

Original Research

Cell Block Examination Is Critical for Sarcoidosis Diagnosis by Endobronchial Ultrasound-Guided Mediastinal Lymph Node Fine Needle Aspiration

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Intrathoracic sarcoidosis is often diagnosed by transbronchial lung parenchymal biopsy (TBBx), however, recent studies suggest endobronchial ultrasound-guided transbronchial fine needle aspiration of mediastinal lymph node (EBUS-FNA) is safer with superior diagnostic yield. We report our experience from 2008 to 2010 with combined EBUS-FNA and TBBx in 61 consecutive patients with clinical suspicion of sarcoidosis. One to three mediastinal lymph nodes (LN) in various locations were sampled using 21/22-gauge needles with on-site interpretation. Additional one to two specimens per site were collected in Normosol® for cell block preparations. A definitive diagnosis of sarcoidosis was made in 51 patients (84%) by EBUS-FNA/TBBx studies (46) and clinical information (5); alternative diagnoses were established in 8 patients (13 %); the last 2 patients remained suspicious for sarcoidosis without confirmatory tissue diagnosis. Of the 46 biopsy (EBUS-FNA and/or TBBx) confirmed cases, 37 (80.0%) were diagnosed by EBUS-FNA. Cell blocks prepared from all 37 patients contained diagnostic material, 10 (27.0%) were interpreted as such by on-site evaluations. The diagnostic yield of LNs at different locations varied, being 100, 68, 50 and 20% in R12, subcarinal, R4, and R11, respectively. A total of 36 patients had both EBUS-FNA and TBBx performed during the same visit. Diagnoses were identical in 15 patients (42 %). TBBx independently identified 9 cases of sarcoidosis. This study indicates that cell block preparation is valuable for EBUS-FNA diagnosis of sarcoidosis. EBUS-FNA and TBBx are effective and complimentary tools for intrathoracic sarcoidosis diagnosis.

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Key Words: Sarcoidosis, EBUS-FNA, cell block, mediastinal lymph node

INTRODUCTION

Sarcoidosis is a multiorgan disease with pulmonary parenchyma and mediastinal lymph nodes affected in nearly 90% and 85% of cases, respectively.¹ Although the etiology of this granulomatous disease remains unknown, current knowledge suggests it results from disordered immune regulation in genetically predisposed individuals after exposure to certain environmental agents.² Tissue confirmation, together with clinical and radiological suspicion, is an integral part of sarcoidosis diagnosis.³ The morphological characteristics of sarcoidosis include nonnecrotizing granuloma as well as exclusion of similarly presenting diseases such as tuberculosis, fungal infection, lymphoma, and metastatic carcinomas. Other pathological features, including multinucleated giant cells, inclusion bodies (Schaumann's, Asteroid, and Hamazaki-Wesenberg) and a high CD4+/CD8+ lymphocyte ratio in bronchoalveolar lavage specimens, can be helpful to establish a diagnosis of sarcoidosis.^{4,5}

Although bronchoscopy with transbronchial lung parenchymal biopsy (TBBx) has been traditionally considered the preferred diagnostic method for sarcoidosis,^{6,7} this may be nondiagnostic in about a third of cases.¹ TBBx is also associated with 7% morbidity including bleeding and pneumothorax. Other invasive and costly procedures to establish a sarcoidosis diagnosis include mediastinoscopy and/or parenchymal wedge biopsy that can have even higher morbidity.³ In the absence of a tissue diagnosis, bronchoalveolar lavage (BAL) and bronchial washing may only provide supportive evidence for establishing a diagnosis.^{3,8}

The development of linear echo endoscopes provides the opportunity for real-time localization and mediastinal lymph nodes aspiration.^{6,7} Both transesophageal ultrasound guided fine needle aspiration (EUS-FNA) and endobronchial ultrasound guided fine needle aspiration (EBUS-FNA) are minimally invasive mediastinal lymph node sampling procedures that have been successfully applied in the nodal staging of non-small cell lung carcinomas, their restaging after chemotherapy and/or radiation, and the diagnosis of

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mediastinal involvement of lymphoma and other extrathoracic malignancy.⁹

Recently several clinical reports have discussed the application of these techniques in the evaluation of sarcoidosis. These early publications suggest the diagnostic yield of EUS-FNA as 82 - 86%, with sensitivity of 86 - 100%;^{1,10-12} the diagnostic yield of EBUS-FNA as 85 - 93%.¹⁰⁻¹² Both EUS-FNA & EBUS-FNA have very low (~1.5%) morbidity rates.¹³ However, differences in nodal access do exist between the two approaches. Hilar nodes and lymph nodes anterolateral to the trachea are more commonly involved in sarcoidosis and are difficult to sample by EUS-FNA.¹³ Reports of EBUS-FNA in sarcoidosis diagnosis are variable in patient selection, size of lymph node sampled, aspiration needle size, tissue processing method, and role of on-site evaluation.^{1,10-13} Recently, Von Bartheld *et al* reported that the addition of cell block analysis to routine Diff-Quick smear examination reduced the false negative rate by 33%.¹ So far few studies have addressed the complementary nature of TBBx and EBUS-FNA in malignancy or sarcoid diagnosis.¹⁴

Here we report our experience with combined EBUS-FNA and TBBx in a total of 61 consecutive patients from 2008 to 2010 with clinical suspicion of sarcoidosis. The prominent diagnostic value of cell block preparations is emphasized. Both EBUS-FNA and TBBx are effective tools for obtaining diagnostic samples and are complimentary in confirming a diagnosis of sarcoidosis.

METHODS

Patients: The study (Protocol # 814049) was approved by University of Pennsylvania Institutional Review Board. Sixty-one consecutive patients (M = 28, F = 33) between the years 2008 and 2010 with clinical suspicion of sarcoidosis were studied. All subjects underwent EBUS-FNA; 36 of these patients also had TBBx performed.

EBUS-FNA & TBBx: An EBUS-FNA bronchoscope (BF-UC160F; Olympus America; Center Valley, PA) was used to visualize mediastinal and hilar lymph nodes. Those considered appropriate for biopsy by the bronchoscopist were sampled under real-time EBUS guidance using a 21- or 22-gauge dedicated needle (NA-201SX-4022-C; Olympus

America; Center Valley, PA). One to three mediastinal lymph nodes were sampled with a maximum of 5 passes/node. On-site interpretation was rendered on 1 - 3 specimens per lymph node site.

In 36 cases, TBBx of lung parenchymal lesions was performed after EBUS-FNA procedure. All TBBx were performed with a standard flexible bronchoscope (BF-1T180, Olympus) and biopsy forceps. The TBBx specimens were fixed in neutral buffered formalin.

On-site FNA sample preparation and evaluation: EBUS-FNA aspirates were expelled from the needle by stylet reinsertion. A portion of the aspirates were smeared on two glass slides; one slide was air-dried and stained with Diff-Quick® procedure while the other was immediately fixed with 95% ethanol for Pap staining. The remaining specimen was rinsed into a specimen cup with Normosol® for ThinPrep® and cell block preparations, paraffin processing and H/E stain. Additional 1-2 aspirates of EBUS-FNA specimens per site were collected in Normosol® for cell block preparations, but not examined on-site.

All cell block slides demonstrating non-necrotizing granuloma underwent acid fast bacilli (AFB) and Gomori-Grocott methenamine silver (GMS) stains. In certain cases, culture for *Mycobacterium Tuberculosis* was also conducted.

Final diagnosis: The final diagnosis of sarcoidosis was based on clinical and radiological suspicion, tissue confirmation of non-necrotizing granulomas, and a follow-up period (ranging 7-36 months, median 24 months).

Data Analysis: To calculate the diagnostic yield of lymph node at a particular location, the number of granuloma positive nodes from that location was divided by the total number of nodes biopsied from that location.

To find the optimal number of needle passes, the cumulative sensitivity for successive needle passes was calculated as follows: the number of patients in whom a particular needle pass gave the first confirmation of granulomas was added to the total already having an EBUS confirmation, and this new total was divided by the total number of patients with a final diagnosis of sarcoidosis.

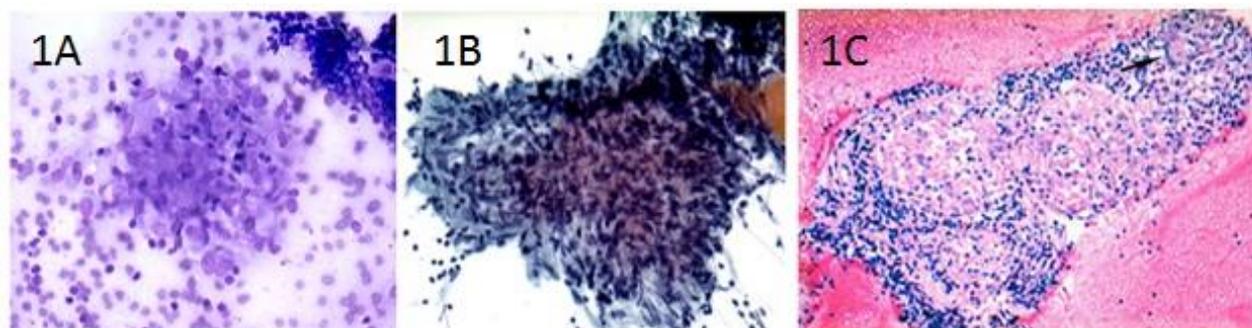


Figure 1. Non-necrotizing granulomas from EBUS-FNA sample; **A:** cell smear and Diff-Quick stain, original magnification X 200; **B:** Cell smear and Papanicolaou stain, original magnification X100; **C:** Cell block and H&E stains, arrow indicates a multinucleated giant cell, original magnification X 100.

Table 1. Number of Endobronchial ultrasound-guided transbronchial fine needle aspiration passes, presence of granulomas and cumulative sensitivity.

| Number of passes | Number of patients | Number of patient with granulomas | Cumulative sensitivity (%) |
|------------------|--------------------|-----------------------------------|----------------------------|
| 1 | 10 | 7 | 70 |
| 2 | 9 | 1 | 80 |
| 3 | 7 | 1 | 90 |
| 4 | 2 | 1 | 100 |

Table 2. Lymph nodes sampled and presence of non-necrotizing granulomas.

| Location | Positive* | Total lymph node | Diagnostic yield of node (%) |
|----------|-----------|------------------|------------------------------|
| L7 | 19 | 28 | 68 |
| R4 | 3 | 6 | 50 |
| R12 | 4 | 4 | 100 |
| R11 | 1 | 5 | 20 |
| L11 | 0 | 1 | 0 |
| L12 | 2 | 2 | 100 |
| R10 | 0 | 1 | 0 |
| R2 | 1 | 1 | 100 |
| L2 | 1 | 1 | 100 |
| Total | 31 | 49 | 63 |

* Presence of non-necrotizing granuloma

Table 3. Comparison of TBBx and EBUS-FNA.*

| | TBBx | EBUS-FNA | Combined 2 approaches | Final diagnosis |
|--|------|----------|-----------------------|-----------------|
| No. of patients with non-necrotizing granuloma | 19 | 22 | 31 | 32 |
| Diagnostic yield (%) | 59 | 69** | 97 | 100 |

*Four patients underwent both EBUS-FNA and TBBx and were reported on-site as "no evidence of granuloma or carcinoma". They were later found to have other diagnoses, including TB, lupus, inflammation, reactive, one case each.

** p = 0.3 by Fischer Exact test

RESULTS

EBUS-FNA

There were 28 males and 33 females with an average age of 48.6 years in our study. EBUS-FNA with on-site smear preparation and interpretation as well as cell block preparation were performed in all 61 patients.

Non-necrotizing granulomas were identified in 37 patients with EBUS-FNA sampling after examination of Diff-Quick® smear, Pap smear, ThinPrep® slides and cell block preparations (**Figure 1**). Of these 37 subjects, cell blocks prepared from all 37 patients contained diagnostic material (100%); however, granulomas were identified on-site in only 10/37 (27%) cases upon examination of the Diff-Quick® stained slides. Pap smear and ThinPrep slides did not reveal

additional granulomas beyond what were identified on the Diff-Quick smear. Of the 10 patients with on-site identification of non-necrotizing granuloma, the cumulative sensitivity of EBUS-FNA with one, two, three and four needle passes was 70%, 80%, 90% and 100%, respectively (**Table 1**).

EBUS-FNA also identified 1 patient each with tuberculosis (confirmed by culture), collagen vascular disease, and acute inflammation. Although the presence of non-necrotizing granulomas were used as primary diagnosis criteria in this study, multinucleated giant cells were common (~ 65%) in the sarcoidosis samples. Other cytologically distinct entities such as asteroid bodies were much less common in our study.

We did not notice significant differences in the samples collected by 21 or 22-gauge needles (including in cell block preparation). Special stains of GMS and AFB were performed in all 37 patients with negative results.

Of the 37 sarcoidosis patients diagnosed by EBUS-FNA, a single lymph node was aspirated in 17, 2 nodes in 14 and 3 in 6 patients. Additional sites were sampled based upon high degree of clinical suspicion with “no evidence of granulomas” among the on-site examined FNA samples.

Of the 37 EBUS-FNA confirmed sarcoidosis patients, the diagnostic yield of LN at different locations varied being 50% in R4, 67.9% in subcarinal, 20% in R11, and 100% in R12 sites (**Table 2**). It is pertinent to point out that R12 lymph nodes are sampled uncommonly.

Combination of EBUS-FNA and TBBx

A total of 36 patients underwent both EBUS-FNA and TBBx

procedures during the same visit; 32 were diagnosed with sarcoidosis; alternative diagnoses were established in 4 patients (including 2 patients in follow-up period). Of these 32 patients, non-necrotizing granuloma were observed in 19 (59%) who underwent TBBx, and in 22 (69%) who underwent EBUS-FNA. Non-necrotizing granulomas were observed in at least one modality in 31 (97%) patients (**Table 3**).

Clinical follow-up and final diagnosis

Patients were followed for 7 – 36 months (median 24 months). During the follow-up period, sarcoidosis was diagnosed in 5 additional cases, discoid lupus in 1 patient, tuberculosis in 1 patient, reactive lymphadenitis in 1 patient, inflammation/pneumonia in 2 patients. The diagnosis of 2 patients was still unclear and are still being followed up clinically. The final diagnoses combining EBUS-FNA, TBBx, and clinical follow-up are summarized in table 4.

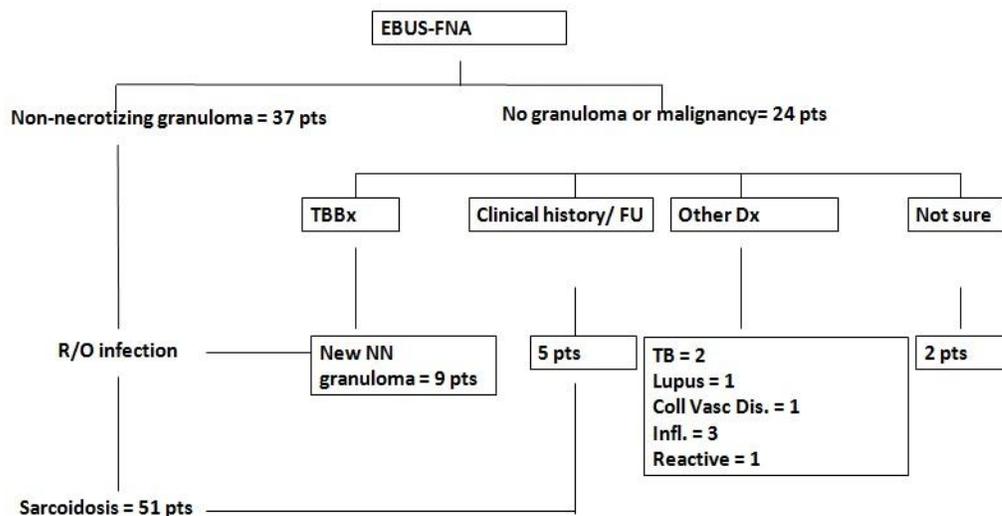


Table 4. Final diagnosis of the 61 patients.

DISCUSSION

Currently, the diagnosis of sarcoidosis is established when there is compatible clinical/radiological evidence together with morphological confirmation.^{2,3} The only clinical situation when diagnosis of sarcoidosis can be made reliably without biopsy is Lofgren syndrome due to its distinct presentation of combined hilar lymphadenopathy, erythema nodosum on the shins, arthritis and fever.¹⁵

A number of bronchopulmonary specimens and diagnostic approaches have been used for sarcoidosis tissue confirmation with variable success rates.^{6-8,10-12} EUS-FNA and EBUS-FNA have recently been used and studied for sarcoidosis diagnosis.^{1,12} However, the pivotal role of cell block preparation in EBUS-FNA diagnosis of non-necrotizing granuloma has not been emphasized. Of the 37 sarcoidosis patients diagnosed by EBUS-FNA in our study, cells blocks contain non-necrotizing granulomas in all patients (100%). In contrast, only 27% patients showed

granulomas in the smears. Von Bartheld *et al* were the first to emphasize the importance of cell block preparation in FNA-based sarcoidosis diagnosis.¹ Among their 18 smear negative patients, 6 (33%) showed non-necrotizing granuloma in cell block analysis. However, only 76 patients in their 101 patient-cohort underwent cell block preparation. It is therefore difficult to calculate the actual yield, sensitivity, or specificity of the cell block preparation in their study. Of the 46 biopsy (EBUS-FNA and/or TBBx) confirmed sarcoidosis patients in our study, 37 patients were diagnosed through examination of cell block preparations; a diagnostic yield of 80.4%. If we consider only the 37 cytology confirmed sarcoidosis patients, all of them (100%) showed non-necrotizing granuloma in cell block preparations. Potentially bronchoscopists could simply put their EBUS-FNA material into a cup and request cell block preparation. Although this may save resource and time for cytopathologist by eliminating the on-site examination, this

type of practice has certain drawbacks including inability to triage specimen, to perform most molecular studies, to increase turnaround time, to delay in patient management and missing the unexpected other disease processes (14% in this study).

Several factors could contribute to the high diagnostic yield of cell block for the sarcoidosis. It has been suggested the epithelioid groups in FNA samples could be disrupted when the aspirates are smeared between two glasses, thus precluding the ability to make an on-site sarcoidosis diagnosis. On the other hand, sedimentation and paraffin-embedding in cell block preparation are not liable to this disruption of the histological structure.¹ In our institution, it is possible for bronchoscopist to collect 1-2 specimens in Normosol® before arrival of the cytopathology team. It is possible that excessive bleeding in the subsequent aspirates compromises the diagnostic yield and on-site interpretation.

The location of lymph node may play role in their diagnostic yield. In our study, the diagnostic yields of different lymph node have varied from 20% in R11, to 100% in R12 station. The sizes of all these nodes were more than 1 cm. These observations suggest the potential advantage of EBUS-FNA over EUS-FNA in accessing hilar lymph nodes for sarcoid diagnosis.¹³ EUS-FNA is an established diagnostic modality for mediastinal lymphadenopathy, with easy accessibility to all mediastinal lymph nodes including L7 and is more effective in reaching lower mediastinal lymph node stations including stations 8.^{13,16} Combination of EBUS-FNA and TBBx in our study reached a diagnostic sensitivity of 90%. This is comparable with results reported in recent literature (range 82% - 87%).^{1,10-12,17,18} In this regard, it is noteworthy that subcarinal lymph nodes constitute only ~ 60% of our targeted lymph nodes whereas a recent study reported EUS-FNA subcarinal lymph node sampling in 97% patients.¹ It is well known that the frequency of mediastinal lymph node involvement in sarcoidosis is variable: from 92% in hilar lymph node to 16% in posterior mediastinal group.¹⁹ The limited number of cases in the present study does not permit us to analyze laterality of lymph node and sarcoidosis diagnosis.

The role of TBBx in sarcoidosis diagnosis is well-established.^{6-7,18,20} Our study is not designed to compare the diagnostic yield of EBUS-FNA and TBBx. TBBx was conducted only in a subset of our patients at the discretion of the bronchoscopists. However, our study does suggest the two approaches are complimentary in reaching diagnosis of intrathoracic sarcoidosis.

It is interesting to note that none of our alternative diagnoses turns out to be malignancy (carcinoma or lymphoma) but we found 2 patients diagnosed as tuberculosis. The lack of malignancy in our cohort may relate to the retrospective nature of our study design and our patient population.

In summary, our study indicates cell block preparation is valuable for EBUS-FNA diagnosis of sarcoidosis. EBUS-FNA and TBBx are effective and complimentary tools for sarcoidosis diagnosis. Laterality (especially right side) and size of the lymphadenopathy and cost effectiveness of EBUS-FNA are areas of interest for further studies.

CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

REFERENCES

1. Von Bartheld MB, Veselić-Charvat M, Rabe KF, Annema JT. Endoscopic ultrasound-guided fine-needle aspiration for the diagnosis of sarcoidosis. *Endoscopy*. 2010 42(3):213-217.
2. Mehrotra R, Dhingra V. Cytological diagnosis of sarcoidosis revisited: A state of the art review. *Diagn. Cytopathol*. 2011;39(7):541-548.
3. Costabel U, Ohshimo S, Guzman J. Diagnosis of sarcoidosis. *Curr Opin Pulm Med*. 2008;14(5):455-461.
4. Rosen Y. Pathology of Sarcoidosis. *Semi Resp Critical Care Med*. 2007;28(1):36-52.
5. Aisner SC, Gupta PK, Frost JK. Sputum cytology in pulmonary sarcoidosis. *Acta Cytol*. 1977;21(3):394-398.
6. Zwischenberger JB, Savage C, Alpard SK, Anderson CM, Marroquin S, Goodacre BW. Mediastinal transthoracic needle and core lymph node biopsy: should it replace mediastinoscopy? *Chest*. 2002;121(4):1165-1170.
7. Miliauskas S, Zemaitis M, Sakalauskas R. Sarcoidosis--moving to the new standard of diagnosis? *Medicina (Kaunas)*. 2010;46(7):443-446.
8. Zaman SS, Elshami A, Gupta PK. Bronchoalveolar lavage cytology in pulmonary sarcoidosis. *Acta Cytol*. 1995;39 (6):1117-2312.
9. Zhang R, Mietchen C, Krüger M, et al. Endobronchial ultrasound guided fine needle aspiration versus transcervical mediastinoscopy in nodal staging of non small cell lung cancer: a prospective comparison study. *J Cardiothorac Surg*. 2012;7:51-56.
10. Trisolini R, Tinelli C, Cancellieri A, et al. Transbronchial needle aspiration in sarcoidosis: yield and predictors of a positive aspirate. *J Thorac Cardiovasc Surg*. 2008;135(4):837-842.
11. Oki M, Saka H, Kitagawa C, et al. Prospective study of endobronchial ultrasound-guided transbronchial needle aspiration of lymph nodes versus transbronchial lung biopsy of lung tissue for diagnosis of sarcoidosis. *J Thorac Cardiovasc Surg*. 2012;143(6):1324-13249.
12. Tremblay A, Stather DR, Maceachern P, Khalil M, Field SK. A randomized controlled trial of standard vs endobronchial ultrasonography-guided transbronchial needle aspiration in patients with suspected sarcoidosis. *Chest*. 2009;136(2):340-346.
13. Cameron SE, Andrade RS, Pambuccian SE. Endobronchial ultrasound-guided transbronchial needle aspiration cytology: a state of the art review. *Cytopathology*. 2010;21(1):6-26.
14. Schwartz LE, Aisner DL, Baloch ZW, et al. The diagnostic efficacy of combining bronchoscopic tissue biopsy and endobronchial ultrasound-guided transbronchial needle aspiration for the diagnosis of malignant lesions in the lung. *Diagn Cytopathol*. 2012 Feb 23. doi: 10.1002/dc.22847:1-7. [Epub ahead of print]
15. Spagnolo P, Sato H, Grunewald J, et al. A common haplotype of the C-C chemokine receptor 2 gene and HLA-DRB1*0301 are independent genetic risk factors for Löfgren's syndrome. *J Intern Med*. 2008;264(5):433-41.
16. Michael H, Ho, S, Pollack B, Gupta M, Gress F. Diagnosis of intra-abdominal and mediastinal sarcoidosis with EUS-guided FNA. *Gastrointest Endosc*. 2008;67(1):28-34.
17. Oki M, Saka H, Kitagawa C, et al. Real-time endobronchial ultrasound-guided transbronchial needle aspiration is useful for diagnosing sarcoidosis. *Respirology*. 2007;12(6):863-868.
18. Iwashita T, Yasuda I, Doi S, et al. The yield of endoscopic ultrasound-guided fine needle aspiration for histological diagnosis in patients suspected of stage I sarcoidosis. *Endoscopy*. 2008;40(5):400-405.
19. Sider L, Horton ES Jr. Hilar and mediastinal adenopathy in sarcoidosis as detected by computed tomography. *J Thorac Imaging*. 1990;5(2):77-80.
20. Morgenthau AS, Iannuzzi MC. Recent advances in sarcoidosis. *Chest*. 2011;139(1):174-182.