The role of EBUS-TBNA for the diagnosis of sarcoidosis – comparisons with other bronchoscopic diagnostic modalities

Takahiro Nakajima a, Kazuhiro Yasufuku b,*, Katsushi Kurosu c, Yuichi Takiguchi c, Taiki Fujiwara a, Masako Chiyo a, Kiyoshi Shibuya a, Kenzo Hiroshima d, Yukio Nakatani d, Ichiro Yoshino a

a Department of Thoracic Surgery, Graduate School of Medicine, Chiba University, Chiba, Japan
b Division of Thoracic Surgery, Toronto General Hospital, University Health Network, University of Toronto, 200 Elizabeth St 9N-957, Toronto ON M5G 2C4, Canada
c Department of Respirology, Graduate School of Medicine, Chiba University, Chiba, Japan
d Department of Diagnostic Pathology, Graduate School of Medicine, Chiba University, Chiba, Japan

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Summary
Background: The diagnosis of sarcoidosis requires both compatible clinical features and pathologic findings as a means to exclude other differential diagnoses. The utility of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) for diagnosis of sarcoidosis has been reported, although its indication remains unclear for cases of suspicious sarcoidosis. To clarify the role of EBUS-TBNA for the diagnosis of sarcoidosis, we compared three diagnostic modalities: EBUS-TBNA, transbronchial lung biopsy (TBLB) and bronchoalveolar lavage fluid analysis (BAL).

Methods: Thirty-eight patients with suspicious sarcoidosis who had enlarged hilar and/or mediastinal lymph nodes on chest CT were retrospectively reviewed. Patients with malignancies or prior established diagnosis of sarcoidosis were excluded. BAL was initially performed followed by TBLB and finally EBUS-TBNA at the same setting. Microbacterial examinations were also performed from all samples.

Results: Pathological findings compatible with sarcoidosis were obtained in 32 patients. The remaining 6 patients were diagnosed as one case each of chronic eosinophilic pneumonia, atypical mycobacterial infection and tuberculosis, and the remaining three were pathologically indefinite cases. Clinically, 35 patients were diagnosed with sarcoidosis. The diagnostic
Introduction

Sarcoidosis is a multi-system disorder of unknown etiology that is characterized by non-caseating epithelioid cell granulomas. Some patients with sarcoidosis develop cardiac or neurological dysfunctions, and 1%–5% of patients with chronic sarcoidosis die of respiratory failure, cardiac or neurological dysfunction. Systemic steroid therapy is used for progressive patients and this therapy should only be used based on a firm diagnosis. In addition to clinical features, a pathological confirmation is crucial for the diagnosis of sarcoidosis and to exclude other possible diseases.

Recently, the efficacy of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) for the diagnosis of sarcoidosis has been reported. A dedicated 22-gauge needle is used for EBUS-TBNA under local anesthesia, which allows cytological as well as histological evaluation of the lesion. However, there are no reports that have compared EBUS-TBNA to conventional bronchoscopic diagnostic modalities, such as transbronchial lung biopsy (TBLB) and bronchoalveolar lavage fluid analysis (BAL).

Materials and methods

Patients

To identify patients for this study, we performed a retrospective chart review of patients who underwent EBUS-TBNA for the period January 2004–April 2008. Patients with suspected or known malignancies or prior established diagnosis of sarcoidosis were excluded. We identified 38 patients with clinical and radiological features suspicious of sarcoidosis and the patients underwent EBUS-TBNA for the tissue confirmation of sarcoidosis. Both chest roentgenogram and computed tomography (CT) were performed prior to bronchoscopic examination. At least 1 enlarged mediastinal or hilar lymphadenopathy >10 mm in the short axis was observed in all patients. Patients were managed on an outpatient basis, unless they were already admitted to the hospital for other reasons. Written informed consent was obtained from all patients included in this study.

Examination procedures

All patients received three diagnostic modalities during the same examination: EBUS-TBNA, TBLB and BAL. All bronchoscopic procedures were performed on an outpatient basis under local anesthesia with mild conscious sedation. Three milliliter of 1% lidocaine was nebulised and 5 ml of 4% lidocaine was sprayed into the pharynx. Two milliliter of 1% lidocaine was administered through the channel during the procedures. The bronchoscope was inserted orally during midazolam-induced conscious sedation. Patients were monitored by ECG, pulse oximetry and blood pressure without the presence of an anesthesiologist. Conventional flexible bronchoscopy (BF-240 and BF-260 bronchovideoscope; Olympus; Tokyo, Japan) was first used for BAL and TBLB.

First, BAL was performed in a standard fashion (50 ml for 3 times, total 150 ml, recovery rate >60%). Then, at least 3 biopsy specimens were obtained by TBLB by independent pulmonologists (KK, YT). TBLB specimen was mainly obtained from the right upper lobe under the fluoroscopy guidance if there were no radiological findings in the lung field (stage I). If there was evidence of pulmonary parenchymal involvement by chest radiographs (stage II), TBLB specimen was obtained from targeted region. Following the conventional bronchoscopy, EBUS-TBNA using a convex probe endobronchial ultrasound bronchoscope (CP-EBUS; BF-UC260F-OL8, Olympus, Tokyo, Japan) was performed (TN, KY, TF, MC).

Diagnosis of sarcoidosis

A diagnosis of sarcoidosis was made if the clinic-radiological findings were supported by pathological tissue demonstrating non-caseating granulomas without necrosis or the cytological specimen demonstrated non-caseating epithelioid cells based on subsequent clinical assessments if a negative culture result was obtained from all samples. Other granulomatous diseases were excluded by reviewing the patient’s history and microbiological results. Cases were classified as “indefinite” if no diagnosis could be made by pathological and biological examinations. All patients were followed up clinically and radiologically for more than 6 months.

EBUS-TBNA procedure

The CP-EBUS is integrated with a convex transducer (frequency = 7.5 MHz) at the tip of a flexible bronchoscope. The outer diameter of the insertion tube of the flexible bronchoscope is 6.7 mm, and that of the tip was 6.9 mm. The direction of view is 35° forward oblique. This CP-EBUS is a linear curved array transducer that scans parallel to the insertion direction of the bronchoscope. Images are obtained by directly contacting the probe or by attaching an inflated balloon filled with saline to the tip, which keeps the probe in contact while sampling the lymph node. The ultrasound images are processed using a dedicated ultrasound scanner (EU-C2000; Olympus) and visualized...
simultaneously along with the conventional bronchoscopy image on the same monitor. This system has an integrated power Doppler mode that allows blood vessels to be identified and avoids inadvertent puncturing. The inner diameter of the working channel is 2.0 mm. A dedicated 22-gauge needle (NA-2015X-4022, Olympus) is used to perform transbronchial needle aspiration. The inner diameter of this needle was nearly equal to that of a conventional 21-gauge needle, which allowed the sampling of histological cores in some cases. The station of lymph nodes was identified according to the International Staging System.4

Lymph node sampling

After the initial puncture, the internal sheath was used to clean out the internal lumen, which became clogged with bronchial membrane. The internal sheath was then removed and negative pressure was applied with a syringe. After the needle was moved back and forth inside the lymph node, the needle was retrieved and the internal sheath was used again to push out the histological core.5

With this method, histological cores as well as cytological specimens could be obtained. The aspirated material was smeared onto glass slides. Smears were air-dried as well as fixed in 95% alcohol. Dried smears were evaluated by an on-site cytopathologist to ensure that the cell material obtained was of adequate quality. Adequate cell material was defined as having a specific diagnosis, such as presence of epithelioid cells without necrosis or the presence of lymphocytes in the specimen. If adequate tissue was not identified by on-site cytology after 3 passes, the procedure was terminated. In addition, Papanicolaou staining and light microscopy were done by an independent cytopathologist who was blinded to the details of the cases. The obtained histological specimens were fixed in formalin before being sent to the pathology department. Aspirated material was also sent for microbiological examination, including special staining for fungi, acid-fast bacilli and cultures for tuberculosis and fungi. In addition, PCR examination was performed for acid-fast bacilli if needed.

Statistical analysis

The diagnostic accuracy rate was calculated using standard definitions. The chi-square test was used for comparison of the two modalities for the correct prediction of sarcoidosis.

Ethics committee approval

The study was approved by the ethical committee of Chiba University, Graduate School of Medicine (No. 220) and written consent was obtained from all patients. All authors read and approved the final manuscript.

Results

Patient characteristics

There were 11 males and 27 females. Their average age was 48.2 years (range 22–77 years). According to the standard scoring system for chest roentgenograms,6 31 patients (88.6%) were classified as stage I and 4 patients were stage II. Pathological and microbiological diagnoses by all bronchoscopic procedures were as follows: 32 cases of sarcoidosis, one case each of chronic eosinophilic pneumonia, atypical mycobacterial infection and tuberculosis, and 3 indefinite cases. BAL was performed for all patients for a supporting diagnosis and the CD4/CD8T cell ratio was evaluated. Serum levels of angiotensin-converting enzyme (ACE) and Gallium 67 scanning were also evaluated for selected cases.7 Finally, 35 patients were clinically diagnosed with sarcoidosis.

Evaluated lymph nodes

A total of 51 lymph nodes were recorded and aspirated. The mean size of the enlarged lymph nodes, as measured by EBUS, was 14.8 mm (range 7.3–30.0 mm). Forty-four (86.3%) enlarged lymph nodes were located in the mediastinal region and the remaining 7 lymph nodes were hilar lymph nodes. The most commonly occurring lymph node station was subcarinal, station 7 (28/51, 54.9%), with a measured mean size of 16.4 mm (range 8–30 mm) (Table 1).

Diagnostic rate

Of 35 patients, 14 (40.0%) had non-caseating granulomas by TBLB. EBUS-TBNA revealed non-caseating granulomas or epithelioid cells in 32 of 35 cases (91.4%; 22 non-caseating granuloma, 25 epithelioid cells). The overall diagnostic accuracy rate for sarcoidosis was significantly better by EBUS-TBNA compared with TBLB (p < 0.001). A CD4/CD8 ratio > 3.5 by BAL was shown in 25 of 35 patients (65.7%). Further analysis was performed following the standard scoring system for chest roentgenograms. For stage I patients, 28 of 31 patients were diagnosed with sarcoidosis by EBUS-TBNA (90.3%, 19 non-caseating granuloma, 22 epithelioid cells), although TBLB could only diagnose 10 patients with sarcoidosis (32.3%) (Tables 2 and 3). However, each modality had 100% accuracy for stage II patients (Table 2). No complications were noted in this study, including pneumothorax, pneumomediastinum or excessive bleeding.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient characteristics.</th>
</tr>
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<tbody>
<tr>
<td>Characteristics</td>
<td>No.</td>
</tr>
<tr>
<td>Patients</td>
<td>38</td>
</tr>
<tr>
<td>Male/female</td>
<td>11/27</td>
</tr>
<tr>
<td>Median age (range), yrs</td>
<td>48.2 (23–77)</td>
</tr>
<tr>
<td>Final diagnosis</td>
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<tr>
<td>Sarcoidosis</td>
<td>32</td>
</tr>
<tr>
<td>Chronic eosinophilic pneumonia</td>
<td>1</td>
</tr>
<tr>
<td>MAC</td>
<td>1</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1</td>
</tr>
<tr>
<td>Indefinite</td>
<td>3</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>31</td>
</tr>
<tr>
<td>Stage II</td>
<td>4</td>
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</table>
Table 2  Lymph node stations assessed by EBUS-TBNA.

<table>
<thead>
<tr>
<th>Station</th>
<th>CT</th>
<th>EBUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short axis in mm (range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#4R (n = 16)</td>
<td>15.2 (9–26)</td>
<td>14.2 (7.3–23.8)</td>
</tr>
<tr>
<td>#7 (n = 28)</td>
<td>16.4 (8–30)</td>
<td>18.0 (10.1–30.0)</td>
</tr>
<tr>
<td>#11 (n = 7)</td>
<td>13.3 (10–18)</td>
<td>12.3 (8.0–16.7)</td>
</tr>
<tr>
<td>Total (n = 51)</td>
<td>14.9 (8–30)</td>
<td>14.8 (7.3–30.0)</td>
</tr>
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</table>

Regional
Mediastinum (n = 44)
Hilum (n = 7)

Discussion

Although many of the stage I and II patients may be asymptomatic, differential diagnosis such as tuberculosis, other granulomatous disorders, Hodgkin lymphomas and malignancy cannot be ruled out with certainty. Therefore, tissue confirmation is crucial in patients with suspicious sarcoidosis. EBUS-TBNA could diagnose sarcoidosis more precisely, especially for stage I cases that showed hilar adenopathies and no infiltrates on chest roentgenograms.6 We previously reported the utility of EBUS-TBNA for the diagnosis of sarcoidosis. However, the indication for EBUS-TBNA in the diagnosis of sarcoidosis has remained unclear, as there are no reports that have compared EBUS-TBNA with TBLB and BAL. Although this study has a limitation due to the small number of patients, EBUS-TBNA is a promising modality for the diagnoses of stage I sarcoidosis.

The diagnosis of sarcoidosis is established when clinical and radiological findings are supported by histological evidence of non-caseating epithelioid cell granulomas. On the other hand, the utility of cytology in the diagnosis of sarcoidosis has been previously reported with the reported sensitivity of over 90%. Granulomas of known causes and local sarcoid reactions must be excluded, thus we examined all aspirated materials for microbacterial examination including special staining for fungi, acid-fast bacilli and cultures for tuberculosis and fungi. In addition, PCR examination was performed for acid-fast bacilli if needed. Furthermore, we excluded the patients with the possibility for other granulomatous diseases such as sarcoid reactions with malignancy and occupational disease (berylliosis) by reviewing the patient’s history.

TBLB is the recommended procedure in most cases. However, the diagnostic yield depends largely on the experience of the person performing the procedure and the number of biopsies.8 Furthermore, TBLB has a risk of pneumothorax and haemoptysis.9 BAL fluid analysis is also performed for sarcoidosis patients as the lymphocyte markers CD4 and CD8 can be used to analyze the CD4/CD8 ratio. Although a ratio > 3.5 substantiates the diagnosis of sarcoidosis,10 BAL is not used for pathological diagnosis.

Elevated serum ACE levels are often observed in sarcoidosis patients.11 However, there are also elevations of ACE levels in other granulomatous diseases, such as tuberculosis and Gaucher’s disease.12 Gallium 67 scanning also shows positive results in sarcoidosis patients. Typical findings of a gallium scan (Panda sign or Lambda sign) can support a diagnosis of sarcoidosis.13 However, these findings are only observed in a limited proportion of patients14 and they are not a pathological diagnosis.

By comparison, up to 90% of sarcoidosis patients present hilar and/or mediastinal lymph node enlargements radiologically.15 Mediastinoscopy has hitherto been the method of choice when TBLB is futile.16 However, mediastinoscopy is invasive, carried out under general anesthesia, costly, requires inpatient care and has a complication rate of 2–3%.17 This has led to the search for a less invasive tool with a high diagnostic yield and minimal complications.

TBA was shown to increase the diagnostic yield to 72% for stage I sarcoidosis.18 However, TBA is performed with “blind” needle aspiration guided by prior CT imaging. Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is a transesophageal approach for mediastinal lymph nodes under ultrasound guidance and the diagnostic yield is also very high.19–21 However, EUS-FNA has a limitation in that it can only access mediastinal lymph nodes that are adjacent to the esophagus. EUS-FNA cannot access the hilar lymph nodes, which are frequently enlarged in sarcoidosis patients.19

EBUS-TBNA was first proved to be clinically useful for evaluation of mediastinal and hilar lymph nodes under local anesthesia and conscious sedation.22 Past reports on EBUS-TBNA have focused on the diagnosis and staging of lung cancer with hilar and mediastinal lymph nodes, and the diagnostic yield is around 90%.5,23–26 Severe complications related to EBUS-TBNA have never been reported.27

Now, we recommend that EBUS-TBNA be added to the conventional bronchoscopic procedures when the patient has suspected stage I sarcoidosis.
Conflict of interest statement

KY received unrestricted grants from Olympus Medical Systems for continuing medical education. Other authors have no conflict of interests to declare.

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