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Serum soluble interleukin-2 receptor level is a predictive marker for EBUS-TBNA-based diagnosis of sarcoidosis

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Abstract. *Background:* Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a widely available diagnostic tool for suspected stage I/II sarcoidosis. Combination of EBUS-TBNA and transbronchial lung biopsy (TBLB) has been proposed as diagnostic procedure in clinical settings. *Objectives:* The aim of this study was to assess the diagnostic yield of combined EBUS-TBNA and TBLB and identify the markers correlated with a high diagnostic rate. *Methods:* We retrospectively analyzed the data of 37 patients with suspected stage I/II sarcoidosis with enlarged hilar or mediastinal lymph nodes on computed tomography (CT) images. These patients had been scheduled to undergo EBUS-TBNA and TBLB. Serum levels of sarcoidosis markers (angiotensin-converting enzyme [ACE], soluble interleukin-2 receptor [sIL-2R], and lysozyme), CT findings, and examination techniques were evaluated as predictive markers for diagnosis. *Results:* Of the 37 patients, 32 had undergone both EBUS-TBNA and TBLB, while the remaining 5 patients had only undergone EBUS-TBNA. The diagnosis was confirmed by TBLB in 16 of the 32 patients (50.0%), EBUS-TBNA in 31 of the 37 patients (83.8%), and combined TBLB and EBUS-TBNA in all patients (100.0%). The serum level of sIL-2R, but not that of ACE or lysozyme, was correlated with successful diagnosis by EBUS-TBNA. *Conclusion:* In patients with stage I/II sarcoidosis, the serum level of sIL-2R is a promising and useful marker for predicting the diagnosis by EBUS-TBNA and reducing the burden of additional TBLB and its possible complications. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (1): 8-16)*

Key words: Sarcoidosis, EBUS-TBNA, TBLB, Soluble interleukin-2 receptor

Introduction

Sarcoidosis is characterized by bilateral hilar and mediastinal lymphadenopathy concomitant with lesions in multiple organs, including the lungs, heart, eyes, and skin. Although its pathophysiology has yet to be fully understood, previous reports have indicated the possibility of abnormalities in immunological regulation (1-3). In many cases, patients with sarcoidosis present with a stable condition with no symptoms. However, some patients often develop progressively worsening pulmonary conditions that could lead to interstitial lung disease with respiratory failure. There is also a possibility that, in addition to pulmonary disorders, other cardiac or neurological lesions might be fatal. Thus, early diagnosis and optimal treatment is essential in such cases.

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Detection of non-caseous granuloma is critical for diagnosis of sarcoidosis. Endoscopic approaches are used for collecting histological samples from the lungs or hilar and mediastinal lymph nodes (4-6). Transbronchial lung biopsy (TBLB) has conventionally been employed as a diagnostic method for sarcoidosis. However, it yields a diagnosis rate of about 50% and is, therefore, not adequate as a definitive diagnostic examination (7-10). In addition, TBLB entails the possibility of complications, including bleeding and pneumothorax. Therefore, further methodological improvement of this approach is an urgent necessity. Recently, a clinical and universally available method that uses endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) for diagnosis of sarcoidosis has been developed. This examination has improved the diagnostic rate of sarcoidosis to 80-90% (11-14). Although EBUS-TBNA entails the risk of mediastinitis as a rare complication, it is usually safe for most patients who are qualified for bronchoscopic examination. These characteristics support its high practicality, although there is room for improvement in some points, including the limited size of tissues and longer examination time (8, 15-17). It has been proposed that EBUS-TBNA and TBLB can be performed during the same examination in order to increase the diagnostic rate. However, this combined approach might require a longer examination time and involve a higher incidence of concomitant complications. Therefore, there is a controversy about whether both methods should be simultaneously applied for patients with suspected sarcoidosis (7, 18-20).

In clinical settings, several biomarkers are used for diagnosing sarcoidosis, including angiotensin-converting enzyme (ACE), soluble interleukin-2 receptor (sIL-2R), and lysozyme (21-24). High serum levels of these markers indicate a definite diagnosis of sarcoidosis. Although ACE is the most commonly used biomarker for diagnosing sarcoidosis, it is not well correlated with active sarcoidosis or the progressive stage of the disease. In fact, lysozyme and sIL-2R are more sensitive markers than ACE (22). Furthermore, sIL-2R is related to the presence of pulmonary manifestations or extrapulmonary organ lesions, which suggests its importance for predicting progressive and complicated disease states. However, a specific marker that is correlated to a high diagnostic rate with combined TBLB and EBUS-TBNA has yet to be identified.

In this study, we analyzed the detection rates of non-caseous granulomas and their relationship with clinical characteristics in patients with suspected stage I/II sarcoidosis scheduled to undergo simultaneous EBUS-TBNA and TBLB. In addition, we investigated which serum marker of sarcoidosis can help improve the diagnostic rate of TBLB and EBUS-TBNA. We also analyzed the relationship of the selected biomarker to disease status on the basis of patient characteristics.

Methods

Subjects

Using a digital data system in our hospital to retrieve patient records for the period of January 2012 to December 2017, we retrospectively identified 37 patients with suspected stage I/II sarcoidosis with enlarged (>10 mm) hilar or mediastinal lymph nodes on computed tomography (CT) images. These patients had been scheduled to undergo combined EBUS-TBNA and TBLB for diagnosis of sarcoidosis. Patients with suspected malignancies or prior established diagnosis of sarcoidosis were excluded on the basis of imaging and serological data. For safety concerns, the patients were managed on an inpatient basis after bronchoscopy. Written informed consent for the examination was obtained from all patients included in this study. For further evaluation, the electronic records of these patients were retrospectively obtained.

Examination procedures

All patients were scheduled to be examined by three diagnostic modalities – EBUS-TBNA, TBLB, and analysis of bronchoalveolar lavage fluid [BALF] – during the same examination. All bronchoscopic procedures were performed under local and systemic anesthesia to keep the patients conscious. Briefly, anesthesia was achieved by nebulization, topical application of lidocaine spray, and intravenous administration of midazolam and pethidine. During the procedure, the patients were monitored for electrocardiogram, pulse oximetry, and blood pressure readings.

First, EBUS-TBNA was performed by using a convex-probe endobronchial ultrasound bronchoscope (BF-UC260F-OL8; Olympus, Tokyo, Japan). Images were acquired by directly contacting the probe or by attaching an inflated saline-filled balloon to its tip, which kept the probe in contact during sampling. A dedicated 22-gauge needle (NA-201SX-4022; Olympus) was used for TBNA. The status of lymph nodes was determined in accordance with the International Staging System (25). The retrieved histological specimens were fixed in formalin and subsequently examined for the presence of non-caseating granuloma in the pathology department.

After EBUS-TBNA, BALF was collected by using a standardized method (50 mL x 3 times; total volume: 150 mL). Then, biopsy specimens were randomly collected from the upper to lower lobes (in order) by TBLB. If CT findings showed any evidence of pulmonary parenchymal involvement, biopsy specimens were collected mainly from the targeted lesion.

Processing of BALF cells

BALF cells were processed for analysis in accordance with standard guidelines as previously described (26). BAL data were available for 36 of the 37 patients included in this study.

Analysis of serum parameters

The serum levels of sIL-2R, ACE, and lysozyme were routinely analyzed during the initial hospital visit, by using commercially available assay kits. For each assay, the normal range was defined on the basis of the manufacturer's recommendations. Normal values of these markers are: 145-519 U/mL for sIL-2R, 8.3-21.4 U/L for ACE, and 5.0-10.2 μg/mL for lysozyme. We only considered the data from patients with suspected sarcoidosis who did not receive any immunosuppressive therapy. The data on sIL-2R, ACE, and lysozyme levels were available for 31, 37, and 29 patients, respectively.

Statistical analysis

Diagnostic accuracy was calculated by using standard definitions. Data were compared by the Mann-Whitney U test. For categorical data, inter-group differences were evaluated by the chi-square test. Correlations between different parameters were determined using Spearman's rank correlation coefficient. P values < 0.05 were regarded as significant.

Ethics committee approval

This study was approved by the ethical committee of Sano Kosei General Hospital (No. 3029).

Results

Combination of EBUS-TBNA and TBLB for detecting granulomas

Table 1 presents the demographic and clinical data of the 37 patients included in this study. To investigate the usefulness of combined EBUS-TBNA and TBLB, we compared the detection rates of non-caseous granuloma among patients who underwent EBUS-TBNA, TBLB, and both. While the detection rates of EBUS-TBNA and TBLB were 83.8% and 50.0%, respectively, the combined examination yielded a higher detection rate of 100.0%. Patients with pathological findings indicating the presence of granulomas were diagnosed as having sarcoidosis. There was an exception in one case, where liver biopsy was needed to confirm the diagnosis in a patient who had concomitant pneumoconiosis with suspected secondary granulomatous changes in the lungs. In addition, 5 patients underwent only EBUS-TBNA; they did not undergo TBLB because of the long examination time and/or a persistent cough, which made it difficult to ensure their safety during the examination.

Relationship between patient characteristics and detection of granuloma by TBLB

To identify a specific marker related to the detection of granulomas by TBLB, we compared patient information, serum levels of sarcoidosis markers, pulmonary or extrapulmonary sarcoidosis lesions, and examination procedures between patients with and without granulomas identified by TBLB (Table 2). There were no statistically significant differences in demographic data between the two groups. The number of patients with pulmonary lesions in the granuloma-positive group was greater than that in

Table 1. Characteristics of the whole study population

Characteristics	
Demographic data	
Men, %	45.9 (17/37)
Age, years	58.5 ± 15.9
Pulmonary involvement, %	59.5 (22/37)
Extrapulmonary involvement, %	27.0 (10/37)
Eye involvement, %	18.9 (7/37)
Skin involvement, %	2.7 (1/37)
Stage of sarcoidosis (I/II), n	15/22
Laboratory data	
ACE concentration, U/L	21.3 ± 7.9
sIL-2R concentration, U/mL	1,155.8 ± 834.3
Lysozyme concentration, μg/mL	12.7 ± 8.8
Bronchoscopic findings	
Implementation of EBUS-TBNA, %	100.0 (37/37)
Lymph-node count targeted in EBUS-TBNA	1:19 2:18
Detection of granuloma in EBUS-TBNA samples, %	83.8 (31/37)
Implementation of BAL, %	97.3 (36/37)
%lymphocytes in BALF, %	32.1 ± 15.5
CD4/CD8 ratio of lymphocytes in BALF	8.6 ± 6.1
Implementation of TBLB, %	86.5 (32/37)
Detection of granuloma in TBLB samples, %	50.0 (16/32)

Abbreviations: ACE=angiotensin-converting enzyme, BAL=bronchial alveolar lavage, BALF=bronchial alveolar lavage fluid, EBUS-TBNA=endobronchial ultrasound-guided transbronchial needle aspiration, sIL-2R=soluble interleukin-2 receptor, TBLB=transbronchial lung biopsy. Values are the proportion of patients in the study group (mean ± standard deviation).

the granuloma-negative group, although the difference was not statistically significant. The serum levels of all three sarcoidosis markers (ACE, sIL-2R, and lysozyme) were higher in the granuloma-positive group than in the granuloma-negative group; however, these differences did not reach statistical significance. The findings of BALF analysis (i.e., percentage of lymphocytes in the total cell population and CD4:CD8 ratio of lymphocytes) were not correlated with the detection rate of granulomas by TBLB. Thus, no marker was identified as being predictive of successful diagnosis by TBLB.

Relationship between patient characteristics and detection of granuloma by EBUS-TBNA

Next, to identify a specific marker for predicting the detection of granulomas by EBUS-TBNA, we compared patient information, serum levels of sarcoidosis markers, pulmonary or extrapulmonary sarcoidosis lesions, and examination procedures between patients who did and did not have granulomas sampled by EBUS-TBNA (Table 3). There were no statistically significant differences in demographic data between the two groups. The number of punctures

Table 2. Comparative analysis of patients with sarcoidosis with and without granulomas detected by TBLB

Characteristics	Granuloma		*P*
	Not detected (n = 16)	Detected (n = 16)	
Demographic data			
Men, %	43.8 (7/16)	50.0 (8/16)	1.00*
Age, years	59.8 ± 15.4	53.1 ± 16.6	.25†
Pulmonary involvement, %	50.0 (8/16)	68.8 (11/16)	.28*
Extrapulmonary involvement, %	31.3 (5/16)	31.3 (5/16)	1.00*
Eye involvement, %	25.0 (4/16)	18.8 (3/16)	.67*
Skin involvement, %	0.0 (0/16)	6.3 (1/16)	.31*
Stage of sarcoidosis (I/II), n	8/8	5/11	.28*
Laboratory data			
ACE concentration, U/L	19.0 ± 6.6	22.6 ± 8.3	.18†
sIL-2R concentration, U/mL	1,018.3 ± 501.8	1,354.0 ± 1,120.8	.33†
Lysozyme concentration, μg/mL	11.3 ± 7.0	15.5 ± 10.7	.25†
Bronchoscopic findings			
Implementation of BAL, %	100.0 (16/16)	100.0 (16/16)	
%lymphocytes in BALF, %	27.5 ± 15.5	36.2 ± 16.1	.13†
CD4/CD8 ratio of lymphocytes in BALF	9.1 ± 7.4	7.7 ± 5.1	.56†

Abbreviations: ACE=angiotensin-converting enzyme, BAL=bronchial alveolar lavage, BALF=bronchial alveolar lavage fluid, sIL-2R=soluble interleukin-2 receptor, TBLB=transbronchial lung biopsy
Values are the proportion of patients in each study group (mean ± standard deviation)
*chi-square test
†Mann-Whitney test

(1 or 2) was not correlated with successful sampling of granulomas. Among the evaluated serum markers of sarcoidosis, sIL-2R (but not ACE or lysozyme) showed higher concentrations in the granuloma-positive group than in the granuloma-negative group (Figure 1A), which suggested its greater sensitivity for predicting successful diagnosis. Thus, this study identified a useful predictive marker for the positive detection of granuloma by EBUS-TBNA.

Characterization of patients with sarcoidosis with high serum levels of soluble IL-2R

To characterize the predictive ability of serum sIL-2R as a marker for choosing EBUS-TBNA as the preferred diagnostic tool for sarcoidosis, we performed receiver operating characteristic curve analysis (Figure 1B). The cutoff index was set at 841 U/mL (area under the curve: 0.7615; Youden index: 0.4538). With this cutoff point, 94.4% of patients with high serum levels of sIL-2R were successfully diagnosed with granulomas by EBUS-TBNA. In contrast, the detection rate of granulomas by EBUS-TBNA in the low-sIL-2R group was only 69.2%.

To investigate the role of sIL-2R in stage I/II sarcoidosis, we compared patient information between those with high and low levels of serum sIL-2R by using the cutoff index (Table 4). Notably, the serum concentrations of the other two markers, ACE and lysozyme, were also higher in the high-sIL-2R group than in the low-sIL-2R group. Furthermore,

Table 3. Comparative analysis of patients with sarcoidosis with and without granulomas detected by EBUS-TBNA

Characteristics	Granuloma		*P*
	Not detected (n = 6)	Detected (n = 31)	
Demographic data			
Men, %	66.7 (4/6)	41.9 (13/31)	.27*
Age, years	47.8 ± 16.9	60.6 ± 15.2	.13†
Pulmonary involvement, %	66.7 (4/6)	58.1 (18/31)	.69*
Extrapulmonary involvement, %	33.3 (2/6)	25.8 (8/31)	.70*
Eye involvement, %	0.0 (0/6)	19.4 (6/31)	.24*
Skin involvement, %	0.0 (0/6)	3.2 (1/31)	.66*
Stage of sarcoidosis (I/II), n	2/4	13/18	.69*
Laboratory data			
ACE concentration, U/L	19.0 ± 8.7	21.8 ± 7.8	.50†
sIL-2R concentration, U/mL	666.2 ± 402.4	1,249.9 ± 867.3	.035†
Lysozyme concentration, μg/mL	11.5 ± 5.4	12.9 ± 9.3	.68†
Bronchoscopic findings			
Lymph-node count targeted in EBUS-TBNA	1:4 2:2	1:15 2:16	.41*
Implementation of BAL, %	100.0 (6/6)	96.8 (30/31)	
%lymphocytes in BALF, %	33.5 ± 14.2	31.8 ± 16.0	.80†
CD4/CD8 ratio of lymphocytes in BALF	6.1 ± 3.3	9.1 ± 6.4	.12†

Abbreviations: ACE=angiotensin-converting enzyme, BAL=bronchial alveolar lavage, BALF=bronchial alveolar lavage fluid, EBUS-TBNA=endobronchial ultrasound-guided transbronchial needle aspiration, sIL-2R=soluble interleukin-2 receptor
Values are the proportion of patients in each study group (mean ± standard deviation)
*chi-square test
†Mann-Whitney test

the serum levels of all three markers were mutually and significantly correlated with each other (Supplementary Figure 1), with the serum levels of sIL-2R and lysozyme being most strongly correlated with each other (r = 0.94; $P < 0.001$). The percentages of patients with values above normal values of these markers were 69.2% and 100.0% for sIL-2R, 7.7% and 66.7% for ACE, and 0% and 61.5% for lysozyme in the low-sIL-2R group and the high-sIL-2R group, respectively. The incidence of pulmonary involvement or stage II sarcoidosis and ratio of CD4:CD8 cells were higher in the high-sIL-2R group than in the low-sIL-2R group; however, these differences did not reach statistical significance.

Discussion

The findings of this study revealed that simultaneous EBUS-TBNA and TBLB is safe, does not involve serious complications, and helps improve the diagnosis rate of sarcoidosis. In addition, our results demonstrated that high serum sIL-2R concentration indicates a better diagnostic rate of sarcoidosis by EBUS-TBNA. The serum levels of ACE and lysozyme – although not associated with successful diagnosis by EBUS-TBNA – were correlated with those of sIL-2R, suggesting a possible additional benefit for sarcoidosis diagnosis.

sIL-2R is closely related to disease activity in

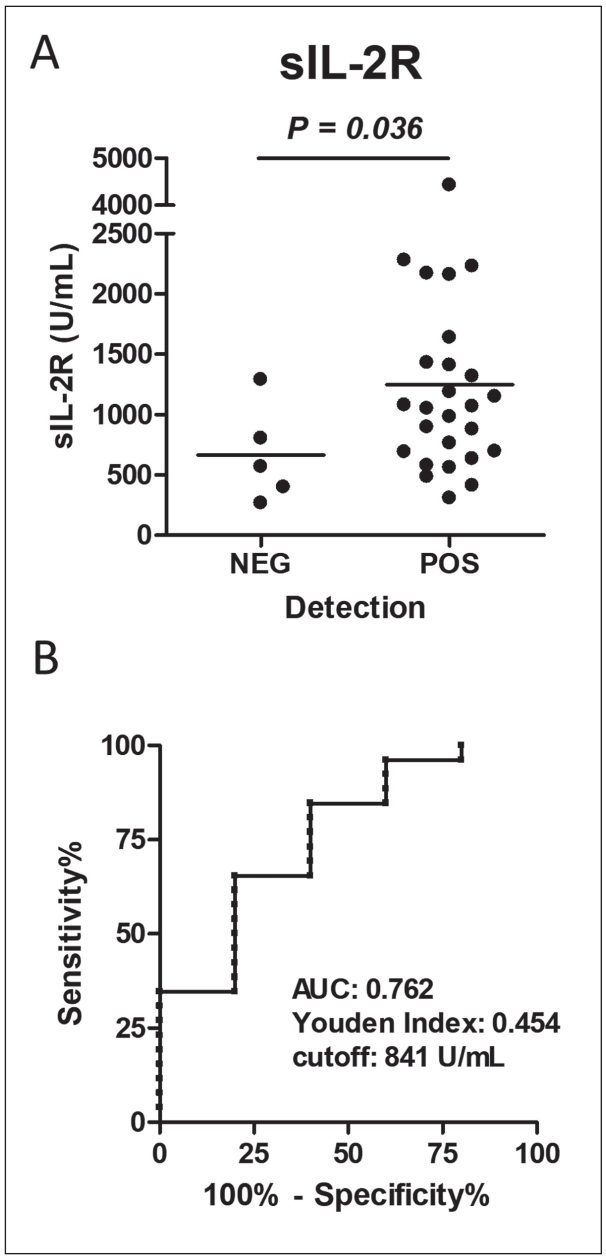

Fig. 1. Relationship between serum sIL-2R level and detection of granuloma by EBUS-TBNA. A) Comparison of serum sIL-2R levels between the granuloma-positive and -negative groups on the basis of EBUS-TBNA findings. B) Findings of receiver operating characteristic curve analysis of the ability of serum sIL-2R level to predict the detection of granulomas in patients with suspected sarcoidosis by EBUS-TBNA. AUC=area under the curve, EBUS-TBNA=endobronchial ultrasound-guided transbronchial needle aspiration, NEG=negative, POS=positive, sIL-2R=soluble interleukin-2 receptor

sarcoidosis (23, 27-33). Previous studies have shown that a high serum concentration of sIL-2R indicates more severe sarcoidosis. Extrapulmonary lesions are also related to elevated levels of serum sIL-2R. These results indicate that many cases of stage I/II sarcoidosis with high serum levels of sIL-2R can progress to stage III/IV. The relationship between lymph-node lesions and serum sIL-2R levels remains unknown. However, this marker is correlated with disease activity in lymphoma, which indicates that it is closely related with other lymphadenopathic diseases, including sarcoidosis. Thus, in patients with stage I/II sarcoidosis, elevated sIL-2R levels suggest the progression of active formation of non-caseous granulomas in lymph nodes.

In addition to sIL-2R, ACE and lysozyme are also employed as serum markers of sarcoidosis. Previous studies have reported that high serum ACE and lysozyme levels might indicate severe disease state in sarcoidosis (23, 34-36). However, our results showed that these markers are not significantly correlated with the detection rate of non-caseous granuloma in lymph nodes by EBUS-TBNA. In fact, our results demonstrated a positive relationship between sIL-2R and ACE/lysozyme levels, suggesting that sIL-2R is a more sensitive serum marker than the other two.

Although EBUS-TBNA is a useful diagnostic method for sarcoidosis, there is room for further improvement. For example, the puncture method has been modified for collecting larger samples for detecting non-caseous granuloma. Changes in needle size and puncture time and the use of rapid cytological analysis are thought to increase the detection rate of non-caseous granulomas (13, 15, 16, 37). It is necessary to improve the diagnostic approach for patients with suspected sarcoidosis who are scheduled for EBUS-TBNA diagnosis. However, knowledge regarding useful serological biomarkers for predicting a higher rate of successful diagnosis by EBUS-TBNA might encourage clinicians to choose this method, which increases the importance of sIL-2R as a biomarker.

In the present study, patient information and clinical data were retrospectively analyzed, which might have led to bias in the findings. Second, there were missing values in the laboratory data, including sIL-2R and lysozyme levels, which might have decreased the statistical power of the markers for

Table 4. Comparative analysis of patients with sarcoidosis with high and low serum sIL-2R levels

Characteristics	Serum sIL-2R level (U/mL)		*P*
	< 841 (U/mL) (n = 13)	≥ 841 (U/mL) (n = 18)	
Demographic data			
Men, %	53.8 (7/13)	38.9 (7/18)	.41*
Age, years	54.7 ± 17.3	59.8 ± 17.2	.43†
Pulmonary involvement, %	46.2 (6/13)	72.2 (13/18)	.14*
Extrapulmonary involvement, %	30.8 (4/13)	22.2 (4/18)	.59*
Eye involvement, %	23.1 (3/13)	11.1 (2/18)	.37*
Skin involvement, %	0.0 (0/13)	5.6 (1/18)	.39*
Stage of sarcoidosis (I/II), n	7/6	5/13	.14*
Laboratory data			
ACE concentration, U/L	16.9 ± 5.4	24.9 ± 8.3	.003†
sIL-2R concentration, U/mL	551.6 ± 169.4	1,592.1 ± 852.9	<.001†
Lysozyme concentration, μg/mL	6.2 ± 1.6	16.6 ± 9.8	.001†
Bronchoscopic analysis			
Implementation of BAL, %	92.3 (12/13)	100.0 (18/18)	
%lymphocytes in BALF, %	35.7 ± 16.0	34.6 ± 15.1	.85†
CD4/CD8 ratio of lymphocytes in BALF	6.6 ± 4.3	9.2 ± 5.2	.16†

Abbreviations: ACE=angiotensin-converting enzyme, BAL=bronchial alveolar lavage, BALF=bronchial alveolar lavage fluid, sIL-2R=soluble interleukin-2 receptor
Values are the proportion of patients in each study group (mean ± standard deviation)
*chi-square test
†Mann-Whitney test

predicting significant differences in diagnosis of sarcoidosis. Third, the laboratory data of healthy controls without sarcoidosis were not collected, which might have caused an improper setting of the cut-off index. Fourth, in some cases, EBUS-TBNA and TBLB were not performed simultaneously, which led to incomplete assessment of the usefulness of the combined examination. Fourth, the number of patients enrolled in this study was small. Therefore, we might have missed some clinically important relationship between the diagnosis rate of sarcoidosis by EBUS-TBNA and the evaluated markers.

In conclusion, this study demonstrated the diagnostic utility of combined EBUS-TBNA and TBLB in enhancing the detection rate of non-caseous granuloma in patients with suspected sarcoidosis. Our findings indicated that serum levels of sIL-2R might predict a higher diagnostic rate by EBUS-TBNA but not TBLB. These findings might help avoid unnecessary examination and possible complications and improve the diagnostic strategy for early-stage sarcoidosis.

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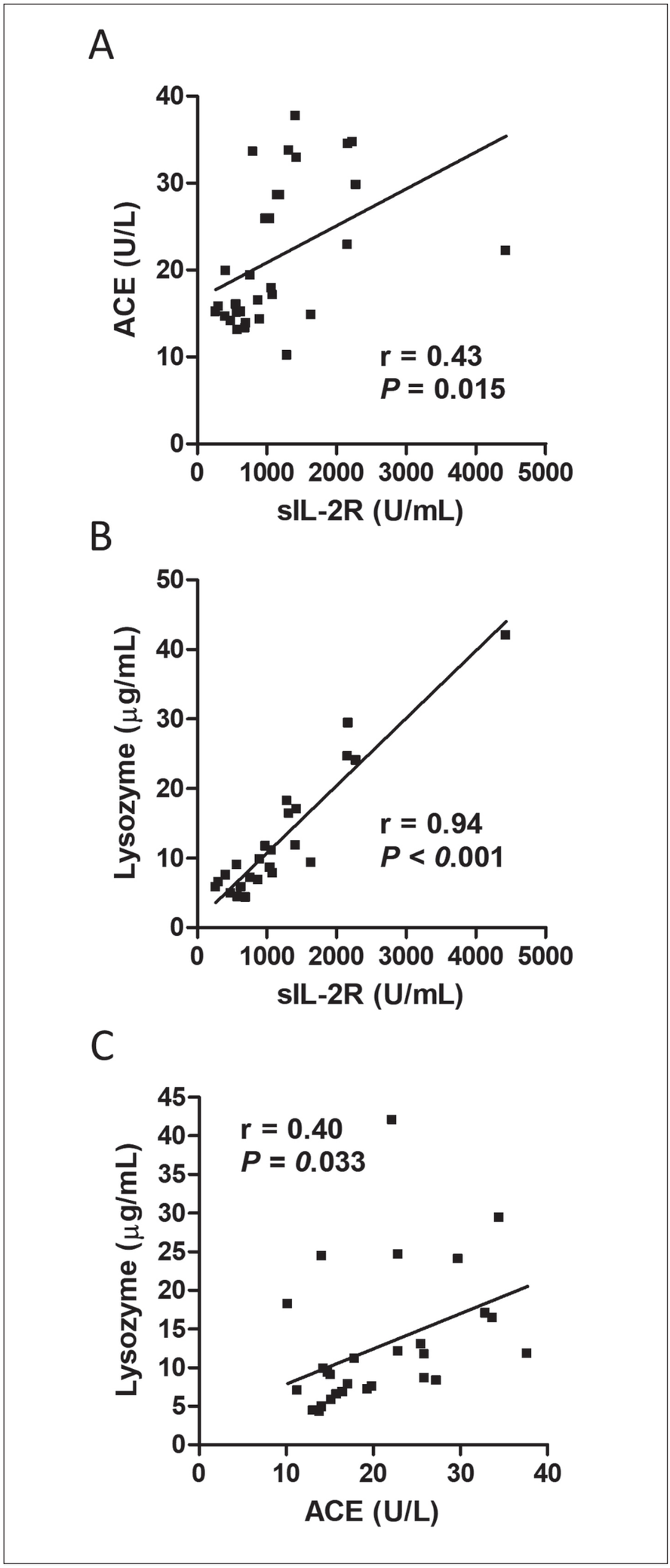

Supplementary Fig. 1. Correlation among the serum levels of sarcoidosis markers (ACE, sIL-2R, and lysozyme). Correlation between the serum levels of ACE and sIL-2R (A), lysozyme and sIL-2R (B), and ACE and lysozyme (C). ACE=angiotensin-converting enzyme, sIL-2R=soluble interleukin-2 receptor