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The utility of endobronchial ultrasound-transbronchial needle aspiration in patients with suspected extra-pulmonary sarcoidosis without thoracic lymphadenopathy

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ABSTRACT

Background: Diagnosis of extra-pulmonary sarcoidosis can be difficult, and a biopsy is usually required. We evaluated the utility of endobronchial ultrasound-transbronchial needle aspiration (EBUS-TBNA) in patients with suspected extra-pulmonary sarcoidosis with thoracic lymph nodes ≤10 mm on chest computed tomography (CT) and no or minimal pulmonary infiltrates.

Methods: The Cleveland Clinic bronchoscopy registry was screened. Patients with thoracic lymph nodes >10 mm on short axis or significant pulmonary infiltrates in the chest CT scan were excluded. Two separate analyses using expert consensus (before and after release of bronchoscopy results) were the reference standard.

Results: 15 patients met the inclusion criteria. 40% had suspected ocular, 33% cardiac and 27% neurologic sarcoidosis. Six patients (40%) had EBUS-TBNA compatible with sarcoidosis. When the reference standard was the consensus diagnosis blinded to bronchoscopy results, the sensitivity, specificity, positive predictive value and negative predictive value of EBUS-TBNA were 56%, 83%, 83%, and 56% respectively. The combination of a positive EBUS-TBNA and BAL CD4/CD8 improved the specificity from 83 to 100%, but the difference was not statistically significant ($p = 0.074$). When the reference standard was the consensus diagnosis with the bronchoscopic results, the sensitivity, specificity, positive predictive value and negative predictive value of EBUS-TBNA were 75%, 100%, 100%, and 78% respectively.

Conclusions: In patients with suspected extra-pulmonary sarcoidosis, the EBUS-TBNA may be useful in the diagnosis of patients with thoracic lymph nodes ≤10 mm and no or minimal pulmonary infiltrates on chest CT. Larger and prospective studies are needed to validate our findings.

1. Introduction

Sarcoidosis is a granulomatous inflammatory and multi-systemic disease of unknown etiology which involves the lungs in more than 90% of patients [1]. Despite this high prevalence of pulmonary involvement, a case-control study including 736 patients with sarcoidosis demonstrated that 14 patients (1.9%) had disease limited to extra-thoracic sites [2]. This group, although relatively small, represents a challenge when it comes to selecting sites for biopsy.

The most common extra-pulmonary organs involved are skin, lymph nodes, eyes and liver [2,3]. Cardiac and neurologic involvement are less frequent [1,2,4–9]. The diagnosis of these extra-pulmonary manifestations can be difficult due to a wide differential diagnosis and nonspecific features of testing, so tissue confirmation is often necessary. Although cardiac and central nervous system biopsies could be obtained, the relatively high risk of these procedures, and the low yield for endomyocardial biopsy have made the evaluation for thoracic involvement a crucial part of the diagnostic process [7–14].

Endobronchial ultrasound-transbronchial needle aspiration (EBUS-TBNA) has been shown to be useful in diagnosing sarcoidosis in patients with thoracic lymphadenopathy on chest imaging. A meta-analysis of 15 studies including mainly patients with Scadding stages 1 and 2 showed a pooled diagnostic yield of 79% (95% CI 71–86%) [15]. In similar populations, EBUS-TBNA had a higher yield than conventional TBNA

Abbreviations: CI, Confidence intervals; CT, Computed tomography; EBUS-TBNA, Endobronchial ultrasound-transbronchial needle aspiration; PET-CT, Positron emission tomography-CT.

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(83.3% versus 53.8%, $p < 0.05$) [16], and endosonography with aspiration of intrathoracic lymph nodes had a higher yield than endobronchial and transbronchial biopsies combined (80% versus 53%, $p < 0.001$) [17].

Although there are no data on EBUS-TBNA in patients with suspected extra-pulmonary sarcoidosis and no thoracic lymphadenopathy on chest computed tomography (CT) scan, these data are available in other diseases such as lung cancer. In three separate studies including patients with no thoracic lymph nodes >10 mm, the yield of EBUS-TBNA in finding occult lymph node metastasis ranged from 8 to 19% [18–20].

Considering the data above, we believe that the best diagnostic approach in patients with suspected extra-pulmonary sarcoidosis and no or minimal abnormalities on chest CT scan has not been fully elucidated. Herein, we evaluated the utility of EBUS-TBNA in patients with suspected extra-pulmonary sarcoidosis with thoracic lymph nodes ≤10 mm in short axis and no or minimal pulmonary infiltrates on chest CT scan.

2. Methods

2.1. Study population

A total of 5383 consecutive patients in the Cleveland Clinic bronchoscopy registry from January 2016 to June 2017 were screened. Of these, 1616 patients underwent an EBUS-TBNA in this period. Sarcoidosis was suspected in 192 patients. Forty-seven patients who had extrapulmonary sarcoidosis as a suspected diagnosis before the bronchoscopy were identified and reviewed retrospectively. Baseline organ involvement was assessed using the World Association of Sarcoidosis and Other Granulomatous Diseases (WASOG) Sarcoidosis Organ Assessment Instrument [21]. Patients with significant pulmonary infiltrates or thoracic lymph nodes >10 mm in short axis in the chest CT scan were excluded. The final study population comprised 15 patients with suspected extra-thoracic sarcoidosis that underwent bronchoscopy, despite no signs of intra-thoracic sarcoidosis on chest CT.

2.2. Tests and reference standard

The following data were extracted from the electronic medical record and de-identified by one of the co-authors (CA): notes from referring physicians (e.g. ophthalmology, neurology and cardiology notes), serum angiotensin-converting enzyme, serum 25(OH) and 1,25(OH)2 vitamin D, serum soluble IL-2 receptor, complete blood count, complete metabolic panel, fungal studies (antibodies, antigens and cultures), chest x-ray, chest CT scan, positron emission tomography-CT (PET-CT), and bronchoscopy results.

PET-CT scan lymph node positivity was defined as standardized uptake value >2.5. The EBUS-TBNA sample was defined as adequate when the final cytology report described a diagnosis other than a non-diagnostic sample. Final EBUS-TBNA cytology results showing non-necrotizing granulomas or multinucleated giant cells were considered compatible with, but not diagnostic of sarcoidosis. Chart review was performed in patients with final EBUS-TBNA results compatible with sarcoidosis to rule out the development of features consistent with an alternative diagnosis during the follow up. Transbronchial biopsy was defined as adequate when alveolated lung tissue was present on histology. Endobronchial biopsy was defined as adequate when bronchial mucosa was present on histology. In our practice, transbronchial and/or endobronchial biopsies are done when EBUS-TBNA rapid on-site cytology evaluation is negative.

Two co-authors (MRN, DAC) with large experience with sarcoidosis reviewed the de-identified data to adjudicate the appropriateness of the initial suspicion of sarcoidosis and the final diagnosis. The final diagnosis was adjudicated for two separate time-points: before and after the release of bronchoscopy results to the two co-authors. Any discordance was resolved with consensus between them. The consensus diagnosis served as our reference standard. We did two separate analyses using two separate reference standards: consensus diagnosis before and after the release of bronchoscopy results to the two co-authors. This allowed us to perform an analysis where our test of interest (EBUS-TBNA) was not part of the reference standard, decreasing the risk of incorporation bias and overestimation of the results.

2.3. Statistical analysis

Descriptive statistics were used to summarize the data. Continuous variables were summarized as mean ± standard deviation or median with range. Categorical variables were summarized as absolute and relative frequencies. To identify baseline variables associated with a final cytology result compatible with sarcoidosis we performed univariate analyses. Due to the small sample size, continuous variables were compared with a non-parametric test (independent-samples Mann Mann-Whitney *U* Test), categorical variables were compared with Fisher's Exact Test and only univariate analyses were performed. Kappa statistics was used to calculate agreement beyond chance between the two expert reviewers during final diagnosis adjudication.

To assess the test characteristics of the EBUS-TBNA, we calculated the following parameters: pre-test probability (prevalence), sensitivity, specificity, positive and negative predictive values, and likelihood ratios. We used 95% confidence intervals (CI) and McNemar's test to compare sensitivities and specificities of different tests. The statistical software SPSS version 21 was used for analysis.

This study was approved by the Cleveland Clinic Institutional Review Board (#17-568).

3. Results

3.1. Baseline characteristics

Fifteen patients were included in the analysis (Fig. 1). The study population consisted of patients with suspected ocular (40%), cardiac (33%) and neurologic (27%) sarcoidosis. Baseline characteristics are shown in Table 1, stratified by EBUS-TBNA result compatible or not compatible with sarcoidosis. Baseline organ involvement according to the WASOG criteria is shown in Table 2. Five patients had non-necrotizing granulomas on final cytology, and one patient had multinucleated giant cells (total of 6 patients [40%] with EBUS-TBNA compatible with sarcoidosis). Fungal and mycobacterial stains were negative. Those 6 patients were followed for a median time of 28 months (IQR 5.25) and no alternative diagnoses were detected. All other 9 patients had benign lymphoid tissue in their samples.

A chest CT scan was performed in all patients and a PET-CT scan in 7 (47%). Bronchoalveolar lavage (BAL) and EBUS-TBNA were performed in all patients, random endobronchial biopsy in three (20%) and random transbronchial lung biopsies in 9 patients (60%).

3.2. Endobronchial and transbronchial biopsies

The median number of endobronchial biopsies was 3 (range 2–10), and all patients had adequate samples. The median number of transbronchial biopsies was 6 (range 5–16), and all patients had adequate samples. No granulomas were found in the endobronchial or transbronchial biopsies. There were no bronchoscopy related complications.

3.3. Reference standard

Before the release of the bronchoscopy results, the final diagnosis adjudication were as follows: expert 1 gave a final diagnosis of sarcoidosis in 9 out of 15 (60%) patients, expert 2 in 13 out of 15 (87%), overall agreement was 73%, and kappa value was 0.375. A final diagnosis of sarcoidosis by consensus before bronchoscopy results was given in 9 out of 15 patients (60%).

After the release of the bronchoscopy results, the final diagnosis

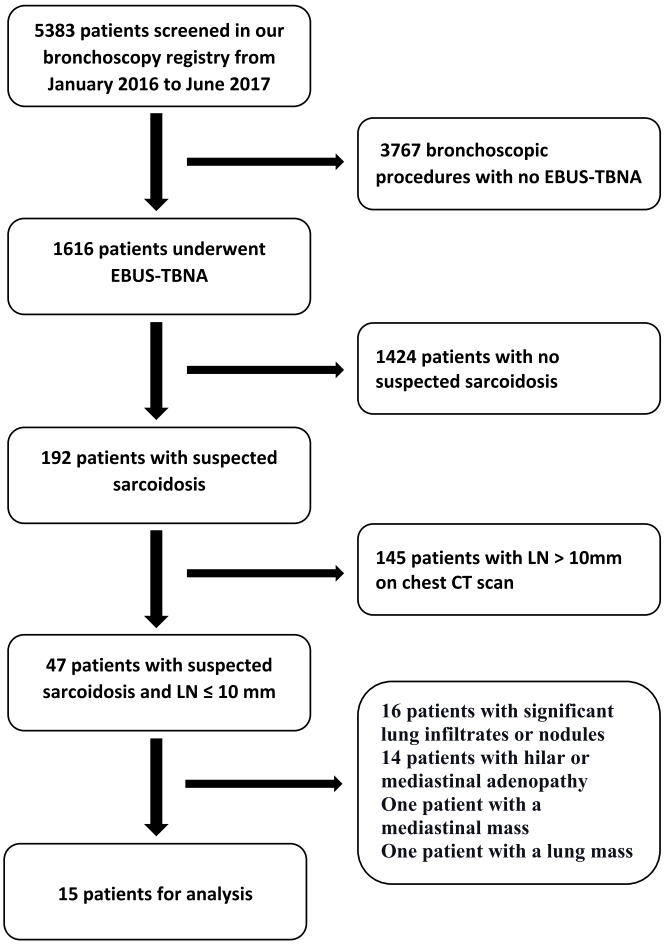

Fig. 1. Flow diagram with the inclusion and exclusion process.
Legend: LN = lymph node; CT = computed tomography.

adjudication were as follows: expert 1 gave a final diagnosis of sarcoidosis in 9 out of 15 (60%) patients, expert 2 in 8 out of 15 (53%), overall agreement was 93%, and kappa value was 0.865. A final diagnosis of sarcoidosis by consensus after bronchoscopy results was given in 8 out of 15 patients (53%).

3.4. PET-CT results and test characteristics

Among the 7 patients with PET-CT, 6 patients had hypermetabolic thoracic lymph nodes. In the one patient with no hypermetabolic lymph node, the EBUS-TBNA was negative. In the 6 patients with hypermetabolic nodes, 4 had EBUS-TBNA consistent with sarcoidosis. Two patients had hypermetabolic nodes but negative EBUS-TBNA results. Diagnostic characteristics of PET-CT scan are outlined in Tables 3 and 4.

3.5. BAL results and test characteristics

Out of 14 patients (one missing data on cell count and differential), three (21%) had BAL lymphocytosis >15%. Six patients (40%) had CD4/CD8 ratio >2.5, and 5 patients (33%) >3.5. Tables 3 and 4 show the diagnostic characteristics of BAL when the reference standard was the consensus diagnosis blinded to the bronchoscopy results. The prevalence of sarcoidosis with this reference standard was 60%.

When the reference standard was the consensus diagnosis with the bronchoscopy results, BAL lymphocytosis >15% had a sensitivity of 29% (95% CI 5–70) and a specificity of 86% (95% CI 42–99). Bronchoalveolar lavage CD4/CD8 ratio >3.5 had sensitivity of 50% (95% CI 17–83) and specificity of 86% (95% CI 42–92). The prevalence of sarcoidosis with this reference standard was 53%.

Table 1
Baseline variables stratified by EBUS-TBNA result compatible or not with sarcoidosis.

Variable	All patients (n = 15)	EBUS-TBNA compatible with sarcoidosis (n = 6)	EBUS-TBNA not compatible with sarcoidosis (n = 9)	*p* value
Age, mean (SD)	51 (14)	61 (12)	45 (11)	**0.036**
Female, n (%)	9 (60)	3 (50)	6 (67)	0.622
White, n (%)	11 (73)	5 (83)	6 (67)	0.604
Current or prior smoker, n (%)	8 (57)	3 (60)	5 (56)	1.000
Organ involvement				0.660
Ocular, n (%)	6 (40)	2 (33)	4 (44)	
Cardiac, n (%)	5 (33)	3 (50)	2 (22)	
Neurologic, n (%)	4 (27)	1 (17)	3 (33)	
LN size by CT, mean (SD)	6.6 (1.2)	7.1 (0.9)	6.3 (1.4)	0.224
LN FDG positive, n (%)[a]	6 (86)	4 (100)	2 (67)	0.429
LN size by EBUS, mean (SD)	7.1 (1.8)	8.1 (1.5)	6.5 (1.7)	0.088
LN sampled per case, mean (SD)	2.6 (0.9)	2.3 (0.5)	2.8 (1.1)	0.388
TBNA passes per LN, mean (SD)	4.4 (1.3)	5.2 (1.5)	3.9 (1.0)	0.066
LN sample adequacy, n (%)	15 (100)	6 (100)	9 (100)	1.000

Legend: EBUS = endobronchial ultrasound; TBNA = transbronchial needle aspiration; LN = lymph node; CT = computed tomography.
Bold is for $p < 0.05$
[a] Only 7 patients had PET scans, 4 of them with EBUS-TBNA compatible with sarcoidosis, 3 of them with EBUS-TBNA not compatible with sarcoidosis.

There was only one patient with lymphocytosis >15% who had no granulomas on EBUS-TBNA, and a separate patient with CD4/CD8 ratio >3.5 who had no granulomas on EBUS-TBNA. None of those two patients were considered to have sarcoidosis on final diagnosis adjudication both before and after bronchoscopy results release.

3.6. EBUS-TBNA test characteristics

The diagnostic characteristics of EBUS-TBNA when the reference standard was the consensus diagnosis blinded to the bronchoscopy results (prevalence of sarcoidosis = 60%) are shown in Tables 3 and 4 Though the combination of a positive EBUS-TBNA *and* BAL CD4/CD8 >3.5 improved the specificity from 83% (95% CI 36–99) to 100% (95% CI 52–100), this difference was not statistically significant (p = 0.074) and a significant overlap of the confidence intervals was noted (Table 4). The combination of a positive EBUS-TBNA *or* BAL CD4/CD8 >3.5 had a sensitivity of 56% (95% CI 23–85%), similar to EBUS-TBNA alone.

When the reference standard was the consensus diagnosis with the bronchoscopy results (prevalence of sarcoidosis = 53%), the diagnostic characteristics of EBUS-TBNA were: sensitivity 75% (95% CI 36–96), specificity 100% (95% CI 56–100), positive likelihood ratio ∞, negative likelihood ratio 0.3 (95% CI 0.08–0.8), positive predictive value 100% (95% CI 52–100) and negative predictive value 78% (95% CI 40–96).

4. Discussion

To our knowledge, this is the first study to evaluate the utility of EBUS-TBNA in patients with suspected extra-pulmonary sarcoidosis without thoracic lymphadenopathy and no or minimal pulmonary infiltrate on chest CT scan. In our analysis, we found EBUS-TBNA final cytology result to be compatible with sarcoidosis in a significant

Table 2
Baseline organ involvement according to the WASOG Sarcoidosis Organ Assessment Instrument.

Variable	All patients (n = 15)	EBUS-TBNA compatible with sarcoidosis (n = 6)	EBUS-TBNA not compatible with sarcoidosis (n = 9)	*p* value
Highly probable ocular sarcoidosis, n (%)	6 (40)	2 (33)	4 (44)	NA
Uveitis, n (%)	6 (40)	2 (33)	4 (44)	NA
Snowball, n (%)	1 (7)	1 (17)	0 (0)	0.333
At least probable cardiac sarcoidosis, n (%)	5 (33)	3 (50)	2 (22)	NA
Reduced EF without risk factors, n (%)	2 (13)	1 (17)	1 (11)	1.000
Spontaneous or inducible sustained VT without risk factors, n (%)	1 (7)	1 (17)	0 (0)	1.000
Patchy uptake on dedicated cardiac PET, n (%)	5 (33)	3 (50)	2 (22)	NA
Delayed enhancement on CMR, n (%)	4 (27)	2 (33)	2 (22)	1.000
T2 prolongation on CMR, n (%)	1 (7)	0 (0)	1 (11)	0.400
Reduced EF without risk factors, n (%)	3 (20)	2 (33)	1 (11)	1.000
Atrial dysrhythmias, n (%)	2 (13)	1 (17)	1 (11)	1.000
Highly probable neurosarcoidosis, n (%)	3 (20)	1 (17)	2 (22)	1.000
Clinical syndrome consistent with CNS granulomatous inflammation plus an abnormal MRI or CSF, n (%)	3 (20)	1 (17)	2 (22)	1.000
Facial palsy, n (%)	1 (7)	0 (0)	1 (11)	1.000
Seizure, n (%)	1 (7)	0 (0)	1 (11)	1.000

Legend: WASOG = World Association of Sarcoidosis and Other Granulomatous Diseases; EF = ejection fraction; VT = ventricular tachycardia; PET = positron emission tomography scan; CMR = cardiovascular magnetic resonance imaging; CNS = central nervous system; MRI = magnetic resonance imaging; CSF = cerebral spinal fluid.

Table 3
Frequency of positive and negative PET-CT scan, BAL and EBUS-TBNA results stratified by final diagnosis.

Test	Result	Final diagnosis: sarcoidosis	Final diagnosis: no sarcoidosis
PET-CT of thoracic lymph nodes (n = 7)	Positive	5	1
	Negative	1	0
BAL lymphocytes (n = 14)	>15%	2	1
	≤15%	6	5
BAL CD4/CD8 (n = 15)	>3.5	4	1
	≤3.5	5	5
EBUS-TBNA of thoracic lymph nodes (n = 15)	Positive	5	1
	Negative	4	5
EBUS-TBNA positive and BAL CD4/CD8 >3.5 (n = 15)	Yes	4	0
	No	5	6

Legend: Reference standard = consensus diagnosis blinded to the bronchoscopy results.

proportion of cases. Furthermore, we demonstrated that PET-CT scan is a sensitive marker of sarcoidosis in this population despite small thoracic lymph nodes, and that a combination of BAL and EBUS-TBNA is quite specific for a final diagnosis of sarcoidosis.

The diagnosis of sarcoidosis is based on a compatible clinical and radiological picture that usually needs a histopathological confirmation [1,13]. The characteristic pathologic findings are the presence of non-necrotizing epithelioid cell granulomas in the absence of another identifiable cause [1,13]. Clinical and radiological features alone could have a high diagnostic accuracy in patients with pulmonary sarcoidosis Scadding Stages I and II, but are less accurate in other scenarios such as in Scadding Stage 0 [1]. Therefore, establishing a diagnosis of extra-pulmonary sarcoidosis in this group of patients with no or minimal findings in the lung parenchyma and no thoracic lymphadenopathy on chest CT scan is particularly difficult due to paucity of biopsy targets. Despite the lack of typical findings on chest CT scan, 60% of the patients ended up having sarcoidosis per our main reference standard (consensus diagnosis blinded to the bronchoscopy results). This represents almost 5% of our patients undergoing bronchoscopy for suspected sarcoidosis at our institution. While this number is relatively small, the true prevalence of this particular situation may be underestimated as some patients may end up never having a diagnostic attempt of any site and are treated empirically.

As demonstrated above, 40% of the EBUS-TBNA final cytology results were compatible with sarcoidosis. Using sarcoidosis specialists blinded to bronchoscopy results as the reference standard, EBUS-TBNA had a sensitivity of 56% and specificity of 83%. While this methodologic approach preserves the independence of the test and makes it statistically robust, it is reasonable to argue that the expert opinion without a biopsy is a not valid reference standard especially in this group of patients. When the sarcoidosis specialists were aware of the bronchoscopy results, the EBUS-TBNA sensitivity and specificity improved to 75% and 100%, respectively. The adjudication of the diagnosis based on all clinical, radiographic, and pathologic data is pragmatic, but including the test in the reference standard introduces bias that can overestimate the accuracy of the test [22,23]. The literature does not provide a clear solution to this problem [24]. We chose to present both analyses, and one could argue that the real effect size could be somewhere in between.

Our study has several limitations. First and foremost, we did not objectively quantify the pretest and posttest probabilities of sarcoidosis. Due to the retrospective nature of the study, we opted for a more simplified approach and classified as “yes” or “no” both suspicion (i.e. pretest probability) and final diagnosis (i.e. posttest probability) of sarcoidosis. We recognize the limitation of this approach since diagnosing a disease is basically a probability estimate. Another limitation was the lack of a multidisciplinary discussion during the final diagnosis adjudication, which is an important step in the diagnostic process of extra-pulmonary sarcoidosis [25]. However, the two expert reviewers had access to the de-identified notes from the referring ophthalmologists, neurologists and cardiologists. Therefore, the impressions from those other subspecialties were always considered. The retrospective nature also increases the risk of bias and overestimation of the results. However, our methodology of blinding the adjudicators of outcome possibly decreases that risk.

The small sample size is another important limitation. As demonstrated with the wide confidence intervals, this small sample size introduces the risk of random error. In addition, statistics relating to sensitivity, specificity, positive and negative predictive values could represent overfitting of the data. However, due to lack of thoracic adenopathy on chest CT in this population with suspected sarcoidosis, we believe that identifying 15 patients that underwent EBUS-TBNA is the largest reported experience to date. We don’t have a protocol in our institution to perform bronchoscopy systematically in those patients, so the decision to refer patients for bronchoscopy was made by the treating physician on a case-by-case basis. Finally, one may question whether all the granulomatous inflammation identified from the nodal samples are indeed due to sarcoidosis. The prevalence of tuberculosis in our area is low making this diagnostic possibility on any of our cases less likely [26–28]. A role of atypical mycobacterial infection in our results also

Table 4
PET-CT scan, BAL and EBUS-TBNA diagnostic characteristics.

Test	Sn, % (95% CI)	Sp, % (95% CI)	+LR (95% CI)	−LR (95% CI)	PPV (95% CI)	NPV (95% CI)
Positive lymph nodes on PET-CT (n = 6/7)	83 (36–99)	0 (0–95)	0.8 (0.6–1.2)	∞	83 (36–99)	0 (0–95)
BAL lymph > 15% (n = 3/14)	25 (4–64)	83 (36–99)	1.5 (0.2–13.0)	0.9 (0.6–1.4)	67 (13–98)	45 (18–75)
BAL CD4/CD8 > 3.5 (n = 5/15)	44 (15–77)	83 (36–99)	2.7 (0.4–18.4)	0.7 (0.3–1.3)	80 (30–99)	50 (20–80)
EBUS-TBNA (n = 6/15)	56 (23–85)	83 (36–99)	3.3 (0.5–21.9)	0.5 (0.2–1.2)	83 (36–99)	56 (23–85)
EBUS-TBNA and BAL CD4/CD8 > 3.5 (n = 4/15)	44 (15–77)	100 (52–100)	∞	0.6 (0.3–1.0)	100 (40–100)	55 (25–82)

Legend: Sn = sensitivity; Sp = specificity; LR = likelihood ratio; PPV = positive predictive value; NPV = negative predictive value. Reference standard = consensus diagnosis blinded to the bronchoscopy results. Prevalence of sarcoidosis = 60%.

seems unlikely, due to the absence of clinical, radiographic and bacteriologic findings of this disease [29,30]. Histoplasmosis, however, is highly prevalent in our region, and it could have played a role in the EBUS-TBNA findings. The lack of culture and pathology findings consistent with histoplasma, the overall clinical picture of the patients, and the lack of histoplasma features during the follow up make this improbable as well [31].

Future directions include many important steps. First, our findings need to be validated in a larger and prospective study. This could provide a more accurate estimate of the prevalence of non-necrotizing granulomas detected by EBUS-TBNA in this population. In addition, it could explore the diagnostic characteristics of PET-CT scan, confirming or rebutting the high sensitivity seen in the present study. Future studies could also explore the role of EBUS-TBNA beyond the presence of multinucleated giant cells or granulomas by utilizing "omics" technologies. The intrathoracic lymph nodes are commonly involved in sarcoidosis, so it would be interesting to study the microenvironment of the so-called "normal" lymph nodes seen in our study's population [32].

In conclusion, our study demonstrates that EBUS-TBNA may be useful in the diagnostic pathway of patients with suspected extrapulmonary sarcoidosis with no thoracic adenopathy and no or minimal pulmonary infiltrates per CT scan. Furthermore, we demonstrated that PET-CT scan in this population may also be useful, despite the lack of typical findings on chest CT. Larger and prospective studies are needed to validate our findings, so we can decide with more confidence if this diagnostic approach is helpful.

Authors contributions

CA had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. CA, MRN, FAA, and DAC contributed substantially to the study design, data analysis and interpretation, and the writing of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Carlos Aravena: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Writing - original draft, Writing - review & editing. **Francisco A. Almeida:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Daniel A. Culver:** Methodology, Writing - original draft, Writing - review & editing. **Manuel L. Ribeiro Neto:** Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Writing - original draft, Writing - review & editing.

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