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Original article

# Reconsideration of the cut-off value of angiotensin-converting enzyme for screening of sarcoidosis in Japanese patients

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ABSTRACT

*Background:* In clinical practice, we frequently experience patients with sarcoidosis who show relatively high but normal values of angiotensin-converting enzyme (ACE). The objective of this study was to reconsider the cut-off value of ACE.
*Methods:* We studied 79 Japanese patients who were diagnosed as having sarcoidosis at our hospital. We excluded patients who had taken steroids or ACE inhibitors and patients with renal impairment. We respectively evaluated ACE values and performed receiver operating characteristic (ROC) analysis from a comparison with data for 299 normal Japanese subjects who showed ACE values in the current Japanese standard normal range (7.0–25.0 IU/L).
*Results:* Patients with sarcoidosis had higher ACE values than those in normal subjects (ACE: 20.3 IU/L [IQR, 16.0–24.4] vs. 15.4 IU/L [IQR, 12.8–18.5]; $p < 0.001$). However, 62 patients (78.5%) had normal ACE levels (cut-off value <25.0 IU/L), and the sensitivity of ACE level for detecting sarcoidosis was only 21.5%. From ROC analysis, a cut-off value of 17.7 IU/L (AUC: 0.727, 95% CI: 0.660–0.794, $p < 0.001$) was the best cut-off value for detecting sarcoidosis and sensitivity increased to 67.0%.
*Conclusions:* The possibility of sarcoidosis cannot be ruled out by using the current Japanese standard value even in patients who have normal ACE levels. Careful interpretation of this biomarker is needed.


## Introduction

Sarcoidosis is a systemic disease characterized by the development of noncaseating epithelioid granulomas in multiple organs. Cardiac involvement was identified in 25–79% of cases at autopsy [1,2], and 47–78% of patients died of cardiac events [3–6]. The lack of established strategies for early diagnosis might lead to poor prognosis.

Since the report by Lieberman [7], serum angiotensin-converting enzyme (ACE) measurements in blood have been commonly used for diagnosis and follow-up in patients with sarcoidosis; however, their diagnostic utility is unclear and controversial due to low levels of sensitivity and specificity [8]. In clinical practice, we frequently experience patients with sarcoidosis who show relatively high but normal values of ACE. The current Japanese standard value of ACE has been determined from healthy controls by simply taking the mean plus 2 standard deviations. The cut-off value of this marker for detecting patients with sarcoidosis has not been fully studied.

The aim of this study was to reconsider the cut-off value of ACE in Japanese patients with sarcoidosis.

## Materials and methods

### Subjects

Between October 2009 and December 2018, we retrospectively investigated 124 patients who were suspected of suffering from sarcoidosis at Kochi Medical School Hospital. We excluded 20 patients who did not meet the diagnostic criteria of sarcoidosis. We also excluded patients who had already received steroid

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therapy and patients with renal impairment (estimated glomerular filtration rate of less than 30 mL/min/1.73 $m^2$), which could affect the ACE value. Data for 87 patients were analyzed.

The diagnosis of sarcoidosis was based on diagnostic standards and guidelines for sarcoidosis-2015 by the Japan Society of Sarcoidosis and Other Granulomatous Disorders [9].

Written informed consent was obtained from all patients in accordance with the guidelines of the Ethics Committee on Medical Research of Kochi Medical School.

### *Healthy controls*

Between 1991 and 1992, 299 Japanese subjects who were chosen by random sampling from mass health examinations were studied by BML (BML Inc., Tokyo, Japan). The current standard value in Japan (7.0–25.0 IU/L) was determined by the mean plus 2 standard deviations of these data.

### *Measurements of biomarkers*

Peripheral blood samples were collected for measurements of biomarkers at the time of clinical evaluation. Serum ACE levels were measured by a colorimetric method using *p*-hydroxyhippuryl-l-histidyl-l-leucine as the substrate: Kasahara's method [10] (BML, Inc.). The normal range is less than or equal to 25.0 IU/L. We defined the level as positive if it was in an abnormal range.

### *Statistical analysis*

Data are expressed as mean ± SD for normally distributed variables and as median with interquartile range (IQR) for non-normally distributed data. Categorical data are expressed as numbers (percentages). Group differences were evaluated using Student's *t* test or the Mann–Whitney *U* test for continuous variables. Pearson's Chi-square test or Fisher's exact test was used for comparisons between categorical variables. Correlation between ACE and other biomarkers was assessed using Spearman's *r* (*r*).

A probability value of $<0.05$ was considered significant. Receiver-operating characteristic (ROC) analysis was performed to calculate sensitivity and specificity, area under the ROC curve, and optimal cut-off with 95% confidence limits.

Statistical analysis was performed using IBM SPSS Statistics, version 21.0 (IBM Corp., Armonk, NY, USA).

## Results

### *Patient characteristics*

Firstly, we analyzed the effects of treatment with ACE inhibitors or angiotensin II receptor blockers on serum ACE levels since ACE inhibitors have been reported to potentially lead to low ACE levels [11]. Fig. 1 shows that ACE levels measured in patients receiving ACE inhibitors were lower in this study. We therefore excluded 8 patients who were taking ACE inhibitors from the 87 patients. Finally, we analyzed 79 patients in order to reconsider the cut-off value of ACE.

Table 1 summarizes clinical characteristics of the patients, of whom 27 were male and 52 were female. The median age of the patients was 65.0 years (IQR, 55.0–71.0) (from 23 to 85 years).

Lungs, lymph nodes, and eyes were involved in many patients. According to the diagnostic standards and guidelines for sarcoidosis, 31 patients (39.2%) were diagnosed as having cardiac sarcoidosis. Fifteen patients had pacemaker implantation for advanced or complete atrioventricular block, two patients had implantable cardioverter defibrillator implantation, and three patients had cardiac resynchronization therapy defibrillator implantation for ventricular arrhythmia and heart failure. Sixteen patients had septal thinning, three patients had left ventricular (LV) aneurysm, two patients had LV hypertrophy, and 22 patients had LV local asynergy.

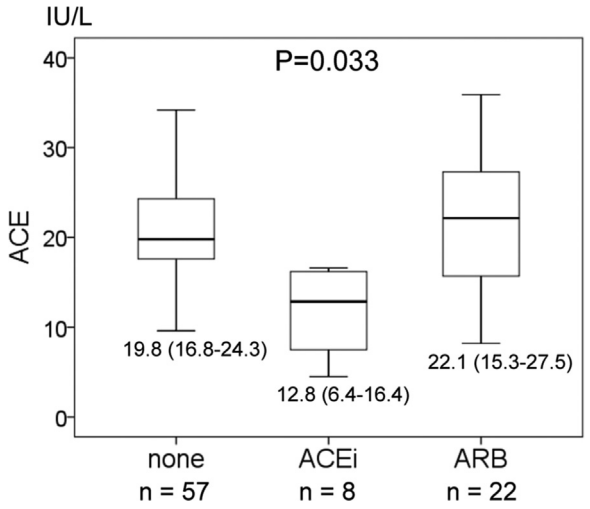


**Fig. 1.** Comparison of serum ACE levels in patients taking an ACE inhibitor or ARB and patients not taking either medication. ACE, angiotensin-converting enzyme; ACEi, ACE inhibitor; ARB, angiotensin II receptor blocker.

**Table 1**
Patient characteristics.

| | All patients (*n* = 79) |
|---|---|
| Age, years | 65.0 (55.0–71.0) |
| Gender, male/female | 27/52 |
| Involved organs | |
| • Lung, *n* (%) | 50 (63.2) |
| • Lymph node, *n* (%) | 43 (54.2) |
| • Eye, *n* (%) | 36 (45.6) |
| • Heart, n (%) | 31 (39.2) |
| • Skin, *n* (%) | 20 (25.3) |
| • Neuro, *n* (%) | 6 (7.6) |
| • Parotid gland, *n* (%) | 2 (2.5) |
| • Liver, *n* (%) | 1 (1.3) |
| Diagnostic group: pathological/clinical | 45/34 |

Data are shown as median (interquartile range) or number (%).

Sarcoidosis was proven pathologically in 45 patients and diagnosed clinically in 34 patients. None of the 79 patients had already received steroid therapy or ACE inhibitor therapy at the time of clinical evaluations.

### *Biomarkers*

In sarcoidosis patients, ACE ranged from 8.2 to 38.3 IU/L (median, 20.3 IU/L [IQR, 16.0–24.4]), lysozyme ranged from 1.7 to 34.8 μg/mL (median, 9.5 μg/mL [IQR, 7.6–12.1]), and soluble interleukin-2 receptor (sIL-2R) ($n = 43$) ranged from 193 to 1960 U/mL (median, 620 U/mL [IQR, 413–883]). ACE values were correlated with lysozyme values and sIL-2R values ($r = 0.526$, $p < 0.001$ and $r = 0.500$, $p = 0.001$, respectively).

The sensitivities of ACE, lysozyme, and sIL-2R on the basis of diagnosis of sarcoidosis were 21.5%, 42.9%, and 65.1%, respectively.

Patients with sarcoidosis had significantly higher ACE values than those in normal controls (ACE: 20.3 IU/L [IQR, 16.0–24.4] vs. 15.4 IU/L [IQR, 12.8–18.5]; $p < 0.001$) (Fig. 2, Table 2). Among sarcoidosis patients, there was no significant difference between the ACE values in the clinical diagnosis group and pathological

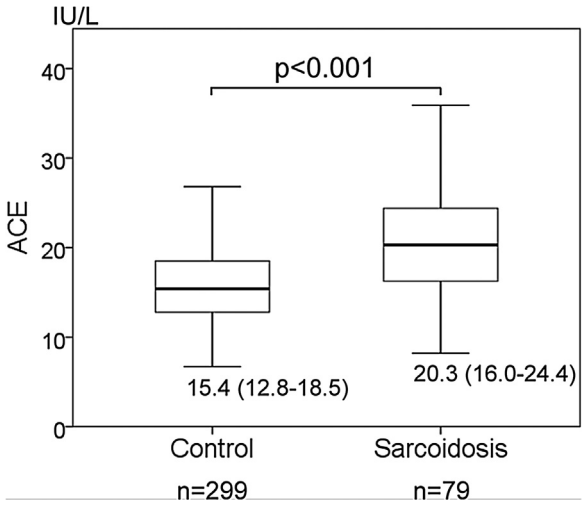


**Fig. 2.** Comparison of serum ACE levels in control subjects and patients with sarcoidosis. ACE, angiotensin-converting enzyme.

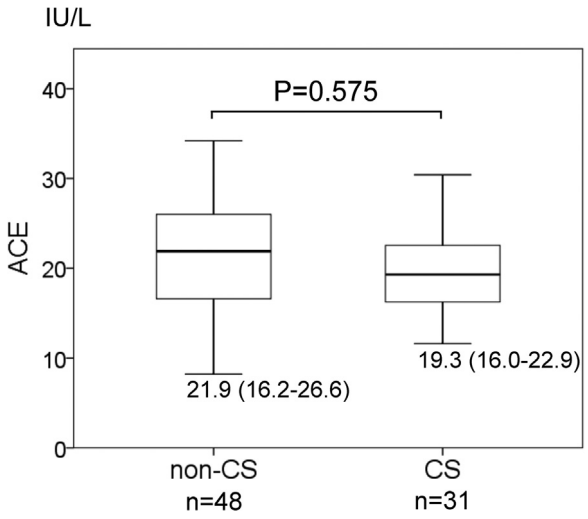


**Fig. 4.** Comparison of serum ACE levels in patients with and those without cardiac sarcoidosis. ACE, angiotensin-converting enzyme; CS, cardiac sarcoidosis.

**Table 2**
Laboratory data of patients with sarcoidosis.

| | Sarcoidosis ($n = 79$) | Standard value |
|---|---|---|
| ACE, IU/L | 20.3 (16.0–24.4) | (7.0–25.0) |
| Lysozyme, μg/mL | 9.5 (7.6–12.1) | (5.0–10.0) |
| sIL-2R, U/mL[a] | 620 (413–883) | (127–582) |
| Calcium, mg/dL | 9.4 (9.2–9.6) | (8.7–11.0) |
| Creatinine, mg/dL | 0.73 (0.59–0.88) | (0.4–0.8) |
| eGFR, mL/min/1.73 m$^2$ | 69.9 ± 21.5 | |

Data are shown as mean ± SD or median (interquartile range).
ACE indicates angiotensin-converting enzyme; sIL-2R, soluble interleukin-2 receptor; eGFR, estimated glomerular filtration rate.
[a] $n = 43$.

diagnosis group (ACE: 20.6 IU/L [IQR, 17.5–24.4] vs. 19.9 IU/L [IQR, 15.4–24.9]; $p = 0.940$) (Fig. 3) or between the ACE values in cardiac sarcoidosis and those without cardiac involvement (ACE: 19.3 IU/L [IQR, 16.0–22.9] vs. 21.9 IU/L [IQR, 16.2–26.6]; $p = 0.575$) (Fig. 4).

Calcium levels, creatinine levels, and estimated glomerular filtration rates were almost in normal ranges (Table 2).

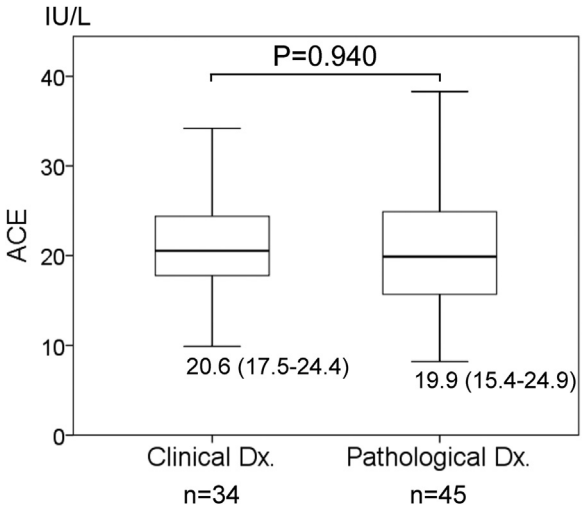


**Fig. 3.** Comparison of serum ACE levels in the clinical diagnosis group and in the pathological diagnosis group. ACE, angiotensin-converting enzyme; Dx., diagnosis.

*Cut-off value of ACE for detecting sarcoidosis*

Although patients with sarcoidosis had significantly higher ACE values than those in healthy Japanese control subjects, 62 patients (78.5%) had normal ACE levels. Table 3 shows the sensitivity, specificity, positive and negative predictive values, and accuracy of ACE on the basis of diagnosis of sarcoidosis. The sensitivity of ACE was 21.5% and negative predictive value (NPV) was 82.1%.

From receiver-operating characteristic (ROC) curves, ACE level of 17.7 IU/L (AUC: 0.727, 95% CI: 0.660–0.794, $p < 0.001$) was the best cut-off value for detecting sarcoidosis (Fig. 5). When we used this cut-off value (≥17.7 IU/L), sensitivity of ACE was 67.0% and NPV was 88.8% in the diagnosis of sarcoidosis (Table 3).

## Discussion

The conventional cut-off level of ACE has been determined deliberately from healthy controls by simply taking the mean plus 2 standard deviations. In the present study, we evaluated ACE values in 79 patients with sarcoidosis and performed ROC analysis from a comparison with data for 299 normal subjects who showed ACE values in the current Japanese standard range. To the best of our knowledge, this is the first study in which the cut-off level of ACE was evaluated from ROC curves in Japanese patients with sarcoidosis. The results of this study indicated that the possibility of sarcoidosis cannot be ruled out by using the current Japanese standard values (7.0–25.0 IU/L) even in patients who show ACE levels in the normal range. The results also indicated that patients with an ACE level above 25.0 IU/L could have sarcoidosis with high probability, based on our result that the specificity of current cut-off value was high (95.3%). From the results of the ROC curves in this study, sensitivity to detect sarcoidosis increased from 21.5% to 67.0% and patients with an ACE value of around 17.7 IU/L still have a possibility of having sarcoidosis. Since ACE measurement is a first screening test for detecting sarcoidosis, we consider that it is important to increase sensitivity to avoid overlooking this treatable disease, even if this new cut-off value brings more false positive cases in normal population. In the near future, we need to develop better diagnostic strategy with using the combination with proposed cut-off values of ACE and other examinations, which leads to the improvement of the diagnostic accuracy.

Bunting et al. reported that ROC curve analysis suggested an upper limit of normal of about 50 U/L for their assay with sensitivity of 63% and specificity of 93% in Caucasian patients with

**Table 3**
Sensitivity, specificity, positive- and negative-predictive value, and accuracy of ACE on the basis of diagnosis of sarcoidosis.

| | Sarcoidosis | Control | | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|---|---|---|
| ACE (≥25.0 IU/L) | | | | | | | | |
| Positive | 17 | 14 | 31 | | | | | |
| Negative | 62 | 285 | 347 | 21.5 | 95.3 | 54.8 | 82.1 | 79.9 |
| | 79 | 299 | 378 | | | | | |
| ACE (≥17.7 IU/L) | | | | | | | | |
| Positive | 53 | 93 | 146 | | | | | |
| Negative | 26 | 206 | 232 | 67.0 | 68.9 | 36.3 | 88.8 | 68.5 |
| | 79 | 299 | 378 | | | | | |

Data are shown as number or percent.
ACE indicates angiotensin-converting enzyme; PPV, positive predictive value; NPV, negative predictive value.

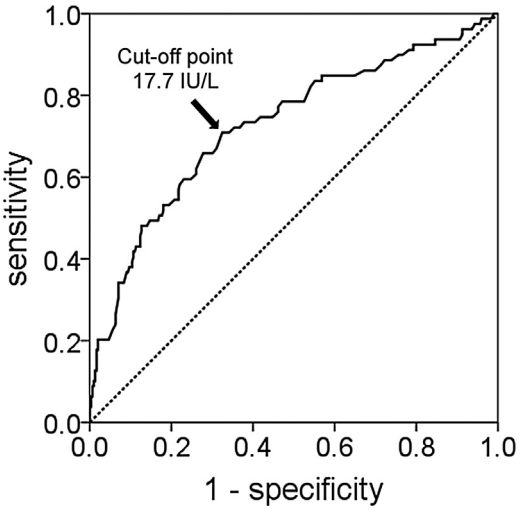


**Fig. 5.** ROC curve analysis of ACE for diagnosis of sarcoidosis. The cut-off point associated with sarcoidosis was 17.7 IU/L. The area under the ROC curve was 0.727 (95% CI: 0.660–0.794, $p < 0.001$). ROC, receiver-operating characteristic; ACE, angiotensin-converting enzyme.

sarcoidosis [12]. In the present study, the cut-off value of ACE was 17.7 IU/mL, which is much lower than that reported by Bunting et al. There are several possible reasons for this difference. First, ACE levels are influenced by ACE gene polymorphisms [13]. Allele I was reported to be associated with low ACE activity. Compared with Caucasians, Japanese have a dominant allele I and are considered to be in a low ACE activity group [14]. Second, the ACE value is influenced by the assay. The variously reported sensitivities are related to the assay method. Third, Sugisaki et al. reported that the mean ACE value was 27.9 ± 31.9 IU/mL in 195 Japanese sarcoidosis patients [15]. On the other hand, in our study, the median ACE value of the patients was 20.3 IU/L [IQR, 16.0–24.4]. A possible explanation for the difference is that ACE levels correlate fairly well with the estimated total bulk of granulomata in the body [16], and while the source of production is not entirely clear, epithelioid and giant cells of sarcoid granulomata have been implicated [17,18]. The difference between the results reported by Sugisaki et al. and our results might be due to the patients' backgrounds.

In our study, there was no significant difference between the ACE values in patients with cardiac sarcoidosis and those without cardiac involvement. We could not identify patients with cardiac sarcoidosis by the ACE values themselves, although cardiac involvement is known to be an important determinant of the prognosis. This might be because ACE levels correlate with the total bulk of granulomata in the whole body, while cardiac involvement is less affected. It is important to develop new biomarkers for appropriate diagnosis of cardiac sarcoidosis. Recently, Fujiwara et al. reported the potential of serum microRNA-126 and -223 as new-generation biomarkers for the differential diagnosis of cardiac sarcoidosis [19,20].

With regard to ACE levels in patients receiving ACE inhibitors, we confirmed that ACE inhibitors potentially lead to low ACE concentrations in peripheral blood samples. Since many patients take ACE inhibitors for hypertension, chronic heart failure, and chronic kidney disease in clinical practice, we need to be careful about interpreting ACE levels for those patients [11].

### *Limitations*

There were several limitations in this study. Firstly, this study was carried out at a single hospital with a relatively small sample size. Secondly, 43% of the patients were in the clinical diagnosis group with no pathological evidence of sarcoidosis. However, in clinical practice, many patients cannot be diagnosed by pathological findings and there was no significant difference between the ACE values in the clinical diagnosis group and pathological group. Finally, although we used control data provided by the current Japanese standard value of ACE, the control subjects had the possibility of having an underlying disease that influences the ACE value.

## Conclusions

The possibility of sarcoidosis cannot be ruled out by using the current Japanese standard value even in patients who have normal ACE levels. Careful interpretation of this biomarker is needed.

## Contributions

Y. Baba, T. Kubo, and H. Kitaoka conceived the idea for the study and planned the investigations. Y. Baba, T. Kubo, H. Kitaoka, Y. Ochi, T. Hirota, N. Yamasaki, H. Ohnishi, T. Kubota, and A. Yokoyama undertook clinical investigations of patients. S. Yamanaka measured biomarkers.

## Funding

None.

## Conflict of interest

None of the authors have conflict of interest to disclose in connection with our manuscript.

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