Correlation between the Diagnostic Yield from the Bronchoalveolar Lavage Fluid Analysis and Clinicoradiological Findings in Sarcoidosis

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Cite this article as: Tokgöz Akyıl F, Ağca M, Öztürk H, et al. Correlation between the Diagnostic Yield from the Bronchoalveolar Lavage Fluid Analysis and Clinicoradiological Findings in Sarcoidosis. Turk Thorac J 2020; 21(1):21-6.

OBJECTIVES: The diagnosis of sarcoidosis is frequently challenging, requiring a search for less invasive, more reliable diagnostic methods. The bronchoalveolar lavage fluid (BALF) analysis has been used in the differential diagnosis of sarcoidosis for many years with a wide sensitivity and specificity rates. The objective of the study is to investigate whether diagnostic performance of the BALF analysis is altered by clinicoradiological findings of patients with sarcoidosis.

MATERIALS AND METHODS: The present study is a retrospective, single-center, observational study, designed in a sarcoidosis outpatient clinic in a training hospital. Patients who had undergone the bronchoalveolar lavage BAL procedure at diagnosis were included in the study. Demographics, clinical and detailed chest X-ray, and high-resolution computed tomography (HRCT) findings at diagnosis were recorded. According to the diagnostic performance, the BALF results were grouped as “diagnostic” and “non-diagnostic,” and recorded parameters were compared between the groups.

RESULTS: Considering the BALF analysis of all the 257 patients, the mean lymphocyte ratio was 41±17.5 (5–80), and the mean CD4/CD8 was 5.5±4.7 (0.1–24.7). The BALF analysis was diagnostic in 56% (n=145) of patients. Diagnostic performance of the procedure did not correlate with any of the demographic data, smoking status, spirometric findings, chest X-ray staging, HRCT findings, and tomography scoring. Extrapulmonary involvement was significantly more frequent in the diagnostic group (66% vs. 34%, p=0.006).

CONCLUSION: BALF results signal sarcoidosis in more than half of the patients. The diagnostic role of BALF is greater in patients with extrapulmonary involvement.

KEYWORDS: Bronchoalveolar lavage, lymphocytes, sarcoidosis

Received: 05.09.2018 Accepted: 18.10.2018

INTRODUCTION

Sarcoidosis is a chronic inflammatory disorder with an unknown cause, affecting mostly the pulmonary and lymphatic systems [1]. The diagnosis of sarcoidosis may be challenging, and clinicians seek less invasive and more reliable diagnostic methods. The bronchoalveolar lavage fluid (BALF) analysis has been used in the generation of differential diagnosis for many years. In sarcoidosis, unknown agents drive the aTh1 immune response, and CD4+ T lymphocytes migrate to affected tissues. As a result, lymphocytosis and altered CD4/CD8 ratios in BALF have been associated with a diagnosis of sarcoidosis [2,3].

To date, many studies have been published on the relationship between the use of BALF and the diagnosis of sarcoidosis. There is a high level of variability in terms of sensitivity, specificity, and cut-off values for CD4/CD8 ratios [4-8]. Several factors, including radiographic stages, tobacco smoking, and corticosteroid treatment, have been described as having an influence on BALF lymphocyte ratios and CD4/CD8 ratios [9-12]. Nevertheless, no systematic evaluation of the diagnostic performance of the BALF analysis has been undertaken.

In this respect, we hypothesize that the extent and severity of airway inflammation can change clinical and radiological features, leading to variations in the diagnostic role of BALF in sarcoidosis. The aim of the present study is to investigate the diagnostic performance of BALF results according to baseline clinical and radiological features.

MATERIALS AND METHODS

The present study is a retrospective, single-center, observational study, designed in a respiratory training and research hospital. Between September 2005 and September 2016, all records of patients with sarcoidosis who presented to the
outpatient clinic were investigated. Patients who had undergone a BAL procedure prior to diagnosis and had sufficient medical data and radiological images at diagnosis were included into the study. Patients with insufficient medical data, incomplete BALF results, and inconvenient BAL performances were excluded (Figure 1). An ethical committee approval was obtained from the local research committee from Sureyyapasa Chest Diseases and Thoracic Surgery Training and Research Hospital (No: 116.2017.092), and verbal informed patient consent was approved by all patients. All patients were diagnosed and followed by expert pulmonologists.

**Figure 1.** Flow chart of patient inclusion

BAL: bronchoalveolar lavage; BALF: bronchoalveolar lavage fluid; HRCT: high-resolution computed tomography

**MAIN POINTS**

- A number of factors such as radiographic stages, tobacco smoking, and corticosteroid treatment, are described to influence BALF lymphocytes and CD4/CD8 ratios.
- More than half of the patients BALF may be diagnostic.
- Patients’ demographics, radiological involvement, spirometric findings do not alter diagnostic performance of BALF.
- In patients whose BALF is diagnostic, extrapulmonary involvement of sarcoidosis is more frequent.
Organization of the Sarcoidosis Outpatient Clinic
The hospital has a sarcoidosis outpatient clinic for patients that have been pre-diagnosed or diagnosed with the disease. The clinic implements a routine follow-up program and clinical filing. BAL is carried out in compliance with published guidelines [13,14] using a flexible bronchoscope (Olympus BF, Type 1T160 or P160, Olympus, Tokyo, Japan) in the area of the most marked radiological abnormality seen on computed tomography, or in the case of diffuse involvement, the middle lobe or lingual is used. At least six aliquots of 20 mL sterile saline are instilled through the bronchoscope and retrieved gently by suction.

Diagnosis is also carried out in line with published guidelines [15]. Once a patient is diagnosed as having sarcoidosis, serum and urine calcium levels, eye examination, echocardiographic and electrophysiological, and abdominal ultrasonography are routinely performed. If the patient has symptoms suggestive of the involvement of any other system, this is evaluated in line with the disease involvement procedure [15].

Data Collection and Study Design
Patient demographic and clinical data were collected. Diagnoses were re-evaluated according to current guidelines [15]. Baseline spirometric findings and serum angiotensin converting enzyme (ACE) (U/L) and serum calcium (mg/dL) levels were recorded. Chest X-ray grading at diagnosis was classified according to the Scadding system [16]. Baseline high-resolution computed tomography (HRCT) findings were evaluated by a radiologist who was blinded to the medical history of the patient, according to the scoring system proposed by Oberstein et al. [17]. Lung parenchyma involvement was evaluated qualitatively as the bronchovascular bundle, intra-parenchymal nodules, septal and nonseptal lines, and parenchymal consolidation (including ground glass opacities). The lung volume affected was quantified using a visual score as follows: 0=no lesions, 1=up to 33%, 2=up to 66%, and 3=more than 66% of the volume affected. Similarly, quantification of focal pleural thickening and enlargement (with a short axis of 1 cm or more considered enlarged) of the lymph nodes, respectively, was carried out as follows: 0=no pathological findings, 1=minor, 2=moderate, and 3=pronounced changes. The total score was calculated.

The presence of any extrapolmonary involvement at the time of diagnosis was recorded.

BALF results were recorded and grouped either as diagnostic or non-diagnostic. Diagnostic BAL was defined as a combination of ≥15% lymphocyte and >3.5 ratio of CD4/CD8 lymphocytes [14]. According to the BALF results, patients were divided into two groups: diagnostic and non-diagnostic. The baseline clinical and radiological findings were compared between groups.

Statistical Analysis
Quantitative data are expressed as the mean±standard (SD) deviation, and qualitative data are expressed as frequencies. Student’s t-test and chi-squared test were used for comparison. All statistical analyses were carried out using a statistical software package (Statistical Package for the Social Sciences for Windows, version 16.0; SPSS Inc.; Chicago, IL, USA). A p-value <0.05 was considered significant.

RESULTS
Of all the 257 patients, 80 (31%) were male, and the mean age was 42±12 years (18-78). Only 25% of the patients had ever smoked (Table 1). At presentation, chest roentgenograms were mainly classified as stage 1 (56%). The mean forced vital capacity (FVC) was 88±14%, and carbon monoxide diffusing capacity (DLCO) was 79±18%. The HRCT findings demonstrated bronchovascular bundles in 34% and parenchymal nodules in 56% of the patients. The average lymph node diameter of the largest lymph node was 15.7±5.6 mm. The mean radiographic total score was 6.2±3 (0-17).

Table 1. General characteristics of the patients

| Gender | Male | 80 (31%) |
|--------|------|----------|
| Female | 177  | 69%      |
| Age (years) | 42±12 | 18–78 |
| Smoking status | | |
| Ever smoker | 65 | 25% |
| Never smoker | 192 | 75% |
| FVC (%) min–max | 88±14 | 41–109 |
| DLCO (%) | 79±18 | 34–110 |
| ACE (U/L) | 66±45 | 5–360 |
| Ca (mg/dL) | 9.6±0.7 | 8.0–17.8 |
| Chest X-ray Stag | | |
| 0 | 3 | 1% |
| 1 | 143 | 56% |
| 2 | 97 | 38% |
| 3 | 14 | 5% |
| Diagnostic method | | |
| Histopathological confirmed | 189 | 74% |
| Other | 68 | 26% |
| BALF analysis | | |
| Lymphocyte % | 41±17.5 | 5–80 |
| Neutrophil % | 16.6±9.7 | 3–80 |
| Eosinophil % | 2.5±1.6 | 0–12 |
| Macrophage % | 40.7±17.2 | 83 |
| CD4/CD8 | 5.5±4.7 | 0.1–24.7 |
| Diagnostic yield of BALF | | |
| Diagnostic | 145 | 56% |
| Non-diagnostic | 112 | 44% |
| Extrapulmonary involvement | | |
| Present | 65 | 25% |
| Absent | 192 | 75% |

ACE: angiotensin converting enzyme; BALF: bronchoalveolar lavage fluid; Ca: calcium; DLCO: carbon monoxide diffusing capacity; FVC: forced vital capacity
Any extrapulmonary involvement was recorded in 65 (25%) patients. The most frequent involvement was dermatologic (n=48, 19%). The eye was affected in 13 patients. Cardiac, neurological, and gastrointestinal involvement and hypercalcemia were recorded in two patients. The BALF analysis revealed an average lymphocyte percent of 41±17.5 (5-80), and the average CD4/CD8 ratio was 5.5±4.7 (0.1-24.7). Lymphocytes were higher than 15% in 233 (91%) of the patients, whereas the CD4/CD8 ratio was greater than 3.5 in 152 patients (59%). BALF was evaluated as diagnostic in 145 (56%) patients (Table 1). The final diagnosis was made by clinical and radiological findings in 68 (26%) and histopathologically confirmed in 189 (74%) patients. Mediastinoscopy was diagnostic in 65 (25%), transbronchial biopsy in 58 (23%), endobronchial mucosa biopsy in 35 (14%), video-assisted thoracoscopic surgery in 12 (5%), endobronchial ultrasonography in 14 (5%), and peripheral lymph node or skin biopsy in 5 (2%) patients. BALF findings were diagnostic in 88 (47%) of histopathologically confirmed diagnosed patients, whereas in 84% of clinically diagnosed patients (p<0.001). The diagnostic performance of the BALF analysis did not correlate with gender, age, smoking status, ACE levels, and spirometric findings (p>0.05, Table 2). A detailed analysis of the smoking status revealed that smokers had lower neutrophil ratios compared to nonsmokers (p=0.04, Table 3). Chest radiography staging did not correlate with the BALF analysis (Table 4). Neither radiologic staging nor tomography findings affected the airway inflammation BALF results. The BALF analysis was more frequently diagnostic in patients with extrapulmonary involvement (p=0.043) (Table 2).

**DISCUSSION**

The present study confirms that the BALF analysis was a useful diagnostic aid in more than half of the patients. The diagnostic performance of the procedure did not correlate with demographics and/or radiological findings. Extrapulmonary involvement at diagnosis is described as a signifier of the BAL procedure for the first time.

Efared et al. [18] have evaluated the diagnostic value of BAL in interstitial lung diseases and have not found a statistically significant relationship between lymphocytes and CD4/CD8 ratios and diagnosis. However, the authors have grouped all diagnoses into discrete groups, and the study population was relatively small. A recent meta-analysis of 17 papers including 999 patients with sarcoidosis and 886 controls has reported that the BALF CD4/CD8 ratio by itself is not sufficient for diagnosis but is helpful in improving diagnosis along with other diagnostic factors [5]. Epidemiological data of Turkish patients revealed a BALF analysis of lone lymphocytic alveolitis of 34% [19]. In a recent epidemiological study in Portugal, 289 sarcoidosis patients underwent bron-
verbal informed consent was obtained from all participants. The authors declared that this study has no conflicts of interest to declare. Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of local research committee and institutional review board. Informed Consent: Verbal informed consent was obtained from patients who participated in this study. Peer-review: Externally peer-reviewed. Author Contributions: Concept - F.T.A., M.A., H.Ö., E.S., İ.E., E.U.B., F.O., R.Y., S.A.B., T.S.; Design - F.T.A., M.A., H.Ö., E.S., İ.E., E.U.B., F.O., R.Y., S.A.B., T.S.; Supervision - F.T.A., M.A., H.Ö., E.S., İ.E., E.U.B., F.O., R.Y., S.A.B., T.S.; Resources - F.T.A., M.A., T.S.; Materials - F.T.A., E.S., İ.E., F.O., R.Y., S.A.B., T.S.; Data Collection and/or Processing - F.T.A., M.A., E.S., İ.E., E.U.B., T.S.; Analysis and/or Interpretation - F.T.A., T.S.; Literature Search - F.T.A., T.S.; Writing Manuscript - F.T.A., T.S.; Critical Review - F.T.A., M.A., T.S.

Conflict of Interest: The authors have no conflicts of interest to declare. Financial Disclosure: The authors declared that this study has received no financial support.

In conclusion, the BALF analysis supports a diagnosis of sarcoidosis in more than half of the patients. Diagnostic role does not correlate with demographics, smoking status or tomography findings. The diagnostic role of BALF is greater in patients with extrapulmonary involvement.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of local research committee and institutional review board. Informed Consent: Verbal informed consent was obtained from patients who participated in this study. Peer-review: Externally peer-reviewed. Author Contributions: Concept - F.T.A., M.A., H.Ö., E.S., İ.E., E.U.B., F.O., R.Y., S.A.B., T.S.; Design - F.T.A., M.A., H.Ö., E.S., İ.E., E.U.B., F.O., R.Y., S.A.B., T.S.; Supervision - F.T.A., M.A., H.Ö., E.S., İ.E., E.U.B., F.O., R.Y., S.A.B., T.S.; Resources - F.T.A., M.A., T.S.; Materials - F.T.A., E.S., İ.E., F.O., R.Y., S.A.B., T.S.; Data Collection and/or Processing - F.T.A., M.A., E.S., İ.E., E.U.B., T.S.; Analysis and/or Interpretation - F.T.A., T.S.; Literature Search - F.T.A., T.S.; Writing Manuscript - F.T.A., T.S.; Critical Review - F.T.A., M.A., T.S.

Conflict of Interest: The authors have no conflicts of interest to declare. Financial Disclosure: The authors declared that this study has received no financial support.
REFERENCES

1. American Thoracic Society/European Respiratory Society/World Association for Sarcoidosis and Other Granulomatous Diseases. Statement on Sarcoidosis. Am J Respir Crit Care Med 1999;160:736-55. [CrossRef]

2. Baughman RP, Lower EE, du Bois RM. Sarcoidosis. Lancet 2003;361:1111-8. [CrossRef]

3. Costabel U. CD4/CD8 ratios in bronchoalveolar lavage fluid: of value for diagnosing sarcoidosis? Eur Respir J 1997;10:2699-700. [CrossRef]

4. Hyldgaard C, Kaae S, Riddervold M, et al. Value of s-ACE, BAL lymphocytosis, and CD4+/CD8+ and CD103+/CD4+ T-cell ratios in diagnosis of sarcoidosis. Eur Respir J 2012;39:1037-9. [CrossRef]

5. Shen Y, Pang C, Wu Y, et al. Diagnostic performance of bronchoalveolar lavage fluid CD4/CD8 ratio for sarcoidosis: A meta-analysis. EBioMedicine 2016;8:302-8. [CrossRef]

6. Marruchella A, Tondini M. Reliability of bronchoalveolar lavage in the routine clinical assessment of patients with sarcoidosis. A retrospective analysis. Panminerva Med 2002;44:257-60.

7. Greco S, Marruchella A, Massari M, Saltini C. Predictive value of BAL cellular analysis in differentiating pulmonary tuberculosis and sarcoidosis. Eur Respir J 2005;26:360-1. [CrossRef]

8. Lee W, Chung WS, Hong KS, Huh J. Clinical usefulness of bronchoalveolar lavage cellular analysis and lymphocyte subsets in diffuse interstitial lung diseases. Ann Lab Med 2015;35:220-5. [CrossRef]

9. Urbankowski T, Knyziak-Medrzycka I, Domagała-Kulawik J, Chazar R. Sarcoidosis and tobacco smoking—clinical picture, diagnostic tests results and bronchoalveolar lavage fluid composition. Pol Merkur Lekarski 2012;32:298-301.

10. Hosier G, Wakił J, Domagała-Kulawik J, et al. Flow cytometric evaluation of lymphocyte subpopulations in BALF of healthy smokers and nonsmokers. Folia Histochem Cytobiol 1999;37:25-30.

11. Danila E, Jurgauskienė L, Malickaitė R. BAL fluid cells and pulmonary function in different radiographic stages of newly diagnosed sarcoidosis. Adv Med Sci 2008;53:228-33. [CrossRef]

12. Danila E, Jurgauskienė L, Norkuniene J, Malickaitė R. BAL fluid cells in newly diagnosed pulmonary sarcoidosis with different clinical activity. Ups J Med Sci 2009;114:26-31. [CrossRef]

13. Costabel U, Guzman J. Bronchoalveolar lavage in interstitial lung disease. Curr Opin Pulm Med 2001;7:255-61. [CrossRef]

14. Meyer KC, Raghu G, Baughman RP, et al. An official American Thoracic Society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. Am J Respir Crit Care Med 2012;185:1004-14. [CrossRef]

15. Baughman RP, Culver DA, Judson MA. A Concise Review of Pulmonary Sarcoidosis. Am J Respir Crit Care Med 2011;183:573-81. [CrossRef]

16. Scadding JG. Prognosis of intrathoracic sarcoidosis in England. BMJ 1961;4:1165-72. [CrossRef]

17. Olsherstein A, Zitzewitz H von, Schweden F, Müller-Quernheim J. Non-invasive evaluation of the inflammatory activity in sarcoidosis with high-resolution computed tomography. Sarcoidosis Vascul Dis Lung Health 1997;14:65-72.

18. Etared B, Elhab-Atsane G, Rabiu S, et al. The diagnostic value of the bronchoalveolar lavage in interstitial lung diseases. J Negat Results Biomed 2017;16:4. [CrossRef]

19. Kiter G, Musellim B, Cetinkaya E, et al. Clinical presentations and diagnostic work-up in sarcoidosis: a series of Turkish cases (clinical diagnosis of sarcoidosis). Tuberk Toraks 2011;59:248-7. [CrossRef]

20. Cardosa AV, Mota PC, Melo N, et al. Analysis of sarcoidosis in the Oporto region (Portugal). Rev Port Pneumol 2017;23:251-8. [CrossRef]

21. Bronchoalveolar lavage constituents in healthy individuals, idiopathic pulmonary fibrosis, and selected comparison groups. The BAL Cooperative Group Steering. Am Rev Respir Dis 1990;141:S169-202.

22. Valeyre D, Soler P, Clerici C, et al. Smoking and pulmonary sarcoidosis: effect of cigarette smoking on prevalence, clinical manifestations, alveolitis, and evolution of the disease. Thorax 1988;43:516-24. [CrossRef]

23. Meyer KC, Raghu G. Bronchoalveolar lavage for the evaluation of interstitial lung disease: is it clinically useful? Eur Respir J 2011;38:761-9. [CrossRef]

24. Heron M, Grutters JC, ten Dam-Molenkamp KM, et al. Bronchoalveolar lavage cell pattern from healthy human lung. Clin Exp Immunol 2012;167:523-31. [CrossRef]

25. Aleksonienè R, Zeleckienè I, Matačiunas M, et al. Relationship between radiologic patterns, pulmonary function values and bronchoalveolar lavage fluid cells in newly diagnosed sarcoidosis. J Thorac Dis 2017;9:88-95. [CrossRef]

26. Kebbe J, Abdo T. Interstitial lung disease: the diagnostic role of bronchoscopy. J Thorac Dis 2017;9(Suppl 10):S996-S1010. [CrossRef]

27. Inomata T, Konno S, Nagai K, et al. Neutrophil predominance in bronchoalveolar lavage fluid is associated with disease severity and progression of HRCT findings in pulmonary Mycobacterium avium infection. PLoS One 2018;13:e0190189. [CrossRef]

28. Okumus G, Musellim B, Cetinkaya E, et al. Extrapulmonary involvement in patients with sarcoidosis in Turkey. Respirology 2011;16:446-50. [CrossRef]

29. Baughman RP, Teirstein AS, Judson MA, et al. Clinical characteristics of patients in a case control study of sarcoidosis. Am J Respir Crit Care Med 2001;164:1885-9. [CrossRef]