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Evaluation of bronchial mucoza and minor salivary gland biopsy in patients with sarcoidosis by flow volume maneuver

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Abstract

Introduction: The aim of our study was to determine predicting endobronchial sarcoidosis by considering minor salivary gland involvement, pulmonary function test, and bronchoscopic images.

Material and Methods: A total of 219 cases of sarcoidosis were retrospectively investigated. All cases were analyzed for age, gender, cigarette consumption, proven sarcoidosis skin biopsy, erythema nodosum, eye examination, minor salivary gland biopsy (MSGB), pulmonary functional evaluation, DLCO, six-minute walk test, angiotensin-converting enzyme level, erythrocyte sedimentation rate, radiologic stages, biopsies for diagnosis, bronchoscopic mucosal appearance, and biopsy results.

Results: A total of 219 cases (M:41, F:178); mean age was 45.9 ± 11.8 (20-75). Erythema nodosum in 67 (30.6%) cases. Radiological stages: Grade 0: 15 (6.9%), Grade 1: 104 (47.6%), Grade 2: 67 (30.5%), Grade 3: 22 (10%), and Grade 4: 11(5%). Of the total cases, 215 (98.1%) were diagnosed with at least one invasive procedure; 155 (70.7%) were from two or more sites. Sarcoidosis involvement of endobronchial mucosa with bronchoscopic imaging was found in 25 (16%); mucosal biopsy was found in 64 (41%); microscopic findings suggestive of sarcoidosis were observed in 30 (46.9%). Forty (18.2%) cases had obstructive pattern in the end airway. Thirty (46.9%) cases were diagnosed with endobronchial sarcoidosis by mucosal biopsy, and terminal airway stenosis was seen in seven (23.3%) cases. In patients diagnosed with endobronchial sarcoidosis by mucosal biopsy, terminal airway stenosis was observed in 23.3%, compared with 18.2% in the overall cohort. Bronchoscopic mucosal biopsy was positive in 25.8% (n= 8) of the cases with positive MSGB. Eight cases had MSGB, and findings were suggestive of sarcoidosis in the bronchoscopic appearance. The number of cases with positive mucosal biopsy results was 62.5% (n=5); a weak, significant correlation was found between positivity of MSGB and bronchoscopic transbronchial lymph node aspiration. There was also a weak, significant correlation between MSGB and bronchoscopic mucosal biopsy positivity. A statistically weak correlation was found between MSGB and bronchoscopic appearance and endobronchial sarcoidosis involvement.

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Conclusion: MSGB positivity can be used to predict bronchoscopic direct examination and mucosal biopsy positivity.

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Introduction

Sarcoidosis is a granulomatous disease of unknown cause that can affect many organs, tissues, and systems. The lungs and intrathoracic lymph nodes are the most commonly affected sites in sarcoidosis. In cases with appropriate clinical and radiological findings, the diagnosis is made by histopathological demonstration of noncaseating granulomas. Multiple organ involvement is essential for diagnosis. However, sarcoidosis can be diagnosed in the presence of any suitable clinical conditions such as hypercalcemia, angiotensin-converting enzyme elevation, together with a biopsy result, when two separate biopsies cannot be performed, and when involvement of two separate organs cannot be proven.¹⁻³

In cases where diagnosis is compatible with sarcoidosis, the first step is to select the appropriate site for biopsy. Fiberoptic bronchoscopy (FOB) and biopsy for the diagnosis of sarcoidosis is an effective and reliable method for the diagnosis of lung sarcoidosis because of its ease of application, generally not requiring general anesthesia, high diagnostic yield, and low complication rates.^{1,2} In the diagnosis of sarcoidosis, lip biopsy shows minor salivary gland involvement. When we compare lip biopsy with bronchoscopy, lip biopsy is one of the invasive procedures to be applied primarily because of its less invasiveness and low complication rates. According to a study conducted in Turkey, half of the sarcoidosis patients showed granuloma structure in lip biopsy, thus reducing the need for more invasive diagnosis.⁴

When we look at the involvement of lungs in sarcoidosis, it is a disease that cannot be ignored (41-71%) with endobronchial involvement. When we look at the findings of endobronchial involvement, erythema, hyperemia, capillary enlargement, irregular thickening, edema, presence of micronodules and macronodules in the mucosa, airway distortion, presence of bronchial or tracheal stenosis, retraction in the large airways, or rarely a mass lesion are the findings of endobronchial sarcoidosis.^{5,6}

In the evaluation of endobronchial mucosal involvement in sarcoidosis, bronchoscopic image and/or mucosal biopsy are considered. The characteristic appearance of mucosal involvement is the presence of nodular appearance. Biopsies taken from nodules are histopathologically noncaseating granulomas.^{1-3,5-7}

In one study, bronchoscopic mucosal biopsy was performed in patients with mucosal involvement with bronchoscopic direct examination, and the biopsy results were positive in 75% of the patients. Endobronchial mucosal biopsy is recommended in patients with findings suggestive of mucosal involvement. In addition, in patients with normal bronchial mucosa and no bronchoscopic findings suggestive of endobronchial sarcoidosis, a positivity of mucosal biopsy was observed in 30%. If bronchoscopy is planned in all patients with a preliminary diagnosis of sarcoidosis, it is also recommended to plan a mucosal biopsy.⁶

Involvement of the mucosa and submucosal glands in sarcoidosis is called endobronchial sarcoidosis. In endobronchial sarcoidosis, obstructive type changes are detected in respiratory function tests. If there is parenchymal involvement because of sarcoidosis, restrictive pulmonary function test (PFT) is performed.^{1,3,7} According to the results of the ACCESS study, the PFTs of the patients are 14% obstructive and 30% restrictive.⁶ According to the study of Kieszko et al., abnormal PFT was observed in more than half of the patients diagnosed with sarcoidosis by endobronchial mucosal biopsy.⁷

Given that endobronchial sarcoidosis often causes obstructive pulmonary changes because of submucosal gland involvement, this study aimed to evaluate whether minor salivary gland biopsy results, in combination with PFTs and bronchoscopic examinations, can predict endobronchial sarcoidosis.

Material and Methods

This retrospective study included patients who were diagnosed and followed up for sarcoidosis between January 2010 and December 2016 at the Department of Pulmonary Diseases, Istanbul University. Cases meeting at least one of the following conditions were included in the study:

1. For the diagnosis of sarcoidosis: appropriate clinical and radiological findings and exclusion of other diagnoses.
2. Demonstration of granulomas without caseification or histopathological findings suggestive of sarcoidosis in clinically suitable cases.

Patient's characterization

The study parameters included age, gender, cigarette consumption, biopsy-proven sarcoidosis skin involvement, erythema nodosum, eye examination, lip biopsy, pulmonary function tests (PFTs), DLCO, six-minute walk test, and serum angiotensin levels. Additional biochemical markers such as angiotensin-converting enzyme (ACE) and erythrocyte sedimentation rate (ESR) were also evaluated. Inflammation markers such as converting enzyme level and erythrocyte sedimentation rate were examined.

Diagnostic methods

While evaluating the PFTs, the values of FEV(1), FVC, FEV(1)/FVC, DLCO, and FEF (25-75%) were considered. They were recorded as a percentage of the expected values for the patient's age, height, and gender. In PFTs, A FEV1/FVC ratio below 70% was considered an obstructive

pattern, a FVC below 80% was considered a restrictive pattern, and a DLCO below 80% was considered a decrease in diffusion capacity. The cases were examined according to their radiological stages and classified into five stages. The results of lip biopsy, bronchoscopic transbronchial biopsy, bronchoscopic lymph node aspiration biopsy, bronchoscopic mucosal biopsy, mediastinoscopy, video-guided thoracoscopic biopsy, transthoracic lung biopsy under computed lung tomography, and open lung biopsy performed for tissue diagnosis were analyzed.

Bronchoscopy

In the cases that underwent bronchoscopy, the macroscopic appearance of the bronchial mucosa was evaluated first. Macroscopic view: they were examined in two groups as normal or abnormal mucosal appearance compatible with sarcoidosis. Findings considered as abnormal mucosal appearance were mucosal edema, hyperemia, mucosal irregularity, increased capillarity, and nodular-granular appearance.

Statistical analysis

Statistical analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean \pm standard deviation for continuous variables and as numbers and percentages for categorical variables. Comparisons between groups were performed using the chi-square test for categorical variables and Student's *t*-test for continuous variables. Correlation analyses between minor salivary gland biopsy positivity, bronchoscopic findings, and PFT results were conducted using Pearson or Spearman correlation coefficients, as appropriate. A *p*-value of <0.05 was considered statistically significant. Written informed consent was obtained.

All patients were diagnosed and followed at the Department of Pulmonary Diseases, Istanbul University,. Ethics Committee approval was obtained prior to the study at Istanbul University (2017/325).

This study is derived from the doctoral thesis of Dr. Zeynep Yegin Katran, completed at the Department of Pulmonary Diseases, Istanbul University, in 2016. The authors declare that they have no conflict of interest. The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request. Written informed consent was obtained from all patients at the time of diagnostic procedures. For this retrospective study, additional consent was waived.

Results

Characteristics of the cases and laboratory values

A total of 219 patients (178 female, 41 male) with a mean age of 45.9 ± 11.8 years were included. About one-fourth of the patients were smokers, with higher smoking rates among males. Detailed demographic data are presented in Table 1.

PPD was measured in 73 of the cases (33.3%), and the mean PPD value was found to be 7.95 ± 10.41 mm in the patients (8.18 ± 9.77 mm in women; 7 ± 13.14 in men). The PPD value was found to be negative in 44 (60.2%) of the cases. The number of PPD negative cases was 34 (57.6%) in women and 10 (71.4%) in men. The number of cases in which Quantiferon was examined was 30 (13.6%). While indeterminate in one of the cases (3.3%), the number of negative cases was 19 (63.3%), and the number of positive cases was 10 (33.3%).

Both PPD and Quantiferon were evaluated in 20 (9.1%) of the cases.

PPD and/or Quantiferon positivity for the differential diagnosis of tuberculosis were observed in 44 (20%) of all cases.

Serum ACE level was measured in 74 (33.7%) of the cases. The mean value was 79.82 ± 58.90 IU/mL (9-278 IU/mL). It was 77.38 ± 59.88 IU/mL in women and 90.28 ± 55.38 IU/mL in men. It was found to be high in 60.8% ($n=45$) cases (>52 IU/mL) (Female 9-278 IU/mL, men 35-209 IU/ml).

Radiological stages

Most patients were stage 1 or 2 (78%). The details can be found in Table 2.

Invasive procedures for diagnosis

A total of 215 (98.1%) of the cases were diagnosed with at least one invasive procedure. Invasive procedures were applied to 155 cases (70.7%) from two or more sites. When all 219 cases were examined, one or more bronchoscopic biopsy procedures were performed in 156 cases (71.2%). When 156 cases who underwent bronchoscopy were

Table 1 Age and smoking characteristics of cases.

	Mean \pm Std	Female Mean \pm Std	Male Mean \pm Std
Age (years)	45.94 ± 11.85	46.80 ± 11.30	42.22 ± 13.51
Cigarette (Pack/Year)	14 ± 20.8	11.34 ± 13.7	22.28 ± 34.28

Physical examination revealed erythema nodosum in 67 cases (30.6%).

Table 2 Distribution of the cases by stages.

	Number of cases (n)	Percent
Stage 0	15	6.9
Stage 1	104	47.6
Stage 2	67	30.5
Stage 3	22	10
Stage 4	11	5
Total	219	100

examined, bronchoscopic lymph node aspiration was performed in 122 (78.2%), mucosal biopsy in 64 (41%), and transbronchial biopsy in 36 (23%) cases. Again, out of all 219 cases, mediastinoscopy was performed in 53 (24.2%), open lung biopsy in 10 (4.3%), video-assisted thoracoscopic surgery in 1 (0.4%), and transthoracic biopsy in 1 case(s) (0.4%) (Table 3).

Invasive procedures for extrapulmonary involvement are evaluated. Among all cases, minor salivary gland biopsy 145 (66.2%), skin biopsy 45 (20.6%), peripheral lymph node biopsy 33 (14.4%), liver parenchyma biopsy 14 (6.1%), splenectomy 6 (2.6%), lumbar puncture (LP) 4 (1.7%), kidney parenchyma biopsy 3 (1.3%), thyroidectomy 3 (1.3%), bone marrow aspiration biopsy 1 (0.4%), and bone biopsy 1 (0.4%) were performed (Table 4).

Results of bronchoscopy and other pulmonary interventions

Findings suggestive of the involvement of sarcoidosis of the endobronchial mucosa with bronchoscopic imaging were detected in 25 (16%) cases. Mucosal biopsy was performed in 64 (41%) of the cases who underwent bronchoscopy, and microscopic findings suggestive of sarcoidosis were observed in 30 (46.9%) cases.

Table 3 Invasive procedures for diagnosis.

	n (%)
Bronchoscopy	156 (71.6%)
Lymph node aspiration	122 (78.2%)
Mucosa biopsy	64 (41%)
Transbronchial biopsy	36 (23%)
mediastinoscopy	53 (24.2%)
Open lung biopsy	10 (4.3%)
Video-assisted thoracoscopic biopsy	1 (0.4%)
TIAB under CT	1 (0.4%)

Table 4 Invasive procedure for extrapulmonary involvement and positive cases.

	Number of cases biopsied among all cases (%)	Number of positive cases in biopsied cases (%)
Lip biopsy	145 (66.2%)	63 (43.4%)
Skin biopsy	45 (20.6%)	44 (97.7%)
Peripheral lymph node biopsy	33 (14.4%)	30 (90.9%)
Liver parenchyma biopsy	14 (6.1%)	14 (100%)
Splenectomy	6 (2.6%)	6 (100%)
LP for neurosarcoidosis	4 (1.7%)	4 (100%)
Kidney parenchyma biopsy	3 (1.3%)	3 (100%)
Thyroidectomy	3 (1.3%)	3 (100%)
Bone marrow aspiration biopsy	1 (0.4%)	1 (100%)
Bone biopsy	1 (0.4%)	1 (100%)

Transbronchial needle aspiration biopsy was performed in 36 (23%) of the cases who underwent bronchoscopy, and the result was found to be compatible with sarcoidosis in 18 (50%) cases. Bronchoscopic lymph node aspiration was performed in 122 (78.2%) cases, and the result was found to be compatible with sarcoidosis in 62 (50.8%) cases (Table 5).

The diagnosis was consistent with sarcoidosis in all 53 (24.2%) cases who underwent mediastinoscopy. In 9 (90%) of 10 (4.5%) cases who underwent open lung biopsy, the result was evaluated as compatible with sarcoidosis. Transthoracic lung biopsy and video-assisted thoracoscopic surgery were performed in one case.

Eye examination

A total of 157 (71.7%) of the cases underwent eye examination; sarcoidosis ocular involvement was detected in 44 (28%) cases.

Lip biopsy

Pathology compatible with sarcoidosis was found in 63 (43.4%) of 145 cases who underwent lip biopsy.

PFTs

Three patients who could not cope with with the flow volume maneuver were excluded from the evaluation. Respiratory function tests were abnormal in 105 (48.6%) of the other 216 cases. Of the 105 cases with abnormal PFT, obstruction was detected in 89 (84.7%), restriction in 3 (2.9%), and mixed-type changes in 13 (12.4%) cases (Table 6).

The percent FEV1 value was $91.28\% \pm 19.28$ (31%-146%). When analyzed in milliliters, it was found to be 2381.86 mL

Table 5 Invasive procedures applied with bronchoscopy and positivity results.

	Number of cases (n) %	Number of positive cases (n), %
Transbronchial biopsy	36 (23%)	18 (50%)
Transbronchial lymph node Aspiration	122 (78.2%)	62 (50.8%)
Mucosa biopsy	64 (41%)	30 (46.9%)

Table 6 Pulmonary function test.

	Number of cases (n), %
Normal PFT	114 (51.4%)
Abnormal PFT	105 (48.6%)
Obstructive PFT	89 (41.2%)
Restrictive PFT	3 (1.4%)
Mixed-type PFT	13 (6%)

± 739.76 mL (590-4640 mL). While the mean FVC percentage value was 95.96 ± 19.26 (27%-144%), it was found to be 2965.95 ± 869.82 mL (660-5310 mL) in milliliters. When we look at the FEV1/FVC ratio, the rate was 81.84 ± 7.51 . When the DLCO was examined, the mean value was found to be $90.40 \pm 22.44\%$. In terms of FEF 25-75%, the mean value was 79.24 ± 23.77 (21-125) percent (Table 7).

Six-minute walk test (6MWT)

The six-minute walk test was performed in 94 (42.9%) of the cases, and desaturation was detected in 14 (14.8%) of the cases.

Discussion

Sarcoidosis has been recognized since the 18th century, and various invasive procedures have been used for its diagnosis. In recent years, however, there has been a growing emphasis on less invasive diagnostic methods. Lip biopsy and bronchoscopy represent such approaches in sarcoidosis.¹⁻⁵

Obstructive respiratory dysfunction usually results from endobronchial mucosal involvement, which is caused by granulomatous inflammation of the submucosal glands.^{3,5,6,8} In this study, we examined the relationship between lip biopsy and endobronchial mucosal findings, supported by bronchoscopic appearance and biopsy results, and we also evaluated PFT outcomes.

Sarcoidosis is more common in women. Müselim et al. reported a female predominance in Turkish patients, which was consistent with our cohort (81.3% female).⁹ Although sarcoidosis is generally considered a disease of younger adults, with a peak between 20 and 29 years,⁹⁻¹¹ our patients were older, with a mean age of 45.9 ± 11.8 years. This finding aligns with Müselim et al., who observed a second peak after age 50 in Turkey.¹¹

Smoking is considered a protective measure against sarcoidosis. Peros et al. found the disease more frequent in nonsmokers.¹² Similarly, 74% of our patients were nonsmokers.

The mean PPD value in our patients was 7.9 ± 10.4 mm, typically negative, consistent with the ACCESS study and supporting its role in differentiating sarcoidosis from tuberculosis. Serum ACE levels were positive in some cases, but serial follow-up was not available. In terms of radiological staging, stages 0 and 1 were more common in our cohort,

whereas ACCESS reported stages 1 and 2 as most frequent. We attribute this to earlier detection at our university hospital, where chest radiographs are frequently performed.

PFTs were abnormal in 48.6% of patients, predominantly obstructive (41.2%). Restrictive (1.4%) and mixed (6%) patterns were rare. Abnormal PFTs increased with advancing radiological stage, consistent with Aleksoniene et al.¹³ End airway obstruction was more frequent in patients with endobronchial sarcoidosis, and mucosal biopsy positivity was higher among those with terminal airway stenosis. This suggests that biopsy should be considered even when mucosal appearance is normal.

Overall, 98.1% of patients were diagnosed histopathologically. Extrapulmonary biopsy rates (28.7%) were similar to the ACCESS study (26.9%).¹⁴ Lip biopsy was positive in 43.4% of our patients, similar to Tabak et al., who reported 48% positivity.⁴ Lip biopsy can therefore reduce the need for additional invasive procedures, although in our study most patients also underwent other biopsies.

Endobronchial involvement has been widely reported, ranging from 27% to 71% in different series.^{5,6,15-20} In our cohort, endobronchial mucosal findings were observed in 22.5%, and mucosal biopsy was positive in 46.9% of such cases, consistent with previous studies.^{15,19,22,23} Transbronchial needle aspiration and bronchoscopic lymph node aspiration also yielded diagnostic rates around 50%. These findings show that mucosal biopsy is a valuable diagnostic tool, particularly when other procedures cannot be performed.

There was a weak but significant correlation between lip biopsy positivity and both bronchoscopic lymph node aspiration ($r=0.156$; $p=0.021$) and mucosal biopsy positivity ($r=0.198$; $p=0.03$). No significant correlation was found between lip biopsy and bronchoscopic appearance ($r=0.103$; $p=0.130$). Although the correlations were weak, our results suggest that lip biopsy positivity may predict other bronchoscopic diagnostic findings.

In this study, terminal airway stenosis was more common in patients with endobronchial sarcoidosis diagnosed by mucosal biopsy. Lip biopsy positivity was found in 43.4% of cases and may reduce the need for additional invasive procedures. Diagnostic yields of mucosal biopsy, transbronchial biopsy, and bronchoscopic lymph node aspiration were comparable. Importantly, a weak but significant correlation was observed between lip biopsy positivity and both mucosal and lymph node biopsy results.

Our findings support the use of lip biopsy as a useful initial diagnostic method in suspected sarcoidosis, especially when less invasive approaches are preferred, consistent

Table 7 Pulmonary function tests according to stages.

	Normal PFT	Obstructive PFT	Restrictive PFT	Mixed SFT	Number of cases
Stage 0	60% (n=9)	33.3% (n=5)	None	6.7% (n=1)	15
Stage 1	54.4% (n=56)	41.8% (n=43)	0.9% (n=1)	2.9% (n=3)	104
Stage 2	47.1% (n=31)	48.4% (n=32)	None	4.5% (n=3)	67
Stage 3	47.6% (n=10)	28.5% (n=6)	9.5% (n=2)	14.4% (n=3)	22
Stage 4	45.4% (n=5)	27.3% (n=3)	None	27.3% (n=3)	11

with recent guideline recommendations and registry-based studies highlighting diagnostic approaches and disease burden.^{21,22,23}

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki. Ethics approval was obtained from the Ethics Committee of Istanbul University (Approval No: 2017/325). Written informed consent was obtained from all patients at the time of diagnostic procedures. For this retrospective analysis, additional consent was waived.

Consent for Publication

All authors have read and approved the final manuscript and consent to its publication.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's Contributions

Both authors contributed equally to the conception, design, data analysis, and writing of the manuscript.

Conflicts of Interests

The authors declare that they have no competing interests.

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