SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2012; 29; 82-89

Feasibility of cytological diagnosis of sarcoidosis with endobronchial US-guided transbronchial aspiration

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Abstract. *Background:* Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) has a high diagnostic value in sarcoidosis if the obtained histological specimen is indicative of a non-caseating epithelioid-cell granuloma. However, EBUS-TBNA in sacoidosis sometimes affords solely cytological specimens. *Objective:* To investigate the relevance of EBUS-TBNA cytology specimens in diagnosing sarcoidosis. *Design:* The study population comprised 72 patients with sarcoidosis and 116 patients who had thoracic malignancies and intrathoracic lymphadenopathy but were eventually proven to be metastasis-free (controls). The EBUS-TBNA samples obtained for these subjects were blindly evaluated for the presence of epithelioid cell clusters by 2 independent cytoscreeners and a pathologist. *Results:* Interobserver variability in the specimen grading was minimal. The sensitivity and specificity were 65.3% and 94.0%, respectively. The sensitivity was high, at 87.5%, for the combined cytological and histological examinations. Of 7 controls whose cytological specimens showed epithelioid cell clusters, 3 were also deemed positive for sarcoidosis on histological examination, which indicated that they had sarcoid reaction to cancer. *Conclusions:* Cytological evaluation of the EBUS-TBNA specimens had higher sensitivity than histological evaluation alone for intrathoracic lymphadenopathy due to sarcoidosis. It should be recognized, however, that up to 6% of patients with thoracic malignancy may have sarcoid reaction in non-metastatic lymph nodes. *(Sarcoidosis Vasc Diffuse Lung Dis 2012; 29: 82-89)*

Key words: sarcoidosis, bronchoscopy, endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), cytology, non-caseating epithelioid cells

Abbreviations

BAL: bronchoalveolar lavage
CI: confidence interval
EBUS-TBNA: endobronchial ultrasound-guided transbronchial needle aspiration
TBLB: transbronchial lung biopsy

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Introduction

Sarcoidosis is diagnosed on the basis of clinico-radiological findings and histological evidence of non-caseating epithelioid-cell granulomas. Traditionally, pulmonary sarcoidosis is diagnosed by transbronchial lung biopsy (TBLB); however, its diagnostic yield in this disease, which is about 40-90%

when 4 or 5 biopsy samples are obtained, depends largely on the expertise of the operator (1-2). If mediastinal and/or hilar lymphadenopathy is detected in imaging studies, biopsy is performed by mediastinoscopy, video-assisted thoracoscopic lung biopsy, or open lung biopsy. Although the diagnostic sensitivity of these procedures is as high as > 90% (3-4), they are invasive and expensive (4).

Recent reports indicate that EBUS-TBNA is a highly efficacious and safe method for diagnosing pulmonary sarcoidosis (5-13). This technique enables both cytological and histological evaluation of the lesion. Since the detection of epithelioid cells in cytological specimens obtained by EBUS-TBNA suggests the presence of non-caseating epithelioid-cell granulomas in the lymph nodes, this modality can be applied in the diagnosis of sarcoidosis. In fact, Nakajima et al (10) and Oki et al (12) reported that both cytological and histological EBUS-TBNA specimens have high sensitivity for sarcoidosis. Epithelioid cells, however, are also detected in the lymph nodes in conditions other than sarcoidosis, namely, lung cancer and infectious diseases such as tuberculosis and mycosis (14). Moreover, it is possible that normal macrophages or histiocytes may be mistaken for epithelioid cells during conventional cytological examination. In other words, it is unclear whether the detection of epithelioid cells in mediastinal and/or hilar lymph nodes by cytological evaluation is specific to sarcoidosis. The most frequent cause of mediastinal and/or hilar lymphadenopathy is metastasis of primary lung cancer or other malignancies. Therefore, if epithelioid cells are detected on cytological evaluation, it is important to determine whether the lymphadenopathy is caused by sarcoidosis or other malignant diseases.

In this study, we obtained mediastinal and/or hilar lymph node cytological samples by EBUS-TBNA from patients with sarcoidosis of stages 1 and 2 and from patients with malignant diseases (mostly primary lung cancer) who were eventually proven to be metastasis-free. These samples were evaluated independently by 2 cytoscreeners and then a pathologist, all of whom were blinded to patient information. Their findings were analyzed to assess the sensitivity and specificity of cytological examination of EBUS-TBNA samples for diagnosing sarcoidosis.

Patients and methods

Patients

The study population comprised 2 groups: sarcoidosis group and control group. The former included patients who were suspected of having sarcoidosis on the basis of the presence of apparent hilar and/or mediastinal lymphadenopathy on CT images and relevant clinical manifestations. These patients had undergone EBUS-TBNA, bronchoalveolar lavage (BAL) fluid analysis, and TBLB during the first evaluation. They were prospectively enrolled in this study between January 2004 and December 2009. Thirty-eight of these patients, who had visited the clinic between January 2004 and April 2008, were the same subjects included in a previous study (10). Although the diagnostic efficacy of EBUS-TBNA for these patients has been reported previously, the sensitivity and specificity of blind cytological evaluation were analyzed for the first time in this study. The control group comprised patients who had thoracic malignancies and showed hilar and/or mediastinal lymphadenopathy on CT images but were eventually found to be free of metastasis on the basis of histological and/or cytological evaluation. These patients were prospectively enrolled in the study between April 2007 and September 2008. Patients in the sarcoidosis group underwent EBUS-TBNA for a couple of enlarged hilar and/or mediastinal lymph nodes, while the control group patients underwent EBUS-TBNA for cancer staging.

Bronchoscopic procedures

EBUS-TBNA was performed as described previously (10). Briefly, a convex-probe-equipped EBUS bronchoscope (CP-EBUS; BF-UC260F-OL8, Olympus, Tokyo, Japan) supported by an ultrasound image processor (model EU-C2000; Olympus) and a dedicated 22-gauge aspiration needle (NA-201SX-4022, Olympus) was used under local anesthesia and mild sedation with midazolam. There was no definite reason to use 22-, instead of 21-gauge, needles in this research. We simply used them just because only 22-gauge needles were available in our institute at the start of this research. We did not compare the efficacy between 22- and 21-gauge needles. Each transbronchial puncture was

performed with a needle equipped with an internal sheath. 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 the histological core. Then, an impulse positive pressure was applied with a syringe to push out remaining material for cytological smear on a glass slide. With this method, both histological cores and cytological specimens were obtained in many cases. For each aspiration, a histological core, when available, was stored and fixed in 10% buffered formalin for subsequent examination, and the remaining aspirated material was mounted on a slide-glass by using the pressure created by pushing the plunger of an empty 20-ml syringe. The slide-glass was then rubbed against another slide-glass to smear the mounted material onto both slide-glasses. One slide-glass was placed in 95% ethanol for fixation and then stained with the Papanicolaou technique. The other was dried for fixation and stained on-site with a rapid Romanowsky-type stain (Diff-Quik®) for immediate examination to ensure that the cell material obtained was of adequate quality. If a positive on-site cytological diagnosis was obtained, no further lymph nodes were sampled. However, this on-site cytological diagnosis and the cytological diagnosis made using Papanicolaou staining were not considered for the clinical diagnosis of sarcoidosis, as stated below. The aspirated material was also dispersed into sterile saline solution for microbiological examinations, including staining and culture for bacteria, fungi, and acid-fast bacilli. In addition, polymerase chain reaction examination was performed for acid-fast bacilli for all the study subjects. The histological diagnoses were made on the basis of the results of hematoxylin and eosin staining. Although it was not essential for enrollment, for most of the patients in the sarcoidosis group, BAL with installation and recovery of 200 ml saline in 4 fractions followed by TBLB consisting of 3–5 biopsies with a conventional bronchoscopy were performed before EBUS-TBNA.

Clinical diagnosis of sarcoidosis for patients without histological proof

For patients without histological proof, the clinical diagnosis of sarcoidosis was established according to the guidelines of the Japan Society of Sarcoidosis and other Granulomatous Disorders (15). Briefly, patients with at least 2 involved organs and 2 or more of the following criteria are diagnosed with sarcoidosis even without histological proof: (1) bilateral hilar lymphadenopathy, (2) elevated level of serum angiotensin converting enzyme, (3) negative result of the tuberculin skin test, (4) marked accumulation of gallium-67 citrate in the involved organs, (5) increased lymphocyte counts and/or elevated CD4/CD8 ratio (considered significant at >15% and >3.5, respectively, for this study,) in BAL fluid analysis, and (6) elevated serum and/or urine calcium concentration.

Cytological evaluation

All slide-glasses (2–6 for each patient) containing the cytological specimens stained with Diff-Quik® and Papanicolaou technique were anonymized by sealing the labels. These samples were then randomly shuffled and evaluated for the presence of epithelioid cell clusters by cytoscreeners who were blinded to the patients' clinical presentation. The results were classified into 5 categories: grade 0, for absence of epithelioid cells; grade 1, for the presence of a few clusters of epithelioid cells not completely distinguishable from histiocytes; grade 2, for the unequivocal presence of epithelioid cell clusters, but less than 10 clusters per slide; grade 3, for the unequivocal presence of abundant clusters of epithelioid cells per slide; and grade X, for inadequate material for evaluation. Figure 1 shows a typical epithelioid cell cluster (A) and a suspected cluster of epithelioid cells, which cannot be completely distinguished from the usual histiocytes (B).

First, 2 certified cytoscreeners - the co-authors NT and FS - screened all the samples of all the 188 patients and graded the samples obtained for each patient. If both cytoscreeners graded a sample as 0, then 0 was considered the final grade for the given sample. All other samples were sent to an expert pathologist and a co-author, KH, for final evaluation with grading.

Statistical methods and ethical considerations

Patients definitively diagnosed with sarcoidosis according to the abovementioned criteria were con-

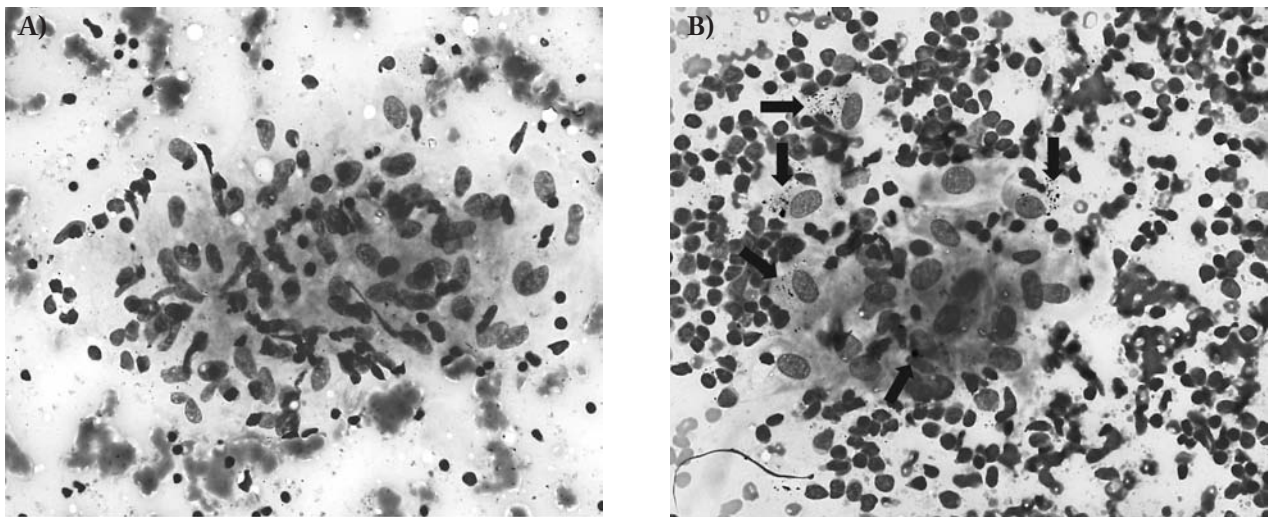

Fig. 1. A typical epithelioid-cell cluster unequivocally indicative of sarcoidosis found in a smear sample obtained by EBUS-TBNA. Cytological samples were classified into grade 2 or 3, depending on the abundance of the clusters, when such typical clusters are found (A). On the other hand, samples with clusters of cells resembling epithelioid cells but indistinguishable from the dust-filled histiocytes (arrows, B) were classified into grade 1 (Diff-Quik stain®, original magnification of 20×)

sidered as "true" sarcoidosis patients. Accordingly, the sensitivity and specificity with the 95% confidence intervals (CI) for cytological evaluation of EBUS-TBNA samples were calculated. The interobserver validity of the cytological evaluation was also assessed in terms of the intra-class correlation coefficient. The correlation between lymph node size and cytological grade was according to Kruskal-Wallis test. The study protocol is consistent with the principle of the Declaration of Helsinki, and was approved by the Institutional Review Board of Graduate School of Medicine, Chiba University (accession #220), and all enrolled patients gave written informed consent for this study.

Role of the funding source

The funding source, the Ministry of Education, Culture, Sports, Science and Technology of Japan, had no role in this study.

Results

Patient characteristics

Seventy-seven and 116 patients were enrolled in the sarcoidosis and control groups, respectively. Among the former group, 72 patients met the criteria for the clinical diagnosis of sarcoidosis and were eligible for further analyses. The diagnostic process for sarcoidosis is shown in Figure 2. All 116 patients in the control group were eligible for further analyses. The patient characteristics for each group are summarized in Table 1. None of the patients had any serious complications during BAL, TBLB, or EBUS-TBNA.

Lymph node stations assessed

Lymph node stations assessed in both the groups are summarized in Table 2. Because of easy accessibility, #3 and #7 mediastinal lymph nodes were predominantly assessed in the sarcoidosis group, whereas more and a wider range of stations were assessed in the control group for the staging of malignancies.

Histological diagnosis of core specimens obtained by EBUS-TBNA and TBLB

Core specimens for histological evaluation were successfully obtained in 57 of the 72 (79.2%) patients and in 104 out of the 116 (89.7%) controls. Non-caseating epithelioid-cell granulomas were found in 52 of the 72 (72.2%) patients and 4 of the 116 (3.4%) controls. Therefore, the sensitivity and specificity of

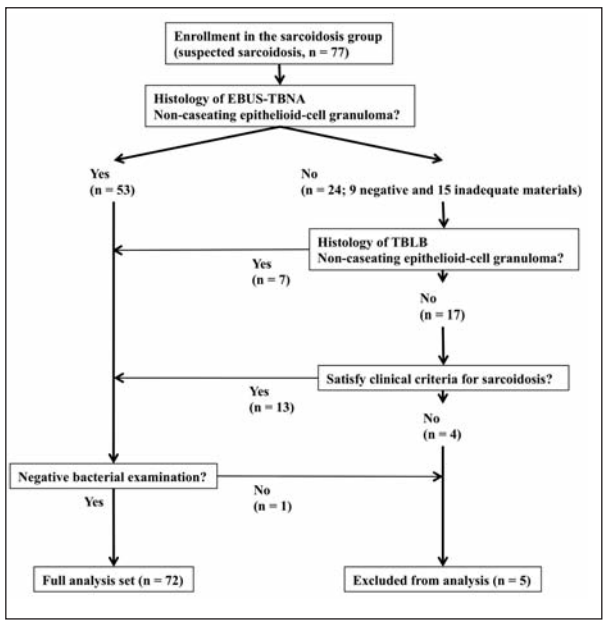

Fig. 2. Process of enrolling patients to the sarcoidosis group. Histological examination of EBUS-TBNA core samples revealed the presence of non-caseating epithelioid-cell granuloma, which is a finding compatible with sarcoidosis, in the specimens of 53 of the 77 patients; among the remaining 24 patients, histological evidence for granulomas were not obtained in the case of 9 patients and the samples were inadequate in the case of 15 patients. Among the 24 patients, 7 patients were diagnosed with sarcoidosis because non-caseating epithelioid-cell granulomas compatible with sarcoidosis were detected on TBLB, and 13 other patients were also diagnosed with sarcoidosis because they met the defined clinical criteria provided in the Patients and methods section. Microbial examination, however, revealed *Mycobacterium tuberculosis* infection in one of the 73 (53 plus 7 plus 13) patients. Thus, 72 patients were included in the full analysis set. On the other hand, 5 patients were excluded from the full analysis set for the following reasons: proven tuberculosis (n = 1), *Mycobacterium avium* infection proven by sputum culture (n = 1), eosinophilic pneumonia proven by BAL fluid analysis (n = 1), and lymphadenopathy of undefined cause (n = 2)

Table 1. Patient characteristics

	Sarcoidosis group (n = 72)	Control group (n = 116)
Median age (range)	53 (22-83) y	72 (36-87) y
Gender Male/Female	23/49	89/27
Stage of sarcoidosis 1/2	48/24	-
Diseases		
Lung carcinoma	-	111
Ad		55
Sq		45
Large		1
NSCLC, NOS		1
SCLC		4
Others		5
Other malignancies	-	5
Colorectal		2
Esophageal		1
Prostatic		1
Mesothelioma		1

Ad: Adenocarcinoma
Sq: Squamous cell carcinoma
SCLC: Small cell carcinoma

Table 2. Number of lymphnode assessed according to station

Station*	Sarcoidosis group (n = 72)	Control group (n = 116)
#1	0	14
#3	27	82
#4	0	35
#7	54	83
#10 (left)	0	4
#10 (right)	0	4
#11s (left)	0	26
#11i (left)	1	3
#11s (right)	9	36
#11i (right)	1	6
#12i (left)	0	1
Total	92	294

* According to UICC version 7

histological evaluation for sarcoidosis were 72.2% and 96.6%, respectively. For patients in the sarcoidosis group, TBLB was performed in 58 (80.6%) patients, and non-caseating epithelioid-cell granulomas were diagnosed in 20 (34.5%) patients. The positive rates according to the disease stage were 27.8% (10/36) in stage 1 and 45.5% (10/22) in stage 2.

Cytological evaluation with EBUS-TBNA

Among the specimens of the 188 patients, those of 104 were independently graded as 0 by both the cytoscreeners. Therefore, the samples of the remaining 84 patients were examined by the pathologist. The results are summarized by group in Table 3. When the cut-off of the cytological evaluation was set at grade 1 and higher, the sensitivity and specificity were 65.3% and 94.0%, respectively. Similarly, when it was set at grade 2 and higher, the sensitivity and specificity were 59.7% and 97.4%, respectively. The intra-class correlation coefficient for interobserver variability between the 2 cytoscreeners was 0.788, and the 95% CI was 0.727 - 0.837. In addition, the variability between cytoscreener 1 and the pathologist and that between cytoscreener 2 and the

Table 3. Cytological grading for epithelioid cell cluster by EBUS-TBNA

Grade	Sarcoidosis group (n = 72)		Control group (n = 116)	
	Number (%)	%, accumulated	Number (%)	%, accumulated
Grade 3	17 (23.6)	23.6	1 (0.9)	0.9
Grade 2	26 (36.1)	59.7	2 (1.7)	2.6
Grade 1	4 (5.6)	65.3	4 (3.4)	6.0
Grade 0*	5 (6.9)	-	19 (16.4)	-
Grade X	5 (6.9)	-	1 (0.9)	-
Grade 0**	15 (20.8)	-	89 (76.7)	-

* A cohort for which the samples classified as grade 0 by the pathologist were classified as grades other than 0 by at least one cytoscreener.
** A cohort whose samples were not sent to the pathologist because both the cytoscreeners classified the sample as grade 0.
See text for definition of the grading

pathologist were 0.800 (95% CI, 0.742 - 0.846) and 0.884 (95% CI, 0.848 - 0.912), respectively.

Table 4 illustrates the correlation between the cytological evaluation and histological diagnosis of the EBUS-TBNA specimens. A high percentage (87.5%; 63/72) of the samples was positive for the detection of epithelioid cell clusters in cytological examination and/or the diagnosis of non-caseating epithelioid-cell granulomas on histological evaluation. Table 5 shows medians and their ranges of the short axis of lymph nodes according to the cytological grades. There was no correlation between the size of lymph node sampled and cytological grade.

Patients with non-caseating epithelioid-cell granulomas in the control group

As stated above (Table 3), cytological evaluation of EBUS-TBNA specimens revealed that 7 patients in the control group had lymphadenopathy with epithelioid cell clusters. All 7 patients were found to have primary lung cancer: 4 were diagnosed with adenocarcinoma and 3, with squamous cell carcinoma. Histological evaluation of the core samples obtained from these 7 patients by EBUS-TBNA confirmed that 2 patients had non-caseating epithelioid-cell granulomas, whereas the remaining 5 had normal lymph node structure. Further, histological evaluation of the EBUS-TBNA specimens from another 2 patients in the control group showed the presence of epithelioid cell clusters but not non-caseating epithelioid-cell granulomas (Table 6). The specificity of cytological, histological, and combined cytological and histological evaluations were 94.0% (109/116), 96.6% (112/116) and 92.2% (107/116), respectively.

Table 4. Correlation of sensitivity of EBUS-TBNA between cytologic and histologic evaluation

		Cytology	
		Positive*	Negative
Histology	Positive	36 (50.0%)	16 (22.2%)
	Negative	11 (15.3%)	9 (12.5%)

* Grade 1 and higher grades; see text

Discussion

In this study, hilar and mediastinal lymph nodes form accumulated 92 and 294 stations, in sarcoidosis and control groups, respectively, were sampled. Sampling lymph nodes with EBUS yielded profound biases as to their station, as shown in Table 2, and size. Although the most frequently affected lymph nodes in sarcoidosis might be hilar and/or intralobar lymph

Table5. Correlation between the size of lymphnode sampled and cytological garading in the sarcoidosis group

	Cytological grade				
	3	2	1	0	x
n	22	30	5	28	7
Median size (range)*	16 (9-27)	17 (9-30)	22 (9-26)	13 (7-28)	18 (11-25)

No correlation found between the size and cytological grade (p=0.324, Kruskal-Wallis test).
* Size of short axis diameter in millimeter, measured by CT

Table 6. Characteristics of the control group patients with epithelioid cells (false positive)

Gender	Age (y)	Histology*1)	Clinical stage*2)	Cytologic stage	Histology of involved lymph node	
					EBUS-TBNA	Surgical
M	78	Ad	T2aN0M0	3	granuloma*3)	granuloma
M	75	Ad	recurrence	2	normal*4)	-
F	72	Sq	T1bN0M0	2	normal	granuloma
M	79	Ad	recurrence	1	granuloma	granuloma
M	72	Sq	recurrence	1	normal	-
M	63	Sq	T2bN3M0	1	normal	-
M	77	Ad	T2aN0M0	1	normal	-
F	77	Ad	T2aN0M0	0	epithelioid cell	normal
M	65	Sq	T1aN0M0	0	epithelioid cell	normal

*1) All patients in this table were with lung cancer. Ad, adenocarcinoma; Sq, squamous cell carcinoma
*2) UICC version 7
*3) Non-caseating epithelioid-cell granuloma
*4) Normal lymph node structure without other specific findings

nodes, they usually located adjacent to pulmonary arteries, and were generally difficult to approach with a relatively wide caliber fiberscope equipped with an echo probe. Therefore, bigger rather than smaller lymph nodes, and ones located central rather than peripheral regions, are preferentially chosen for sampling. In addition, specific sites such as #4 were not sampled because #3 or #7 station is much earlier to sample, as shown in Table 5. On the other hand, they were relatively evenly sampled in the control group, because the aim of sampling in the control group was lymph node staging. It should be also emphasized that on-site cytology influenced the amount of sample available for the screeners and pathologist, because sampling were usually repeated until positive results by on-site cytology were obtained.

The present study revealed that the cytological evaluation of EBUS-TBNA samples is feasible for the diagnosis of stage 1/2 sarcoidosis; the presence of epithelioid cell clusters in the aspirate is the diagnostic hallmark, unless there are other specific findings, for example, positive results of microbial examination. All cytological samples were reviewed by cytoscreeners who were blinded to the patients' clinical information; their findings were then classified into 5 categories, including those for no evidence of epithelioid cells and for inadequate materials. The interobserver validity among 2 cytoscreeners and the pathologist was adequately high.

Although previous studies (10, 12) have suggested the advantages of the cytological evaluation of EBUS-TBNA specimens, the specificity of the detection of epithelioid cells in the cytology specimens has not been assessed. In addition, since epithelioid cells might be difficult to differentiate from the usual macrophages, there is scope for some bias in the diagnosis; for example, when the cytoscreeners or pathologists are aware that the patient's clinical information is suggestive of sarcoidosis, the chances of them detecting epithelioid cells in the samples are higher than those when they are blinded. In the present study, therefore, all samples from both groups were randomly shuffled and evaluated blindly by 2 independent cytoscreeners; samples that were classified as grade 1 or more by at least 1 cytoscreener were then analyzed by a blinded pathologist. This sample selection process mimics the actual course of diagnostic workup for biopsied specimens in clinical settings, and the blind evaluation might minimize diagnostic bias. The results of the evaluations, with high specificity and sensitivity, were encouraging. Epithelioid cell clusters were found in 7 patients in the control group; in 2 of them, the cell clusters were confirmed by the histological evaluation of both EBUS-TBNA and surgical specimens, and in another patient, the clusters were confirmed by histological evaluation of the surgical specimens. Moreover, the histological examination of the EBUS-TBNA specimens revealed epithelioid cell clusters in another 2 patients in the control group as shown in Table 6. These findings imply that these patients had sarcoid reaction to cancer (16-18) or a non-specific phenomenon. In fact, Steinfort et al. found sarcoid reaction in the surgically dissected lymph nodes obtained from 4.3% (8/187) of the investigated patients with non-small-cell lung cancer (17). This percentage is similar to that observed in the present

study (6.0% or 7/116). Put together, these findings indicate that epithelioid cell clusters are present in several percent of non-metastatic mediastinal and/or hilar lymph nodes of patients with malignant diseases, although its precise mechanism is not known.

Similar to the findings of previous studies on the application of histological evaluation of EBUS-TBNA to the diagnosis of sarcoidosis (5-13), cytological evaluation alone yielded an excellent sensitivity of 65.3% in the present study. The diagnostic sensitivity of histological evaluation alone was 72.2%, which was increased by 15.3% in combination with cytological evaluation, resulting in as high as 87.5%. Traditional TBLB was performed in 58 patients in the sarcoidosis group, and its sensitivity was 34.5%. The difference in the sensitivities of EBUS-TBNA and TBLB is quite reasonable, because the former was attempted for all patients whose CT images showed evidence of enlarged lymph nodes, while the latter was performed for even patients whose CT images failed to show lung parenchymal lesions. EBUS-TBNA is routinely utilized for on-site cytology, whereas TBLB cannot be used for obtaining cytological specimens because the samples may be damaged if they are subjected to smear preparation. This might also explain the high sensitivity of EBUS-TBNA.

In conclusion, the combination of the detection of epithelioid cell clusters on cytological evaluation and non-caseating epithelioid-cell granulomas on histological examination of EBUS-TBNA specimens has high sensitivity and specificity for sarcoidosis at stages 1 and 2. Although they are highly specific to sarcoidosis, it should be also emphasized that epithelioid cell clusters are present in non-metastatic mediastinal and/or hilar lymph nodes of several percent of patients with malignant diseases.

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