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Determination of immunological biomarkers in sarcoidosis and their relation to disease activity

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Abstract

Sarcoidosis is a multisystem granulomatous disease of unknown origin. Ninety percent of patients with sarcoidosis have lung involvement. The onset can be acute or non-acute and the severity of sarcoidosis ranges widely from asymptomatic patients with accidental radiographic findings to patients with severe organ involvement. This case control analytic prospective study was conducted at the Chest Clinic, Al Zahraa hospital, to assess the diagnostic value of serum soluble interleukin 2 receptor (sIL-2R), cluster of differentiation 4 (CD4)/CD8 ratio and CD103 in sarcoidosis. We investigated the value of serum sIL-2R using ELISA and blood CD103, blood CD4/CD8 ratio using flow cytometry for 30 cases of sarcoidosis in different stages and 30 control persons to detect their use as a marker for diagnosis. We found a significant increase in sIL-2R in the sarcoidosis group as compared to the control group (p<0.0001), while there was a significant decrease in CD103/CD4 in sarcoidosis group as compared to the control group (p <0.001). In conclusion**,** sIL-2R and CD103 can be used as diagnostic markers for sarcoidosis.

Keywords: Sarcoidosis, sIL-2R, CD103, CD4/CD8 ratio.

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Introduction

Sarcoidosis is a multisystem disorder of unknown etiology. Some cases are attributed to genetic factors, an inflammatory response by specific antigens including self-antigen, and autoimmune involvement.[1] The onset can be acute or non-acute and the severity of sarcoidosis ranges widely from asymptomatic patients with accidental radiographic findings to patients with severe organ involvement.[2]

Sarcoidosis is a relatively uncommon disease with heterogeneous onset and manifestations. The American Thoracic Society (ATS) has designated three criteria for the diagnosis of sarcoidosis: (1) characteristic clinical and radiologic presentation; (2) evidence of noncaseating granulomas in one or more tissue samples; and (3) exclusion of alternative causes of granulomatous disease.[3] Early diagnosis of sarcoidosis is challenging, and thus diagnosis is often delayed.[4] Evaluation often requires

extensive diagnostic testing and invasive tests, such as histological sampling of the affected tissue.[5] There is a need for safe, easy, and reliable biomarkers to establish diagnosis and prognosis of sarcoidosis. Sarcoidosis is characterized by an over-immune response given to an unknown antigen. The process starts with monocyte/macrophage activation, followed by the formation of granuloma, and ends with remission or fibrosis. Associated with these phases, serum levels of some biochemical markers increase in patients with sarcoidosis.[6] In sarcoidosis it is likely that there is a complex interplay between adaptive and innate immunity**,** represented by T-lymphocytes and monocyte-derived macrophages.

Angiotensin-converting enzyme (ACE) is an acid glycoprotein that converts angiotensin I into angiotensin II. It is produced mainly by activated alveolar macrophages and elevated in sarcoidosis patients. ACE is the only biomarker mentioned in the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) international guidelines for the diagnosis and follow up of sarcoidosis patients, but its sensitivity and specificity are low.[7]

Eurelings et al., 2019 concluded that serum soluble interleukin 2 receptor (sIL-2R) is a sensitive biomarker and superior to ACE in establishing the diagnosis of sarcoidosis and can be used to rule out sarcoidosis in patients suspected of sarcoidosis.[5]

In patients with sarcoidosis, numbers of cluster of differentiation (CD)103 CD4+ T-cells in broncho-alveolar lavage (BAL) fluid are significantly lower than in other lung diseases. Therefore, differential expression of CD103 on CD4+ T-cells could serve as a diagnostic marker for sarcoidosis.[8]

The interleukin 2 (IL-2) receptor (IL-2R) α-chain (IL-2Rα) is being shed upon immune activation. Therefore, increased levels of sIL-2R are considered as an indication of an on-going immune response which could be used to monitor immune-mediated diseases. In sarcoidosis T helper cells and alveolar macrophages are the most likely sources of sIL-2R.[9]

It is unclear if sIL-2R has immune-stimulatory, immunomodulatory, or no functional effects since conflicting results have been reported. Several potential mechanisms of sIL-2R's biological functions include IL-2 sequestration, prolonging IL-2 half-life, preventing activation of resting T cells or increasing affinity of IL-2Rβ for IL-2.[10] The most likely function of sIL-2R is to modify IL-2 signaling. Increased levels of sIL-2R could either promote disease processes, represent an ineffective attempt to resolve the inflammation or have no effect at all.[11] The present study was conducted to assess the diagnostic value of sIL-2R, CD4/CD8 ratio and CD103 in sarcoidosis.

Subjects and Methods

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine for Girls, Al Azhar University (approval no. 202006268). An informed verbal consent was obtained from each study participant before included in the study. This was a case control analytic prospective study performed at the Chest Clinic in Al Zahraa hospital, included 60 subjects of both sexes. Their ages ranged from 31 to 65 years.

The selected subjects were divided into 2 groups, Group 1 (Patients group), included 30 patients with sarcoidosis (6 males and 24 females) recruited from Chest Outpatients Clinic at Al Zahraa University Hospital during the period from May to September 2021. Diagnosis of sarcoidosis was based on clinicoradiographic findings compatible with the diagnosis, histologic confirmation of granulomatous inflammation, exclusion of known causes of granulomatous disease. The second group, Group 2 (control group), included 30 age and sex matched apparently healthy individuals.

Inclusion criteria included patients with pulmonary sarcoidosis at any stage, regularly monitored at the Chest Clinic at Al Zahraa University Hospital. Exclusion criteria included patients who received immunosuppressive medication as immunosuppressive medication, which can reduce sIL-2R levels; patients with hyperthyroidism, diabetes, liver disease, hematological or systemic malignancy; patients with pulmonary diseases other than pulmonary sarcoidosis. Furthermore, exclusion from the

control group included subjects with hypertension, hyperthyroidism, diabetes, liver disease, hematological or systemic malignancy and smokers.

Methodology

All patients were subjected to full medical history and clinical examination. Diagnosis of sarcoidosis was based on diagnostic standards and guidelines for sarcoidosis-2015 by the Japan Society of Sarcoidosis and Other Granulomatous Disorders.[12]

Blood samples collection and laboratory investigations

A peripheral venous blood sample (4 ml) was withdrawn from each study subject. Of these, an aliquot of 2 ml was collected in an EDTA tube for examination of CD103, CD4 and CD8 by flow cytometry and for complete blood count (CBC). The second aliquot (2 ml) was collected in serum separator tube, centrifuged at 959 xg for 10 min and stored frozen at – 30 °C until used for examination of serum sIL-2R by an enzyme linked immunosorbent assay (ELISA). CBC was performed using an automated blood count system (Hematological Sysmex, XE-21N, Kobe, Japan), according to the manufacturer's instructions.

Flow cytometry assay

Flow Cytometry was conducted in the Clinical Pathology Department of Al-Zahraa Hospital, Al-Azhar University using four-color fluorescence-activated cell sorting (FACS) cytometer (FACS Calibur, BD, Biosciences, San Jose, USA). Cell Quest Pro software (BD Biosciences, San Jose, USA) was used for data analysis. A fresh blood sample (50 ul) was incubated with 5 ul of the following reagents: peridinin chlorophyll protein complex (PerCP)-conjugated anti-human CD8, PE-conjugated anti-human CD4 (Cat. no. MA1-19793), APC conjugated CD3 and FITC-conjugated anti-human CD103, for 10 min at room temperature. Then a lysis reagent (BD, Biosciences, San Jose, USA), for destruction of RBCs, was added for 8 minutes before cells were washed with FACS buffer and centrifuged at 500xg. Fifty thousand events were acquired for analysis.

Initial gating on typical mature lymphocytes expected area on forward scatter/side scatter (FS/SS) dot plot. T lymphocytes were identified along CD3APC/SS graph by being CD3+. Within the CD3 + T cell population, the subset of CD4 + T helper cells on lower right region (R3) area while CD8+ T cytotoxic population was detected on upper left region (R4). On another quadrant histogram representing CD103 on the X axis versus SS on Y axis the expression of 103+ population was identified on each subset in the lower right quadrant (Figure, 1). Data were expressed as detection CD4/CD8 ratio, percentage of positive population of CD103 on each subset and mean fluorescence intensity (MFI) of CD103 on each T subset (Figure, 1).

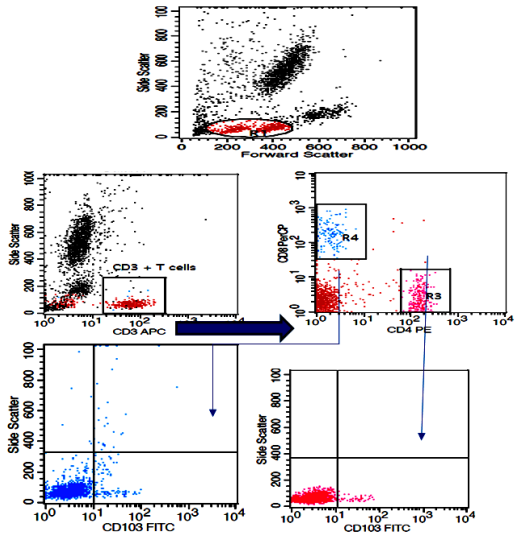

Figure 1. Illustrates gating strategy of CD103 expression on T helper and T cytotoxic and method of detection CD4/CD8 ratio.

ELISA test for determination of sIL-2R

The serum level of SIL-2R was determined using a commercial ELISA kit (Cat. No E0205Hu, Bioassay technology laboratory, China), according to manufacturer's instructions. The kit has sensitivity of 2.75 pg/ml and the standard curve ranged: 5-1000 pg/ml. Finally, the serum level of ACE was collected from the hospital patients' records.

Statistical analysis

The collected data were revised, coded, tabulated, organized, and analyzed using the Statistical Package for the Social Sciences (SPSS)

version 20.0. Parametric data were expressed as mean± SD and non-parametric data expressed as number and percentage. Numerical data were summarized using medians and ranges while categorical data were summarized as numbers and percentages. The independent t-test was done to compare between two groups. Pearson Correlation Coefficient was done to assess the relation between different studied parameters in the same group. A *p*-value of ≤0.05 was considered significant. The receiver operating characteristic (ROC) curve was plotted to analyze recommended cut-off values for IL-2R. The area under the (AUC) curve denotes the diagnostic performance of the marker. At a selected cutoff value for IL-2R, the sensitivity, specificity, positive predictive value, and negative predictive value were determined.

Results

The study patients had an age range between 34 to 65 years, compatible with the control group 31 to 62. The gender distribution of controls was compatible to the patient group (female/male: 21/9, 70/30%). There was an increased prevalence of sarcoidosis in females than males [female/male: 24/6, 80/20%], Table 1.

Table 1. Demographic data of studied groups.

Demographic data		Sarcoidosis (N=30)	Control (N=30)
Age (years)	Range	34-65	31– 62
	Mean ± SD	47.2±8.4	42.1 ± 8.1
Sex	Male No (%)	6 (20%)	9 (30%)
	Female No (%)	24 (80%)	21 (70%)

The studied variables are presented in Table 2. There were 16.7 % smokers among our study patients. There were 96.6 % (29/30) of cases present with pulmonary sarcoidosis, 83% with lymphadenopathy, 33% with hepato-splenomegaly, 26.6% with skin lesion, 23.3% with cardiac disease, 13.3% with eye lesion and 6.6% with thyroid lesion (66.6% of patients had extra thoracic manifestation), Table 3.

Table 2. Variable data of the 30 Sarcoidosis patients.

Variable	Parameter's data
Disease duration (months)	(3-240 M)
Duration of cortisone intake (months)	(2-24 M)
Smokers	(5/30)
HRCT stage of sarcoidosis (I/II/III/IV)	(1/25/3/1)
Diagnosis of sarcoidosis	Biopsy (28/30) BAL (2/30)
Lymphadenopathy	25/30
Pulmonary manifestations	29/30

M: month; HRCT: High resolution computed tomography.

Table 3. Extra-pulmonary involvement of the 30 Sarcoidosis patients.

Extra-pulmonary involvement	Parameter's data
Hepatosplenomegaly (HSM)	10/30
Skin lesion	8/30
Eye lesion	4/30
Thyroid diseases	2/30
Heart diseases	7/30

There was a significant increase in monocyte, ACE and IL-2R in sarcoidosis group as compared to control group. While there was a significant decrease in CD103/CD4 in sarcoidosis group as compared to control group, Table 4. There was a significant positive correlation between IL-2R and monocytes, ACE and CD4, but significant negative correlation between IL-2R and CD103/CD4, Table 5 and Figure 2. The ROC curve of IL-2 R for discriminating sarcoidosis cases and controls showed sensitivity of 96.7 % and specificity of 88%, Table 6 and Figure 3.

Table 4. Comparison of different Laboratory parameters among studied groups**.**

Laboratory Parameters	*p*- value
TLC x10^3/ul	NS
Lymphocyte x10^3/ul	NS
Monocyte x10^3/ul	<0.001
ACE (U/L)	<0.001
sIL-2R (pg/ml)	<0.0001
CD4	<0.001
CD8	NS
CD4/CD8	NS
CD103/CD4	<0.001
CD103/CD8	NS

P > 0.05 is not significant (NS). ACE: angiotensin converting enzyme.

Table 5. Correlation between sIL-2R and different Laboratory Parameters in sarcoidosis group.

Laboratory Parameters	IL-2R	
	r	*p* value
TLC x10^3/μl	-0.002	NS
Lymphocyte x10^3/μl	0.140	NS
Monocyte x10^3/μl	0.428	0.001
ACE (U/L)	0.791	<0.0001
CD4	0.387	0.002
CD8	-0.148-	NS
CD4/CD8	0.078	NS
CD103/CD4	-0.385	0.002
CD103/CD8	0. 143	NS

P > 0.05 is not significant (NS).

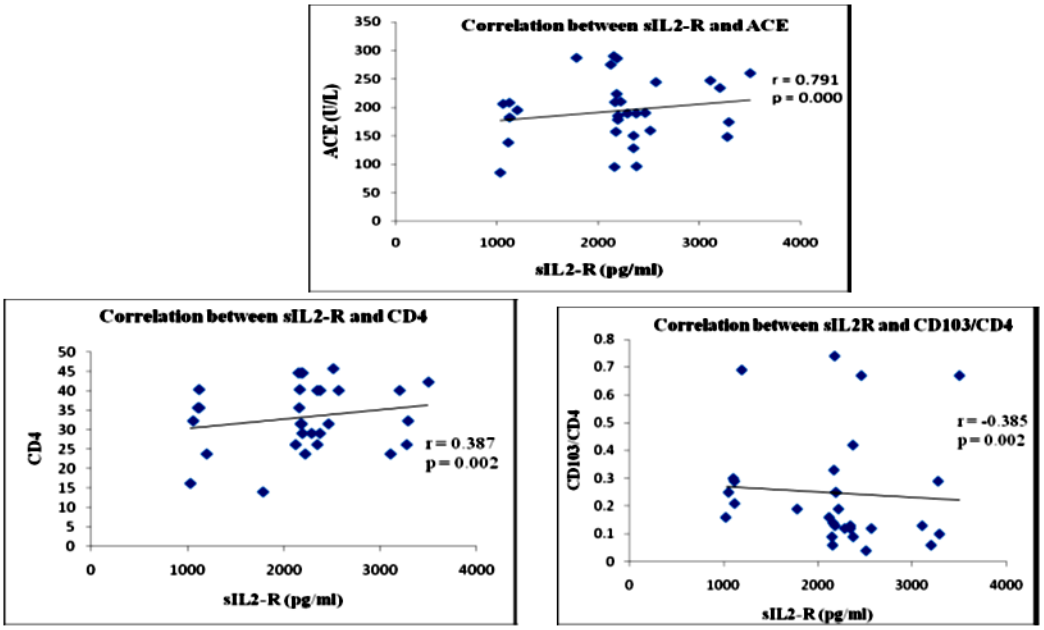

Figure 2. Correlation between sIL-2R and ACE, CD4 and CD103/CD4.

Table 6. Sensitivity, Specificity, PPV and NPP of the best selected cut off value to differentiate between Sarcoidosis cases and controls.

Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (95% CI)	*p* value
925	96.7(%)	88(%)	88(%)	96.7(%)	0.915 (0.827-1.007)	< 0.001

PPV: Positive Predictive Value, NPV: Negative Predictive Value, AUC: Area under the curve, CI: Confidence Interval.

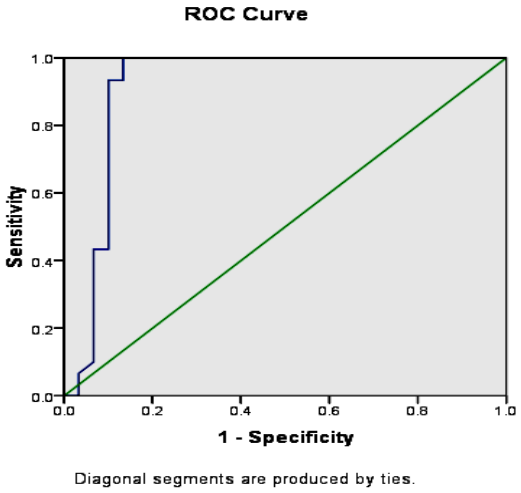

Figure 3. Receiver operating characteristic (ROC) curve of IL-2 R for discriminating Sarcoidosis cases and control.

Discussion

In the present study we intended to assess the value of sIL-2R, CD4/CD8 ratio and CD103 as diagnostic parameters for sarcoidosis. Our study showed age range between 34 to 65 years with increased prevalence of sarcoidosis in females than males. These results come in agreement with those of Refat et al., 2020,[13] reported the age range of 32 to 62 year, 50 % of them were below 40 years. They also found that females were more affected (73.3%) than males (26.7%). This observation was supported by data of Baughman et al., 2016,[14] found a higher incidence of sarcoidosis in females (75%) than males. Such finding suggested hormonal effects on the activity of sarcoidosis.

There were 16.7 % smokers among our patients this comes in the same line with many studies that demonstrated a lower incidence of

sarcoidosis in smokers.[15,16,17] Some higher incidence was reported by Urbankowski et al., 2012,[18] they found that 42.6% of sarcoidosis patients were smokers.

In the present study, there were 96.6 % (29/30) of cases presented with pulmonary sarcoidosis, 66.6% of them had extra thoracic manifestation. This result agreed with that of Sève et al., 2021,[19] reported that intrathoracic involvement occurred in 90% of patients with symmetrical bilateral hilar adenopathy and/or diffuse lung micronodules. Among extrapulmonary manifestations, skin lesions, uveitis, liver or splenic involvement, peripheral and abdominal lymphadenopathy and peripheral arthritis were the most frequent with a prevalence of 25–50%.[19]

The present study showed a significant increase in monocyte, ACE and IL-2R in sarcoidosis group as compared to control group, while there was a significant decrease in CD103/CD4 in sarcoidosis group as compared to the control group. This significant increase in monocytes coincided with findings of Lepzien et al., 2021,[20] they reported that monocytes/monocyte-derived cells were increased in blood and BAL of sarcoidosis patients compared to healthy controls. Interestingly, high frequencies of blood intermediate monocytes at time of diagnosis were associated with chronic disease development.

In agreement with our study observation, Kobak et al., 2020[21] found increased serum ACE level in 54.5% of patients and Bernardinello et al., 2021[22] reported increased level of ACE in the sarcoidosis group**.** Similarly, Wang et al., 2022[23] reported that the mean value of serum ACE in sarcoidosis patients was 30:80 ± 56:61U/L, which was significantly higher than that in non-sarcoidosis patients (28:07 ± 14:11 U/L, *p*=0:001). A study by Lawrence et al., 1988[24] reported on soluble IL-2R in serum and concentrated BAL. They found that measurement of sIL-2R in sera was more useful than that in BAL. These preliminary findings suggested that measurements of sIL-2R in serum samples may indeed be of use in the management of patients with sarcoidosis**.** High serum sIL-2R levels were associated with acute or active disease, with disease progression over the next 6 months in untreated patients, or in patients requiring long-term therapy; however, a similar study reported negative correlations.[6]

The main finding of the current study was the significant increase in sIL-2R among the sarcoidosis patient group compared to the control group ($p<0.05$). This coincided with data reported by Uysal et al., 2018,[25] they stated that the sIL-2R levels had very good diagnostic value in differentiating between the control and patients. It was an accurate parameter in the assessment of sarcoidosis and correlated with active disease**.**

In agreement with our study, Nguyen et al., 2017[26] also reported that sIL-2R levels correlated with disease activity, particularly in patients with extrapulmonary disease. We also found an increased level of sIL-2R among patients in stage III (parenchymal involvement in addition to nodal enlargement) more than patients in the other stages. This result agreed with the findings of Eurelings et al., 2019,[5] who stated that there was a significant correlation between serum sIL-2R levels and chest radiograph stages.

Tanrıverdi et al., 2016[27] reported that BAL CD4/CD8 ratio were significantly higher in sarcoidosis than other diffused parenchymal lung diseases. The best cut off value of CD4/CD8 was 1.34 with sensitivity and specificity of 76.4% and 79.4%, respectively. The cut off values of CD4/CD8 of >3.5 and >2.5 had specificity 95.9% and 95.3%, respectively and sensitivity of 52% and 41%, respectively.

Another study demonstrated that a CD4/CD8 ratio above 3.5 showed a high specificity of 93–96% for sarcoidosis, although the sensitivity was low (52–59%).[28] Some other authors doubted the clinical utility of this ratio based on the observation that is highly variable. These authors found that only 42% of 86 patients with biopsy-proven sarcoidosis had a BAL ratio greater than 4.0, and that 12% even had an inverted ratio below 1.0, reflecting a predominance of CD8+ T-lymphocytes.[28]

In our study, there was no differences in peripheral blood CD8 and CD4/CD8 ratio between patients and normal controls. These results agreed with those of Iida et al., 1997,[29]

who stated that CD8+ cells and the CD4/CD8 ratio in peripheral blood were similar in patients and normal control subjects.

Concerning CD4 we found significant increase in the sarcoidosis group more than the control group this finding coincided with that of d'Alessandro et al., 2021,[30] who found relative increases in the number of CD4+CXCR5+CD45RA−Th cells in the peripheral and alveolar compartments of sarcoidosis patients with respect to peripheral cell distribution in controls.

Our study findings also coincided with those of Iida et al.,1997,[29] who reported that the percentage of CD4+HLA-DR+ cells in peripheral blood was significantly higher in patients than in normal control subjects ($p<0.05$). Integrin CD103 can promote T-cell migration into the epithelium and is involved in the retention of lymphocytes in the mucosa. Constant CD103 expression can reflect antigen persistence in the lung tissue. Our results showed a significant decrease in CD 103/CD4 in sarcoidosis patients compared to the control group. This result coincided with that observed by Aleksonienė et al., 2021,[31] who reported that the number of CD4+CD103+ T cells was significantly higher in the control group compared with patients at stage I, II and III sarcoid.

Kolopp-Sarda et al., 2000[32] found that a high CD4+/CD8+ ratio combined with a low CD103+CD4+/CD4+ ratio allowed to distinguish sarcoidosis from other interstitial lung diseases with a 96% sensitivity. Similarly, Mota et al., 2012[33] found a 98% sensitivity. However, two other studies showed a lower performance of the CD103+CD4+/CD4+ ratio as a diagnostic marker for sarcoidosis.[34,35]

Bretagne et al., 2015[36] reported that the use of combined CD4+/CD8+ and CD103+CD4+/CD4+ ratios resulted in modest increases of the AUC to 76% for sarcoidosis versus all causes of lymphocytic alveolitis, and to 78% for sarcoidosis versus other interstitial lung diseases. This remained lower than the AUC of the CD4+/CD8+ ratio alone (79%), suggesting that the contribution of the CD103+CD4+/CD4+ ratio itself was low.

In our study, there was a significant positive correlation between IL-2R and monocytes, ACE and CD4 but significant negative correlation between IL-2R and CD103/CD4. Our results coincided with those of Nguyen et al., 2017,[26] who reported that serum sIL-2R levels were correlated with levels of ACE. In the same line Grutters et al., 2003,[37] found a positive correlation between sIL-2R level measured in serum samples obtained at presentation of disease and the number of CD4 T lymphocytes in the BAL samples. This finding is corroborative to the concept that the sIL-2R concentration is an index of T-cell activation and brings support to its role as a marker for disease activity in sarcoidosis. Moreover, it suggests that the serum sIL-2R test might be useful in evaluating the intensity of T-helper cell alveolitis in sarcoidosis, which is one of the important characteristics of this disease.[37]

In the present study, the ROC curve analysis of IL-2 R for discriminating our sarcoidosis cases and controls showed sensitivity of 96.7 % and specificity of 88%. These results coincided with those of Eurelings et al., 2019[5] who confirmed that compared to ACE, sIL-2R had higher sensitivity (88% versus 62% with ACE) and higher specificity (85% versus 76% with ACE) in identifying people with sarcoidosis. In conclusion, serum sIL-2R levels were significantly elevated in sarcoidosis and can be used for diagnosis and as a marker of disease activity in sarcoidosis patients as ACE. Decreased levels of CD103/CD4 in blood can also be used for diagnosis of sarcoidosis.

Author Contributions

RSI, EKM; made the statistical analysis. ASH, EKM; performed the laboratory work. EMM; examined the patients. EKM; collected samples. All authors participated in writing and reviewing the paper.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine for Girls, Al Azhar University (approval no. 202006268).

Informed consent

An informed verbal consent was obtained from each study participant before included in the study.

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