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Principal component analysis of clinical characteristics of pulmonary sarcoidosis with or without extrapulmonary lesions: a single-center observational study in Japan

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To the editor,

Sarcoidosis is a systemic granulomatous disease with a prevalence of 1–160 per 100,000 population (1). The extrapulmonary involvement is often seen and can be fatal (2). Therefore, it is important to diagnose and manage them at an early phase of the disease (3). According to previous reports, extrapulmonary lesions are more common in women with higher serum levels of angiotensin converting enzyme (ACE), calcium, and IgG (2,3). However, the details of the relationship between these clinical information and extrapulmonary lesions in sarcoidosis remains unknown.

Hence, we compared the clinical characteristics of patients with pulmonary sarcoidosis with extrapulmonary lesions and those without them. In addition, we performed principal component analysis (PCA) using serum markers and bronchoalveolar lavage fluid (BALF) findings.

Of the patients who underwent bronchoscopy at the National Defense Medical College Hospital between April 2006 and September 2020, a total of 59 patients who were histologically diagnosed with sarcoidosis based on the clinical guideline on sarcoidosis were included in this study (3). The diagnosis of extrapulmonary lesions was confirmed by imaging examinations and consultation with specialists of each organ. Radiological findings were assessed blindly by two respiratory specialists. Moreover, serological and BALF findings necessary for the diagnostic criteria of sarcoidosis were collected retrospectively. For statistical analysis, GraphPad Prism version 10.1.1 (GraphPad, San Diego, CA) was used. Fisher's exact test and Mann-Whitney U-test were used for the analysis of qualitative and quantitative variables, respectively. For PCA, we extracted serum markers and BALF findings and plotted the scores for each principal component (PC) to discriminate the presence and absence of extrapulmonary lesions. This study was approved by the Ethical Committee of National Defense Medical College (No.4313, 25 November, 2020).

Among 59 participants, 36 (61.0%) had extrapulmonary lesions (Table 1). Although age was not significantly different between the groups, the proportion of female patients was significantly higher in the group with extrapulmonary lesions than in the group without them (24 [66.7%] vs. 8 [34.8%], p=0.03). The frequency of extrapulmonary lesions in the eye, skin, heart, nerve, and muscle were 27 (75%), 12 (33.3%), 7 (19.4%), 4 (11.1%), and 4 (11.1%), respectively. Of the four nervous lesions,

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Table 1. Clinical characteristics and investigatory findings of patients with pulmonary sarcoidosis with or without extrapulmonary lesions.

	Without extrapulmonary lesions (n=23)	With extrapulmonary lesions (n=36)	*p* value
Clinical characteristics			
Age, median (IQR)	53.0 (31.0–69.0)	56.0 (45.0–66.5)	0.48
Male, n (%)	15 (65.2)	12 (33.3)	0.03
Current smoking, n (%)	6 (26.1)	9 (25.0)	>0.99
Extrapulmonary lesions			
Eye, n (%)	N.A.	27 (75.0)	N.A.
Skin, n (%)		12 (33.3)	
Heart, n (%)		7 (19.4)	
Nerve, n (%)		4 (11.1)	
Muscle, n (%)		4 (11.1)	
Kidney n (%)		3 (8.3)	
Lymph node, n (%)		3 (8.3)	
Stage			
0, n (%)	2 (8.7)	2 (5.6)	0.64
I, n (%)	5 (21.7)	4 (11.1)	0.29
II, n (%)	15 (65.2)	28 (77.8)	0.37
III, n (%)	1 (4.3)	2 (5.6)	>0.99
IV, n (%)	0 (0)	0 (0)	>0.99
Serological findings			
Serum total calcium (mg/dL), mean±SD	9.5±0.4	9.5±0.6	0.67
Serum lysozyme (ug/mL), mean±SD	8.6±2.3	13.3±7.8	<0.01
Serum ACE (U/L), mean±SD	24.7±9.9	33.2±13.3	<0.01
Serum sIL2R (U/mL), mean±SD	705.6±332.8	1,234.7±731.2	<0.01
Chest X-ray findings			
BHL, n (%)	21 (91.3)	32 (88.9)	>0.99
CT findings			
Thickening of the bronchovascular bundles, n (%)	8 (34.8)	20 (55.6)	0.18
Nodules, n (%)	19 (82.6)	29 (80.6)	>0.99
Ground glass opacification, n (%)	9 (39.1)	13 (36.1)	>0.99
BALF findings			
Total cell count ($\times 10^5$/mL), mean±SD	1.9±1.7	1.7±1.0	0.81
%Lymphocyte (%), mean±SD	30.8±15.9	24.2±17.1	0.11
%Macrophage (%), mean±SD	68.2±15.5	74.1±17.4	0.15
%Neutrophil (%), mean±SD	0.6±1.1	1.3±2.2	0.22
%Eosinophil (%), mean±SD	0.4±1.3	0.4±0.9	0.30
CD4/CD8 ratio, mean±SD	5.4±3.3	6.6±4.5	0.29

Abbreviations: ACE: angiotensin converting enzyme; BALF: bronchoalveolar lavage fluid; BHL: Bilateral hilar adenopathy; CT: computed tomography; sIL2R: serum soluble interleukin-2 receptor.

three were central and one was peripheral. Serological tests showed significantly higher serum lysozyme (normal range: 5.0-10.2 ug/mL), ACE (normal range: 7.7-29.4 U/L), and soluble interleukin-2 receptor (sIL2R; normal range: 157-474 U/mL) levels in the group with extrapulmonary lesions than in the group without them (13.3±7.8 vs. 8.6±2.3, $p<0.01$; 33.2±13.3 vs. 24.7±9.9, $p<0.01$; and 1234.7±731.2 vs. 705.6±332.8, $p<0.01$, respectively). There were no significant differences in current smoking history and radiological and BALF findings between the two groups.

In PCA, the analysis included variables such as ACE and sIL-2R levels in serum, total cell counts in BALF, and the percentages of eosinophils, lymphocytes, neutrophils, macrophages, and the CD4/8 ratio in BALF. PC1 and PC2 were identified through both the screen plot and parallel analysis (Figure 1A). The cumulative variance accounted for by PC1 and PC2 reached 56.1% (Figure 1B). In PC1, macrophage ratio exhibited a positive direction while lymphocyte ratio and total cell count in BALF showed negative associations. In PC2, positive trends were observed for total cell counts, eosinophil ratio, and macrophage ratio in BALF. Conversely, negative associations were identified between CD4/8 ratio and neutrophil ratio in BALF, and serum ACE and sIL-2R levels (Figure 1C). Additionally, the PC scores of both the extrapulmonary and pulmonary groups for each PC were assessed using rank tests.

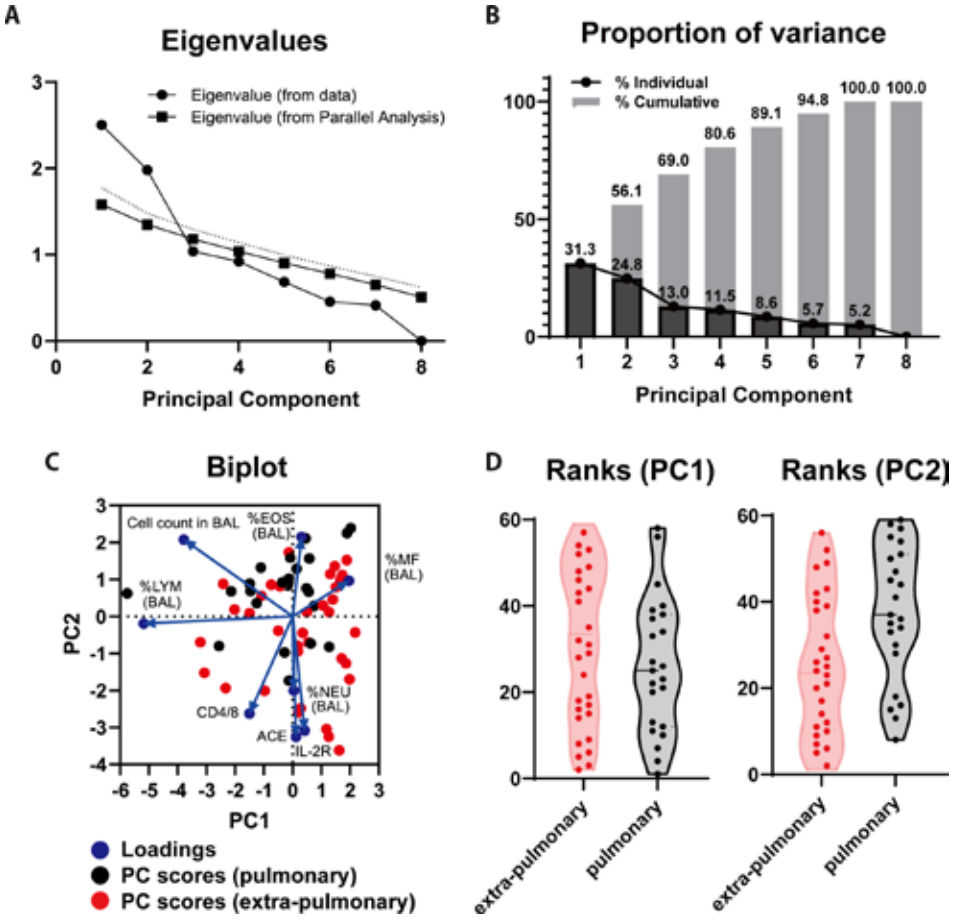

Figure 1. Principal component (PC) analysis; screen plot and parallel analysis (A), proportion of variance (B), biplot (C), and ranks test for each component (D). (A) The eigenvalues derived from the data are higher than those obtained from parallel analysis only for PC1 and PC2. (B) The proportion of variance explained by PC1 is 31.3%, while that for PC2 is 24.8%. (C) Red and black circles depict patients with and without extrapulmonary lesions, respectively. The direction in which the vectors point indicates a correlation with each PC clustered in the area. The length of the vector indicates the squared multiple correlation relationship between the fitted value of the variable and the variable itself. (D) The violin plot illustrates the distribution of the population based on the order of PC scores for each group concerning PC1 and PC2. A higher rank in the plot corresponds to a higher PC score.

There appears to be a slight prevalence of extrapulmonary cases displaying high PC scores in PC1. Conversely, within PC2 it distinctly shows an abundance of extrapulmonary cases exhibiting lower PC scores (Figure 1D).

In this study, we found that cell fractions in BALF, particularly neutrophils and eosinophils, may be useful in predicting the presence or absence of extrapulmonary lesions in sarcoidosis. Clinical characteristics including sex and serum markers in sarcoidosis with extrapulmonary lesions were consistent with previous reports (2,3). Serum markers may have been elevated via multi-organ granulomatous reactions (4). Furthermore, ocular complications were the most common complications in the study participants, which is typical for this disease (3).

Based on our PCA results, PC1 weakly indicates that the macrophage and lymphocyte ratio could contribute to diagnosis of extrapulmonary and pulmonary cases, respectively. Conversely, in PC2, pulmonary cases might be distinctly characterized by the eosinophil ratio, whereas extrapulmonary cases could be strongly characterized by serum ACE and IL-2R levels, CD4/8 ratio, as well as the neutrophil ratio. Previous study has reported that the number of neutrophils in BALF may be a marker of progressive

disease state in patients with sarcoidosis (4). Others have reported that neutrophil and eosinophil count in BALF may also be associated with progressive pulmonary sarcoidosis (5). This may be explained by the possible association between type 2 inflammation and the development of pulmonary lesions. Eosinophils and other type 2 cytokine-producing cells may be involved in this process. In contrast, extrapulmonary lesions develop mainly via type 1 inflammation (6). Thus, localized type 2 inflammation in the lung may have a protective effect on the progression of extrapulmonary lesion development.

The limitations of our study include the small number of patients. Therefore, acquiring additional data from a larger sample size is necessary for a more comprehensive analysis. Additionally, since sarcoidosis is a systemic disease, we cannot entirely exclude patients with undetected extrapulmonary manifestations.

In conclusion, this study employs PCA to assess the clinical characteristics of pulmonary sarcoidosis with or without evidence of extrapulmonary lesions, utilizing laboratory and BALF findings, which suggests that the serum markers and the cell fractions in BALF may be useful in predicting the progression of extrapulmonary lesions in sarcoidosis. Further investigation is needed to elucidate the relevance of this finding to the pathogenesis of sarcoidosis.

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