoidosis and other diseases have reported higher neutrophil percentages, up to 40% [17, 39, 42]. This discrepancy may be explained by methodological differences between respective studies [30, 43, 44]. Many authors who applied a nebulisation procedure of similar length to the presented study, and included thorough and multiple cycles of oral cavity cleaning, observed even lower neutrophil counts than in the presented material [4, 38, 40, 44].

Altered osmolarity inside the respiratory tract after inhalation of hypertonic saline solution causes transient hyperreactivity of the bronchial tree and may induce bronchospasm during the procedure of sputum induction [25, 45, 46]. In order to avoid this phenomenon, inhalation of a bronchodilating agent precedes inhalation of saline solution under the procedure of sputum induction. The most commonly used agent is salbutamol [8, 27], which was demonstrated to more efficiently prevent bronchospasm during sputum induction [47] and had no effect on differential cell count in the obtained samples [48, 49]. Following such a procedure, sputum induction is safe and well tolerated by healthy subjects and by patients with chronic obstructive pulmonary disease, asthma, or interstitial lung diseases [50–53].

The sputum induction procedure was well tolerated in the presented study. The observed PEF decrease during induction was statistically significant, but the greatest registered mean decrease in this parameter was 20.43 l/min (4.44% of the expected value), and occurred after the third nebulisation cycle. The procedure was interrupted due to dyspnoea or PEF decrease of > 20% from baseline in two patients, and symptoms quickly disappeared after administration of another dose of salbutamol.

Conclusions

Sputum induction is a safe diagnostic method, well tolerated by patients with sarcoidosis. The evaluation of IS differential cell counts does not seem to be of much importance for disease diagnostics. However, observation of increased lymphocyte percentages in sputum samples can be of use in the assessment of disease activity, especially in patients with interstitial lung changes.

Conflict of interest

The authors declare no conflict of interest.

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