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Diagnostic value of CD4+, CD8+ and CD103+ lymphocytes in mediastinal lymph node specimens obtained via endobronchial ultrasonography for sarcoidosis

Soner Umut KÜVER¹(ID)
Fatma TOKGÖZ
AKYIL²(ID)
Selahattin ÖZTAŞ³(ID)
Baran GÜNDOĞUŞ³(ID)
Nurcan ÇETİN⁴(ID)
Tülin SEVİM³(ID)

¹ Department of Intensive Care, Faculty of Medicine, Marmara University, Istanbul, Turkey

¹ Marmara Üniversitesi Tıp Fakültesi, Yoğun Bakım Bilim Dalı, İstanbul, Türkiye

² Clinic of Chest Diseases, Canakkale State Hospital, Canakkale, Turkey

² Çanakkale Devlet Hastanesi, Göğüs Hastalıkları Kliniği, Çanakkale, Türkiye

³ Department of Chest Diseases, Health Sciences University, Süreyyapaşa Chest Diseases and Thoracic Surgery Training and Research Hospital, Istanbul, Turkey

³ Sağlık Bilimleri Üniversitesi, Süreyyapaşa Göğüs Hastalıkları ve Göğüs Cerrahisi Eğitim ve Araştırma Hastanesi, Göğüs Hastalıkları Kliniği, İstanbul, Türkiye

⁴ Department of Biochemistry, Düzen Laboratories Group, Istanbul, Turkey

⁴ Düzen Laboratuvarlar Grubu, Biyokimya Bölümü, İstanbul, Türkiye

ABSTRACT

Diagnostic value of CD4+, CD8+ and CD103+ lymphocytes in mediastinal lymph node specimens obtained via endobronchial ultrasonography for sarcoidosis

Introduction: The aim of the present study is to investigate the diagnostic value of the CD4+, CD8+ and CD103+ lymphocyte sub-groups in mediastinal lymph nodes, as an adjunctive marker in sarcoidosis.

Materials and Methods: The present study is a single-center, prospective cohort study designed in a reference center for chest diseases. Forty-six patients who underwent endobronchial ultrasound (EBUS)-guided mediastinal lymph node sampling with a preliminary diagnosis of sarcoidosis were enrolled. The different lymphocyte subgroups were counted by flow cytometry in lymph node biopsy samples. Based on the final diagnosis, subjects were divided into two groups: sarcoidosis and non-sarcoidosis. Lymphocyte subset analysis were compared between the groups.

Results: The final diagnoses were sarcoidosis in 31 (67%) and non-sarcoidosis in 15 patients (33%). The total cell counts, lymphocyte ratios, CD8+ T lymphocyte ratios and CD4/CD8 ratios were similar in both groups ($p > 0.05$). CD4+ T lymphocyte rates were higher in patients with sarcoidosis ($p = 0.017$). CD103 subset analysis revealed significantly lower CD103+CD4+, CD103+CD8+ lymphocytes and CD103+CD4+/CD4+ ratios in sarcoidosis ($p = 0.008$, $p = 0.048$, $p = 0.014$, respectively).

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Yazışma Adresi (Address for Correspondence)

Dr. Fatma TOKGÖZ AKYIL
Çanakkale Devlet Hastanesi,
Göğüs Hastalıkları Kliniği,
ÇANAKKALE - TÜRKİYE
e-mail: fatmatokgoz86@gmail.com

Conclusion: Cytological examination of EBUS-guided lymph node samples may provide substantial findings in differential diagnosis of sarcoidosis. Patients with sarcoidosis have higher CD4+ lymphocytes, lower CD103+CD4+ lymphocytes and CD103+CD4+/CD4+ and CD103+CD8+ lymphocyte ratios. These subsets of lymphocytes in lymph node biopsies may be novel predictors of sarcoidosis diagnosis.

Key words: CD103; endobronchial ultrasonography; sarcoidosis

ÖZ

Sarkoidozda endobronşiyal ultrasonografi eşliğinde mediastinal lenf nodu örneklemelerinde CD4+, CD8+ ve CD103+ T lenfositlerin tanısal değeri

Giriş: Bu çalışmanın amacı, lenf nodu örneklemelerinin flow sitometrik analizinde CD4+, CD8+ ve CD103+ lenfosit alt gruplarının, sarkoidozda yardımcı bir belirteç olarak tanısal değerini araştırmaktır.

Materyal ve Metod: Çalışma tek merkezli, prospektif kohort çalışmasıdır. Ocak 2017-Eylül 2017 tarihleri arasında ayırıcı tanısında sarkoidoz düşünülen endobronşiyal ultrasonografi (EBUS) eşliğinde lenf nodu örneklemesi yapılan 46 hasta çalışmaya dahil edildi. Lenf nodu örneklerinde, farklı lenfosit alt grupları flow sitometri sayıldı ve karşılaştırıldı. Son tanıya göre hastalar sarkoidoz ve sarkoidoz dışı olmak üzere iki gruba ayrıldı. Lenfosit alt grup analizleri her iki grup arasında karşılaştırıldı.

Bulgular: Son tanı 31 (%67) hastada sarkoidoz, 15 (%33) hastada sarkoidoz dışı (akciğer kanseri, antrakoz, pnömokonyoz, pulmoner emboli, pnömoni) idi. Total hücre sayıları ve lenfosit oranları, CD8+ T lenfosit yüzdeleri ve CD4/CD8 oranları her iki grup arasında benzer bulunmuştur ($p > 0.05$). CD4+ T lenfosit oranları sarkoidoz hastalarında daha yüksek saptanmıştır ($p = 0.017$). Sarkoidoz hastalarında, CD103+CD4+ ve CD103+CD8+ lenfosit yüzdeleri daha düşük tespit edilmiştir (sırasıyla, $p = 0.008$ ve $p = 0.048$). CD103+CD4+/CD4+ oranı sarkoidoz hastalarında daha düşük bulunmuştur ($p = 0.014$).

Sonuç: EBUS ile alınan lenf nodu materyallerinin sitolojik incelemesi sarkoidozun ayırıcı tanısında önemli ipuçları sağlayabilir. Sarkoidoz hastalarında CD4+ lenfosit hücre sayıları daha yüksek olsa da CD4/CD8 oranının tanıya katkısı sınırlıdır. Düşük CD103+CD4+ ve CD103+CD8+ T lenfositlerin sarkoidoz tanısında belirleyici olacağı saptanmıştır.

Anahtar kelimeler: CD103; endobronşiyal ultrasonografi; sarkoidoz

INTRODUCTION

Sarcoidosis is a granulomatous disease with unclear etiology which can involve all organs and systems, particularly the lung and lymphatic system (1). The characteristic histopathologic finding is non-caseating epithelioid-cell granulomas with central CD4+ lymphocytes and peripheral CD8+ lymphocytes. Local coagulation necrosis can be observed in rare cases (2,3).

A reliable diagnosis require the presence of compatible clinical and radiological evidence, histopathological noncaseating granulomas and exclusion of other granulomatous diseases (1). Despite surgical biopsy and other diagnostic methods, a reliable final diagnosis may be challenging (4-7). Thus, novel diagnostic markers are still under investigation.

Endobronchial ultrasound (EBUS)-guided fine needle aspiration biopsy of mediastinal lymph nodes can be beneficial in the histopathologic diagnosis of sarcoidosis (8-14).

Previously, bronchoalveolar lavage fluid (BALF) analysis has demonstrated lower CD103 expression in CD4+lymphocytes in sarcoidosis (15). CD103+CD4+ cells to CD4+ cells in the BALF has

been suggested to contribute differential diagnosis of interstitial lung disease (16-18). In terms of EBUS-guided mediastinal lymph node samples, only a limited number of studies have focused on the role of CD4+ and CD8+ lymphocytes (19,20). Still, the diagnostic role of CD103+ cells in mediastinal lymph node specimens were not investigated previously.

The aim of the present study is to investigate the diagnostic contribution of CD4+, CD8+ and CD103+ lymphocyte sub-group analyses EBUS-guided mediastinal lymph node samples in sarcoidosis.

MATERIALS and METHODS

The present study is a prospective, single center cohort study, carried out in a training and research hospital between January 2017 and September 2017. The study was approved by the Ethics Committee and the study complied with the ethical principles of the Declaration of Helsinki (Kartal Kosuyolu Yüksek İhtisas Training and Research Hospital No: 2016/4/6). Informed consent was obtained from all patients.

Patient Selection Criteria and Data Collection

Patients were selected from EBUS center of our institution. Patients who were referred to the EBUS center



with a differential diagnosis of sarcoidosis based on current guidelines were included in the study (21). Those who were not eligible for the procedure and who refused to participate in the study were excluded (Figure 1).

Diagnostic criteria for sarcoidosis were: the presence of a necrosis-free granulomatous inflammation in the histopathological biopsy specimens with compatible clinical radiological findings and the exclusion of other granulomatous inflammatory diseases. For the diagnosis of patients with no granulomatous inflammation in the biopsy and for those who decline another invasive procedure, BAL fluid with a lymphocytic characteristic; CD4/CD8 of > 3.5 ; and clinical follow up being compatible with the sarcoidosis were accepted (1).

Demographics, radiological findings, sampled lymph node stations, histopathological findings of the EBUS samples, further diagnostic procedures if any, final clinical diagnosis were recorded.

Total cell count, total lymphocytes (%), CD4+ and CD8+ lymphocytes, CD4/CD8 rates, CD103+, CD4+ and CD103+CD8+ cells and ratios of CD103+CD4+/CD4+ and CD103+CD8+/CD8+ cells were calculated.

The lymph node was sampled via EBUS (Fujifilm EB-530US) under general anesthesia after at least eight hours of fasting. The sampled specimen was placed in 10 cc saline, and the materials were delivered to the laboratory in accordance with the cold chain rules.

In the lymph node samples, a flow cytometric analysis was performed to determine the differential cell count and the lymphocyte phenotype (BD FACSCanto II [4+2+2]). The material homogenized by needle aspiration was counted in the Sysmex XN-1000 body module, and cell counts ($/\text{mm}^3$) were obtained. The sample was first washed with a phosphate buffer and then concentrated and evaluated in terms of percentage and total numbers of CD4, CD8, CD3 and

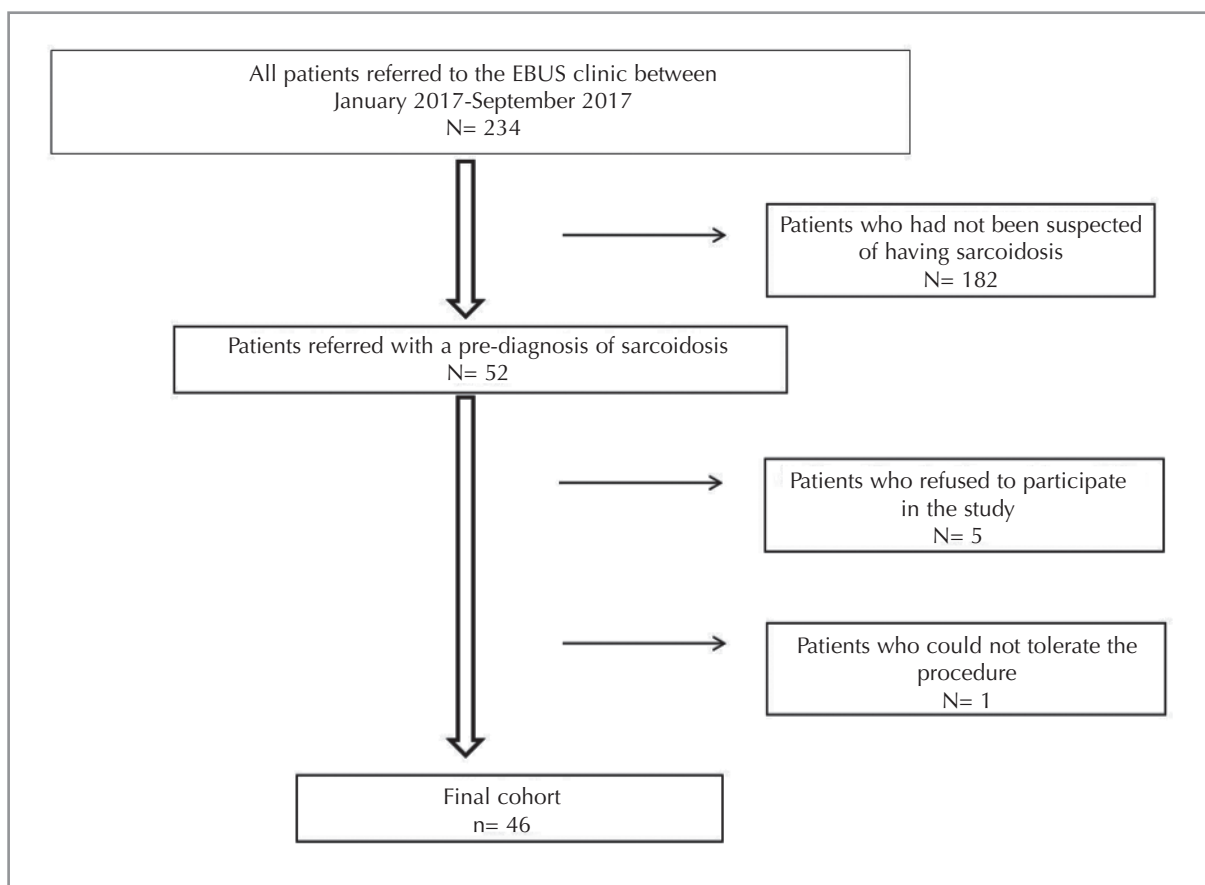


Figure 1. Flowchart of the patient inclusion.

CD103 through the fluorescent-coated surface antibodies in the flow cytometry.

Study Design

The patients included in the study were divided into two groups according to their final diagnosis as sarcoidosis and non-sarcoidosis. Cellular analysis of and lymphocyte samples were compared between the groups.

Statistical Analysis

All data are expressed as mean±standard deviation. A Chi-square and Student's t-test were used to evaluate the data obtained from inter-group comparison. The diagnostic values of the statistically significant parameters were evaluated in accordance with the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), and from the cut-off values obtained from receiver operating characteristic (ROC) curves. All statistical analysis was carried out using statistical software package system (SPSS for Windows, version 16.0; SPSS Inc., Chicago, IL, USA). A p value of < 0.05 was considered significant.

RESULTS

Of all the 46 patients included, 21 (46%) were males and the mean age was 46 ± 15 (24-76). Any parenchymal lesion was observed in 24 (61%) patients on thorax computed tomography (CT). The most frequently sampled lymph nodes were lower paratracheal, subcarinal and hilar lymph nodes (80%, 80% and 76%, respectively).

In the cytological examinations, the mean total cell count was 33561 ± 27331 , of which $20\% \pm 18$ consisted of lymphocytes. CD4+ lymphocytes were $40\% \pm 18$ while CD8+ lymphocytes were $14\% \pm 11$ (Table 1).

Additional invasive procedures were required in five patients (mediastinoscopy in 3, peripheral lymph node biopsy in 1 and thoracotomy in 1 patient). The final diagnoses based on the clinical and histopathological findings were sarcoidosis in 31 (67%) patients and non-sarcoidosis in 15 (33%) patients. The non-sarcoidosis diagnoses were primary lung cancer (n= 10), pneumoconiosis, pneumonia and pulmonary embolism and anthracosis (n= 2). Of the patients who had undergone mediastinoscopy, two were diagnosed as sarcoidosis and one as reactive lymphoid hyperplasia. Patient with excised peripheral lymph node biopsy was also diagnosed as sarcoidosis. The last patient who had undergone open lung biopsy was diagnosed as malignancy.

Of the thirty-one patients with sarcoidosis, 17 (55%) were female and 16 (57%) had any parenchymal lesion. There was no statistical relationship between the final diagnoses and gender or the presence of parenchymal lesions ($p > 0.05$).

Comparison of the cytological examination according to the final diagnosis revealed similar total cell count ($p = 0.723$). Although lymphocyte rates were higher in patients with sarcoidosis, no statistical significance was found ($22 \pm 16\%$ and $16 \pm 23\%$, respectively, $p = 0.349$). CD4+T lymphocyte rates

Table 1. Lymphocyte subgroup analysis according to final diagnosis

	All patients (n= 46)	Sarcoidosis (n= 31)	Non-sarcoidosis (n= 15)	p
Cell count	33.561 ± 27.331	35.649 ± 30.749	32.551 ± 25.999	0.723
Lymphocyte (%)	20 ± 18	22 ± 16	16 ± 23	0.349
CD4+ (%)	40 ± 18	44.5 ± 13	31.3 ± 23	0.017
CD8+ (%)	14 ± 11	12.7 ± 4	17.2 ± 17	0.176
CD4+/CD8+	3.5 ± 2.1	3.8 ± 2	2.8 ± 3	0.143
CD103+CD4+ (%)	0.76 ± 0.99	0.50 ± 0.56	1.3 ± 1	0.008
CD103+CD8+ (%)	2.0 ± 3.3	1.4 ± 1.9	3.4 ± 5	0.048
CD103+CD4+/CD4+	0.05 ± 0.15	0.013 ± 0.014	0.13 ± 0.26	0.014
CD103+CD8+/CD8+	0.14 ± 0.19	0.11 ± 0.17	0.21 ± 0.22	0.110
CD103+CD4+/CD103+CD8+	0.63 ± 0.67	0.58 ± 0.72	0.75 ± 0.85	0.495

were higher in patients with sarcoidosis ($p= 0.017$). CD8+T (%) lymphocytes ($p= 0.176$), and CD4/CD8 ratios were similar in both groups ($p= 0.143$).

The CD103+CD4+ (%) lymphocytes and CD103+CD8+ (%) lymphocytes were significantly lower in the sarcoidosis group ($p= 0.008$ and $p= 0.048$, respectively). Patients with sarcoidosis had lower CD103+CD4+/CD4+ ratios ($p= 0.014$). No significance was observed in CD103+CD8+/CD8+ and CD103+CD4+/CD103+CD8+ ratios ($p= 0.110$, $p= 0.495$) (Table 1).

In terms of CD103+CD4+ cells, the sensitivity and specificity for a cut-off value of < 0.95 were 90% and 53%, respectively. The PPV was 80% and NPV was

72% and the accuracy was 80%. The area under the curve (AUC) value for this cut-off point was 0.746 ($p= 0.007$) (Figure 2).

The AUC value for the CD103+CD8+ cells was 0.713 ($p= 0.02$). With a cut-off < 2.45 , sensitivity and specificity was 90% and 47%, respectively. PPV, NPV and accuracy were 78%, 70% and 80%, respectively (Figure 3).

DISCUSSION

The current study has shown that flow cytometric analysis of EBUS-guided lymph node materials provide considerable findings in diagnosis of sarcoidosis. Contrary to the BAL fluid analysis, CD4/CD8 ratio is not found as a determinant. Hereby, lymph node biopsy materials were studied for the first time in the means of CD103+ lymphocyte subgroups. CD103+CD4+, CD103+CD8+ lymphocytes and CD103+CD4+/CD4+ ratios were found as contributors to the diagnosis.

The frequent incidence of alveolar lymphocytosis in sarcoidosis suggests that local immunocellular response is increased in sarcoidosis. Kantrow et al. (4) have detected lymphocytosis in 56 (65%) patients including 86 patients with histopathologically diagnosed sarcoidosis. Lymphocytosis in BALF can also present in hypersensitivity pneumonia and in other interstitial lung diseases other than sarcoidosis. In this regard, lymphocytosis in the BALF is not enough to support a diagnosis of sarcoidosis. In our study, no significant difference was found between diagnosis and lymphocyte percentages. The design of the present study is not sufficient to compare the lymphocytosis in BALF and lymph nodes. However, we assume that further studies may enlighten this issue.

On the other hand, CD4+ T lymphocytes were increased in the BAL fluid of patients with sarcoidosis in previous studies. This finding has been useful in differentiation of sarcoidosis from other lymphocytic diseases, including hypersensitivity pneumonia and lymphoma (4). Similarly, a significant increase was noted in the lymph node CD4+ T lymphocyte ratio in the group with sarcoidosis when compared to the non-sarcoidosis group. Although there was no significant difference in the lymphocyte percentages of the two groups, the presence of CD4+ T lymphocytosis in favor of sarcoidosis drew attention to the importance of this finding.

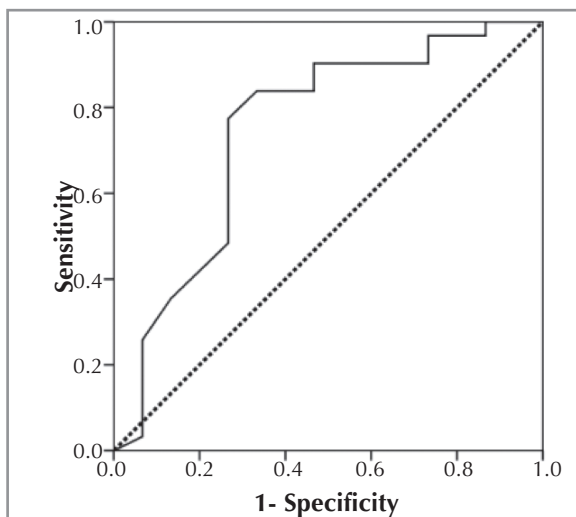


Figure 2. ROC curve for CD103+CD4+ cells.

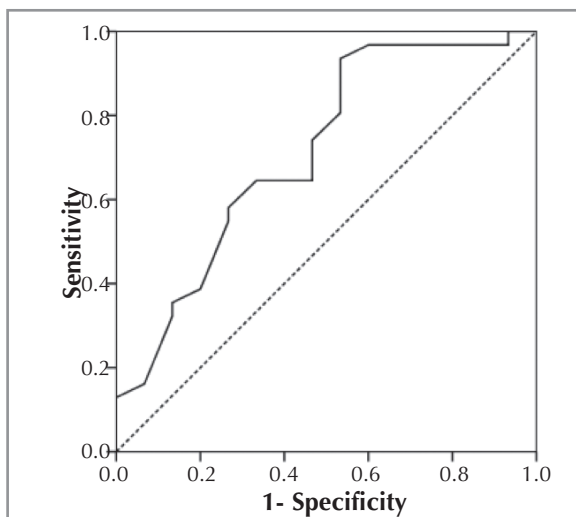


Figure 3. ROC curve for CD103+CD8+ cells.

In current study, no significant increase was observed in CD4/CD8 ratios in lymph nodes in sarcoidosis. However, high rates were observed in one-quarter of the cases diagnosed with non-sarcoidosis diseases.

In line with our results, Ruiz et al. had analyzed 23 sarcoidosis and seven non-sarcoidosis patients and were obtained similar findings. In their study, in which lymph node and BAL CD4/CD8 ratios were examined, the increased CD4/CD8 ratio was found to be 61% in sarcoidosis and 43% in non-sarcoidosis (20). In a study by Oda et al. BAL and lymph node CD4/CD8 rates of sarcoidosis were compared, the mean values were found to be 6.1 and 3.6, respectively (19). These findings indicate that high CD4/CD8 ratios in a lymph node cytologic analysis have only limited role to contribute to diagnosis of sarcoidosis.

In several studies, CD103 cells, which are produced by the intraepithelial lymphocytes in the bronchial mucosa, is shown to have a diagnostic role in sarcoidosis. In a study by Kolopp-Sarda et al., the combined use of the CD4+/CD8+ (≥ 2.5) and CD103+/CD4+ (< 0.31) ratios in the BALF analysis was identified as a promising new instrument for sarcoidosis, with 96% sensitivity (15). On the other hand, in a study by Heron et al. 119 patients with alveolar lymphocytosis were evaluated and concluded that the combined use of CD103+CD4+/CD4+ (< 0.2) and CD4/CD8 (> 3.0) ratios in the BALF is useful in differentiating sarcoidosis and other interstitial lung diseases (16). Similarly, in the study of Mota et al., the CD103+CD4+/CD4+ (< 0.25) ratio showed a high specificity (91%) in the diagnosis of sarcoidosis (17). In the present study, the diagnostic role of CD103+ cells in lymph node specimens was investigated for the first time. To the best of our knowledge, a T lymphocyte analysis, which produced CD103 in the lymph node, has never before been carried out. Similar to the BALF analysis, a significant decrease was observed in CD4+ T lymphocytes producing CD103 and CD8+ T lymphocytes in the lymph node. Although we were unable to compare the numerical values with other studies due to the different methods used, the decrease in the ratio of CD103+CD4+/CD4+ was determined to be statistically significantly decreased.

These findings emphasize that a CD103 cell analysis have a potential diagnostic role, independently of the CD4/CD8 ratio in patients with sarcoidosis. The clin-

ical significance of these markers in granulomatous diseases such as tuberculosis would be clarified in novel studies.

The most important limitation of the study was our inability to compare the diagnostic values of CD4/CD8 ratio and T lymphocytes producing CD103 as combined indices, due to the limited number of patients in the study. The second limitation of the study is the lacking information of detailed demographics, bronchoalveolar lavage findings, stages of the disease and extrapulmonary involvement of these patients. Due to the design of the study, these variables were not recorded at the initial phase of the study. However, the strengths of the study are the careful patient selection and the reliability of the laboratory analysis of CD103 cells in the lymph node for the first time.

In conclusion, a cytological examination of lymph node materials obtained via EBUS provide important data in differential diagnosis of sarcoidosis. Although the CD4+ lymphocytes are higher in patients with sarcoidosis, the CD4/CD8 ratio makes only a limited contribution to the diagnosis. Low CD103+CD4+T and CD103+CD8+ lymphocytes may be promising biomarkers of sarcoidosis.

CONFLICT of INTEREST

The authors indicated no potential conflicts of interest.

AUTHORSHIP CONTRIBUTIONS

Concept/Design: All of authors.

Analysis/Interpretation: All of authors.

Data Acquisition: All of authors.

Writing: SUK, FTA, TS

Critical Revision: SUK, FTA

Final Approval: All of authors.

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