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Original Article

Is Induced Sputum A Useful Noninvasive Tool in the Diagnosis of Pulmonary Sarcoidosis?

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Abstract

OBJECTIVES: In patients with pulmonary sarcoidosis, the provocation of sputum expectoration through the inhalation of hypertonic saline has been investigated as an alternative diagnostic tool for invasive procedures. We aimed to assess the diagnostic value of induced sputum (IS) by observing its cell distribution in patients with a confirmed histopathological diagnosis of sarcoidosis.

MATERIALS AND METHODS: In this prospective, cross-sectional study, we compared the IS results of 20 patients with a histopathologically confirmed pulmonary sarcoidosis diagnosis and 24 healthy volunteers. The percentages of macrophages, lymphocytes, neutrophils, and eosinophils in IS and the CD4/CD8 ratio were compared.

RESULTS: The percentage of lymphocytes in IS was significantly higher in the pulmonary sarcoidosis patients compared to the control group (41.6% vs 8.9%, p<0.001). There were no significant differences in the other IS cell percentages and CD4+/CD8+ ratio between the groups. Sputum induction was well tolerated.

CONCLUSION: Sputum induced by the inhalation of hypertonic saline is a safe, inexpensive, less invasive, and easily repeated method and can be a valuable alternative to other invasive methods in the diagnosis of pulmonary sarcoidosis.

KEYWORDS: Clinical problems, diagnostic methods, interstitial lung disease

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INTRODUCTION

The provocation of sputum expectoration through saline inhalation was first described in 1958 by H. Bickerman. This induced sputum (IS) technique was used for obtaining cytological material from the lower respiratory tract in lung cancer patients [1] and for the diagnosis of pulmonary infections and airway inflammation in different pulmonary conditions [2-5].

Sarcoidosis is characterized by hilar lymphadenopathy, pulmonary infiltration, and ocular and cutaneous lesions. It is described as a systemic granulomatous disease mostly affecting young adults. The causes of sarcoidosis are not precisely known. The diagnosis of pulmonary sarcoidosis is mainly based on the radiological futures and the detection of non-necrotic granulomas in histopathological materials [6]. Pulmonary sarcoidosis can be diagnosed by bronchoalveolar lavage (BAL) analysis or by histopathological evaluation of biopsies through fiberoptic bronchoscopy (FOB), video-assisted thoracoscopy (VATS), or open lung biopsy. These procedures are firstly invasive and are not reliable with regard to detecting granulomas in the obtained sample. FOB is the least invasive, but it is not free of complications. The diagnostic accuracy of FOB in sarcoidosis diagnosis is approximately 85%. However, it is not always possible to perform FOB in patients with suspected sarcoidosis. Some patients may not agree to undergo FOB due to its inconvenience [7-9]. Hence, clinicians need noninvasive techniques, such as IS, for the diagnosis of pulmonary sarcoidosis among clinicians.

The pathophysiology of pulmonary sarcoidosis is based on alveolitis caused by macrophages and Tcell lymphocytes [10]. Bronchoalveolar lavage analysis shows inflammation of the interstitium and can be considered a diagnostic tool. In previous studies, lymphocytes, CD41 lymphocytes, and activated macrophages have been found to be high in the BAL of patients with pulmonary sarcoidosis [11,12]. In recent studies, the use of IS as an alternative to BAL was investigated in sarcoidosis patients [13-16]. The results of studies in this regard are contradictory, and the efficiency of this method has not yet been clearly established.

We aimed to investigate the diagnostic value of IS by observing the cell distribution in the sputum of patients with a confirmed histopathological diagnosis of sarcoidosis.

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MATERIAL AND METHODS

Study Population

This prospective, controlled study was performed in a pulmonary clinic of a university hospital. The ethics committee of the institution approved the study Gazi University Research Ethics Committee of Medical Faculty (19.01.2009 and No: 19). The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Informed consent was obtained from all the participants of the study.

Patient Characteristics

Inclusion criteria: Twenty patients newly diagnosed with pulmonary sarcoidosis and not undergoing any treatment along with the following criteria were included in the study:

- Older than 18 years of age
- Absence of other respiratory diseases (such as; asthma, chronic obstructive lung diseases, bronchiectasis, tuberculosis, lung cancer and non-sarcoidosis intertitial lung diseases)
- Non-smokers
- Absence of symptoms of respiratory tract infection in the previous 4 weeks
- No treatment with oral or inhaled corticosteroids or antibiotics in the previous 3 months
- Forced expiratory volume at 1 second (FEV_1) above 60%
- Oxygen saturation above 90% at rest and absence of hypoxemia

The control group included 24 healthy volunteers meeting the abovementioned inclusion criteria [14].

Sarcoidosis Diagnosis and Staging

Pulmonary sarcoidosis was diagnosed based on the recommendations adopted by an international panel of experts [17] and confirmed histopathologically. The staging of patients' sarcoidosis was performed radiologically using a chest X-ray.

Sputum Induction and Processing

Sputum was induced through the inhalation of aerosols of hypertonic saline as described in another study [18]. The sputum samples were processed by selecting the mucus plugs and mixing with four parts 0.1% dithiothreitol and Dulbecco phosphate buffered saline (PBS), filtered through a 48-mm nylon mesh, and centrifuged at 3000 rpm for 10 min at 4 C. The cell pellet was resuspended in PBS. The filtered cells were diluted in RPMI 1640 medium and cytospin slides were prepared using a cytocentrifuge (Shandon, Thermofisher Scientific) for 5 minutes at 1000 cycles. The cytospin slides were stained using the Giemsa stain. Samples containing less than 20% squamous cells were considered eligible for the study [19].

Pulmonary Function Tests

Pulmonary function tests (Sensormedics Vmax Series 20C Respiratory Analyzer, Yorba Linda, USA) were performed according to the American Thoracic Society's guidelines [20]. Forced expiratory volume at 1 second, forced vital capacity (FVC), FEV_1/FVC ratio, carbon monoxide diffusion capacity (DLCO), vital capacity (VC), and total lung capacity (TLC) of the participants were recorded.

Total Cell Count and T-cell Phenotyping in the Induced Sputum

Total cell count per microliter of the processed sample was assessed using flow cytometry (Cell Dyn 3200, Abbott, USA), and two cytospin slides were prepared using 300 mL solution each (Cytospin 3, Shandon Southern Instruments, USA). Cytospin slides were spotted using the May-Grunwald-Giemsa stain and analyzed by an experienced microbiologist blinded to patients' and control group data. A differential cell count was performed combining all the cells in the cytospin slides, including at least 400 non-squamous cells. Thereafter, the counts of macrophages, eosinophils, lymphocytes, and neutrophils were reported [21,22].

A T-cell subpopulation analysis was carried out using flow cytometry with the FACSCalibur™ system (Becton Dickinson, USA) and TriTEST CD4/CD8 monoclonal antibodies (Becton Dickinson, USA) according to the manufacturer's instructions. The monoclonal antibody panel allowed us to assess the following T-cell subpopulations: CD4+ (helper T cells) and CD8+ (cytotoxic T cells). The anti-CD4, and anti-CD8 antibodies were conjugated using fluorescein isothiocyanate and phycoerythrin [23].

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS® IBM Corp.; Armonk, NY, USA) version 21.0 program. Age was presented as mean ± standard deviation (SD). Data were expressed as frequency distribution and percentages. The Mann–Whitney U tests were used for comparing the differences between the groups. P values of <0.05 were considered statistically significant.

RESULTS

In total, 44 participants were enrolled in the study (20 patients, 24 healthy volunteers); 72% in the sarcoidosis group were females, and the mean age of the patient population was 41±12 years. The characteristics of patients and controls are shown in Table 1.

The sarcoidosis cases were confirmed through histopathology (FOB in 15 patients, mediastinoscopy in 3, VATS in 1, supraclavicular lymph node biopsy in 1). Four (20%) patients had stage I disease and 16 (80%) patients had stage II disease.

The control group included 24 healthy volunteers (18 were women; age range, 21–72 years).

Patients with sarcoidosis were older than the control subjects and had lower FVC, VC, and TLC, but these differences were not statistically significant. The FEV_1 (88.6% vs 101.2%, p=0.020), FEV_1/FVC ratio (79.8% vs 87.8%, p=0.001), and DLCO (68.0% vs 138.0%, p=0.04) values of sarcoidosis patients were statistically lower than the control group. All the patients and controls tolerated sputum induction well without any adverse events. The sputum samples were adequate for all the participants.

The cell counts in the IS from patients and volunteers are reported in Table 2. The lymphocyte ratio in the IS of the patient group was significantly higher than the control group (41.8% vs 8.90%, p<0.001). However, there were no difference between the groups in terms of the CD4/CD8 ratio (4.5% vs 31%, p=0.058; Table 2, Figure 1).

Table 1. Characteristics of examined groups

Parameters		Patient group n=20	Control group n=24	p
Age (mean, years)		42.00±11.47	39.45±17.39	0.579
Sex (%)	Female	14 (70)	18 (75)	0.818
	Male	6 (30)	6 (25)	
PFT	FEV_1 (%)	88.55±18.55	101.17±15.27	0.02
	FVC (%)	93.35±17.82	99.97±14.96	0.195
	FEV_1/FVC	79.75±5.33	87.84±8.78	0.001
	DLCO (%)	68.05±23.43	132.17±38.78	0.04
	VC (%)	89.58±30.19	97.30±14.66	0.342
	TLC (%)	94.23±22.60	106.17±11.53	0.058

PFT: pulmonary function test; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; DLCO: carbon monoxide diffusion capacity; VC: vital capacity; TLC: total lung capacity

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

	Patient group n=20	Control group n=24	p
Macrophage (%)	32.9±24.5	28.9±25.3	0.678
Eosinophil (%)	4.6±2.8	5.6±3.4	0.727
Lymphocyte (%)	41.6±11.8	8.9±8.7	<0.001
Neutrophil (%)	34.7±12.8	15.5±10.2	0.175
CD4/CD8 ratio	4.5±4.4	3.1±2.2	0.334

SD: standard deviation

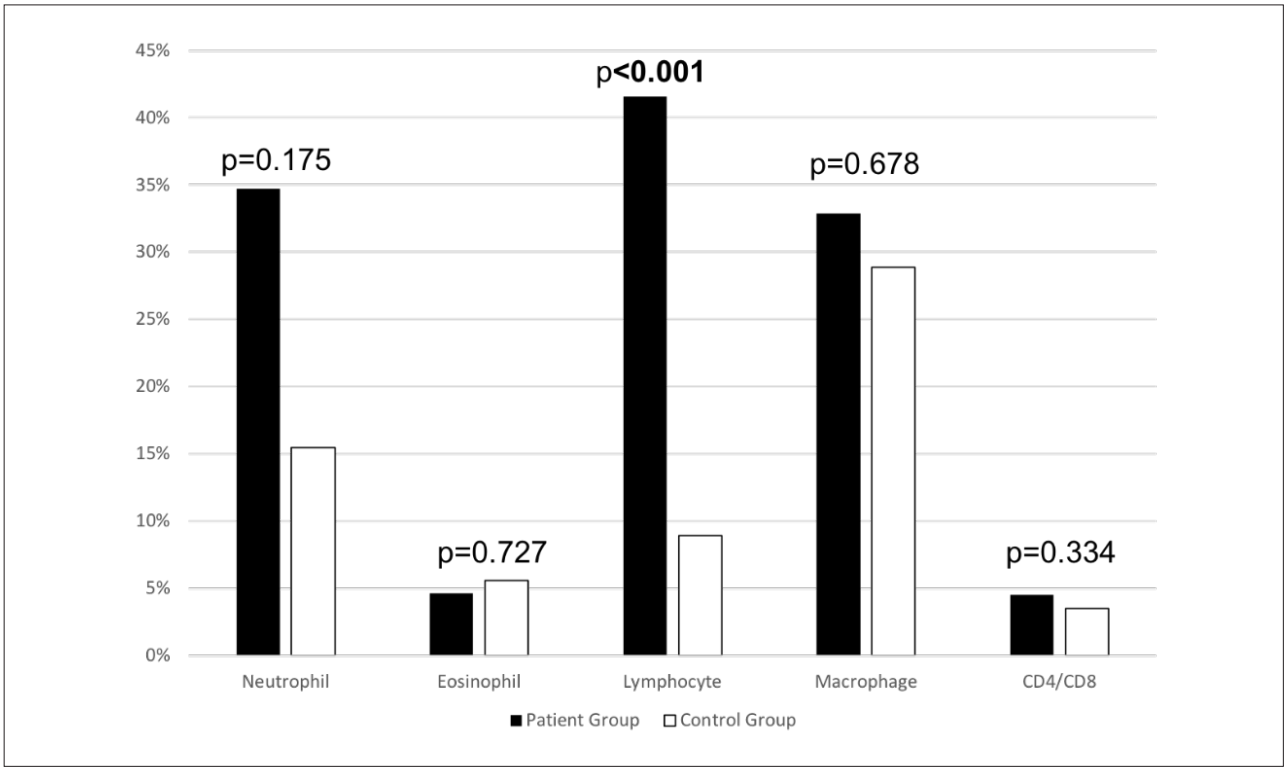


Figure 1. Differential cell counts in the IS of the groups
IS: induced sputum

DISCUSSION

In this study, we showed that the IS samples from newly diagnosed, untreated patients with pulmonary sarcoidosis had significantly more lymphocytes compared to healthy volunteers. To the best of our knowledge, this is the first study from our country to compare cellular distribution with Tcell subtyping in the IS of sarcoidosis patients.

Bronchoalveolar lavage is a useful diagnostic tool for collecting cells and secretions from particularly the lower respiratory tract [24]. BAL specimens, which show inflammatory response in interstitial lung diseases, have been used as a diagnostic tool in many pulmonary diseases including sarcoidosis [25]. Although clinicians can obtain cells from the peripheral part of the lung, it is a relatively invasive method and can only be applied in appropriate conditions. Currently, the only noninvasive method to directly study cellular inflammatory processes in the lower respiratory tract is the examination of sputum. IS provides a cellular sample from the central region of the bronchial tree. Therefore, it can be considered an unsuitable tool for the diagnosis of pulmonary sarcoidosis. However, it has been proven that the 20-minute IS procedure can be used to obtain sputum samples from the distal part of the lung [26]. Furthermore, lymphocytic infiltration in sarcoidosis can be involved not only in the interstitium but also in the bronchial epithelium [27,28]. This relatively cheap, less invasive, and easily repeatable procedure can be a precious diagnostic alternative to FOB and BAL.

Induced sputum was first studied as new diagnostic method for sarcoidosis diagnosis by D'Ippolito et al. [13]. They examined IS of 15 newly diagnosed, untreated sarcoidosis cases and 12 healthy volunteers. They found that patients with sarcoidosis had a significantly higher number of total cells (65.1% vs 30.1%, p=0.01) and lymphocytes (9.4% vs 3.8%, p=0.05) compared to the healthy volunteers, while the number of macrophages was significantly lower (60.4% vs 69%, p=0.05) [13]. After this study, many studies have been conducted to investigate the importance of IS in the diagnosis and clinical followup of sarcoidosis cases.

The lymphocyte count in IS in the sarcoidosis group was 41.6% in our study, which was comparable to that obtained in other studies ranging from 0.4% to 67.1% [13-15]. Lymphocyte counts and CD4/CD8 ratio in BAL specimens are is still evaluated and recommended in the clinical assessment of patients with pulmonary sarcoidosis [29]. Although, the lymphocyte count in sarcoidosis cases was higher than those of the control group, no significant difference in the CD4/CD8 ratio was found between the groups. We attributed this result to the small sample size of our study population. Also, our study groups were homogenous with regard to smoking and absence of additional lung diseases. Previous studies that found a higher CD4/CD8 ratio in the IS of sarcoidosis patients, which mainly compared this ratio with non-sarcoidosis interstitial lung diseases (NS-ILD) [23,30,31]. In our study, we compared CD4/CD8 ratio in the IS of sarcoidosis with healthy subjects, and we could not find any difference between CD4/CD8 ratios of the groups. We attributed this difference to the high level of CD4/CD8 ratio in the control subjects.

Similar to our results, researchers could not find any difference in the lymphocyte counts between sarcoidosis and control groups in another study [14]. The authors stated that the lower lymphocyte count was because of the higher number of the patients with non-active sarcoidosis than active cases [14]. In our study, we had 16 patients in the active stage.

In a study by Fireman [30], the differential cell counts in the IS specimens of the pulmonary sarcoidosis patients and NS-ILD patients were compared. Two-thirds (62.5%) of their 67 patients were in Stage 0-II and one-third (37.3%) were in Stage III-IV. They showed that pulmonary sarcoidosis patients' IS had a statistically and significantly higher percentage of lymphocytes (19.7% vs 15.0%, p=0.04) and macrophages (36.4% vs 29.0%, p=0.017) and a lower percentage of neutrophils (38.3% vs 48.5%, p=0.017) and eosinophils (2.8% vs 7.2%, p=0.041) compared to the NS-ILD group. Furthermore, they showed that the CD4/CD8 ratio in sarcoidosis patients was significantly higher than the NS-ILD group (5.7 vs 2.1 p<0.0001) (30). In our study, although we compared IS results of the sarcoidosis patients with the healthy control group, we detected a higher lymphocyte count in the sarcoidosis group but no differences in the neutrophil and eosinophil counts.

In previous studies, the number of neutrophils in IS has been reported to be over 40% [31,32]. Although not statistically significant (p=0.175), the rate of neutrophils in the sarcoidosis group was higher than that of the control group (34.7% vs 15.5%) in our study, but it was still below 40%.

Recently, in a study by Porzezińska et al. [14], the neutrophil counts were found similar in both active and non-active sarcoidosis patients and the healthy group, similar to our results. Authors explained this result with the methodological difference between studies. Also, it was thought that the reason of the lower number of neutrophils in their study was due to the exaggerated number of mouth-cleaning cycle during the procedure [14].

There are some limitations of our study. Firstly, the number of enrolled patients was relatively small. Previous studies regarding IS in sarcoidosis patients also included a small number of patients, and most of them were retrospective studies. Despite the small sample size of our study, we recruited all the patients prospectively and applied IS during pulmonary sarcoidosis diagnosis. However, our control group was recruited retrospectively from our archives [21].

In conclusion, in our study, similar to previous studies, included patients with newly diagnosed sarcoidosis who had a higher lymphocyte count than the healthy control group. Induced sputum may be a useful tool to diagnose sarcoidosis noninvasively. Future large scale studies are warranted to confirm and expand on our findings.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gazi University School of Medicine (19 January 2009/ 19).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – N.K., M.S., A.K., E.F.; Design – A.B., N.K., M.S.; Supervision – N.K., M.S., A.K., E.F.; Resources – F.Y., A.K., E.F.; Materials – A.B., M.S.; Data Collection and/or Processing – A.B., F.Y., N.K., M.S.; Analysis and/or Interpretation – A.B., F.Y.; Literature

Search – A.B., F.Y.; Writing Manuscript – A.B., F.Y., N.K.; Critical Review – N.K., M.S., E.F.

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