Respiratory Medicine (2010) **104**, 1336–1343

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/rmed

# Association between CD14 gene polymorphisms and disease phenotype in sarcoidosis

**Zvi G. Fridlender [a,*,1], Asaf Schwartz [a,1], Martin Kohan [a], Gail Amir [b], Mendel Glazer [c], Neville Berkman [a]**

[a] *Institute of Pulmonology, Hadassah-Hebrew University, Medical Center, POB 12000, Jerusalem 91120, Israel*
[b] *Department of Pathology, Hadassah-Hebrew University, Medical Center, POB 12000, Jerusalem 91120, Israel*
[c] *Rokah Pulmonary Institution, Kupat Cholim Clalit, Jerusalem, Israel*

Received 28 October 2009; accepted 29 March 2010
Available online 28 April 2010

**KEYWORDS**
Sarcoidosis;
CD14;
Polymorphism;
Genotype–phenotype association

**Summary**
Although the etiology of sarcoidosis is unknown, genetic susceptibility has been demonstrated. Granuloma formation is a key feature in the pathophysiology of sarcoidosis and Crohn's Disease, raising the possibility that these diseases share common pathogenetic pathways. An association between sarcoidosis and the protein "CD14", a molecule that is part of the lipopolysaccharide (LPS) cell surface receptor complex, has been suggested.

In the current study we evaluated the CD14 gene promoter *159 C → T* polymorphic site and soluble CD14 levels in a cohort of 74 sarcoidosis patients compared to 85 healthy controls. We further sought to identify correlations between clinical phenotype, specific genotypes and soluble CD14 levels.

We found the TT genotype to be more prevalent in the sarcoidosis patient group than in controls ($p = 0.03$). Serum levels of soluble CD14 were higher in the sarcoidosis patients ($p = 0.001$). Within the patient cohort, CC homozygous patients presented at an older age with milder disease as assessed with the SAC score, longer time to diagnosis, and less impairment of pulmonary function tests.

Our study suggests a role of CD14 in the pathogenesis of sarcoidosis, and a clinical phenotype-genotype association. Further mechanistic and epidemiologic studies are needed in order to establish the specific role of CD14 in the etiology, pathogenesis and clinical phenotype of sarcoidosis.


* Corresponding author. Tel.: +972 50 5172444.
*E-mail address:* fridlender@hadassah.org.il (Z.G. Fridlender).
[1] These authors contributed equally to this manuscript.

0954-6111/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2010.03.029

## Introduction

Sarcoidosis is a systemic inflammatory granulomatous disease that can involve almost every organ system in the body. The course of the disease is highly variable and unpredictable, ranging from a mild self-limiting form to a severe debilitating and even fatal disease.[1,2] The etiology of sarcoidosis is unknown, however, genetic susceptibility has been demonstrated, with evidence of familial aggregation and racial variance in disease incidence.[3–6]

The characteristic pathological feature of sarcoidosis is the non-caseating granuloma. The mechanism of granuloma formation involves the activation of Th1 helper cells by Antigen Presenting Cells (APC's), and secretion of monocyte chemotactic cytokines.[1–3] Formation of granulomas is also an essential process in the pathophysiology of Crohn's Disease, raising the possibility that these two diseases share common pathogenetic pathways.[3,7]

CD14 is a myeloid-monocytic marker antigen, expressed as either a 55 kDa membrane bound protein (mCD14), or as a 48 kDa soluble plasma protein (sCD14).[8] It acts as part of the lipopolysaccharide (LPS) cell surface receptor complex. CD14 binding leads to the activation of NF$\kappa$B, followed by up-regulation of inflammatory mediators.[8] Despite significant progress in understanding the origin of soluble CD14 (sCD14), its physiological function remains largely unknown.[9] On one hand, sCD14 has been shown to induce an inflammatory response in non-myeloid cells lacking the membrane bound form of the protein.[10] In contrast, sCD14 has been shown to have beneficial properties in protection against LPS-induced endotoxin shock, by acting as a competitive inhibitor of LPS binding to mCD14.[9]

The presence of a single nucleotide polymorphism (SNP) in the proximal promoter of the CD14 gene, *159 C → T,* has been shown to be related to higher plasma levels of sCD14 (TT), and lower levels of IgE.[11] This suggests a role for CD14 in diverting the immune response from a Th2 towards Th1 phenotype.[11,12] An increased frequency of the T allele has been reported in Crohn's disease.[13–15]

A possible relationship between Sarcoidosis and CD14 has been suggested previously.[16–19] DNA from Gram-negative bacteria and elevated levels of LPS in Bronchoalveolar Lavage (BAL) have been observed in sarcoidosis patients.[19] Furthermore, The $-159$ *C→T* polymorphism in the CD14 gene has been shown to increase TNF-$\alpha$ production in response to LPS and Gram-positive and Gram-negative bacteria.[20]

In the current study we evaluated the CD14 *159 C → T* polymorphic site and determined soluble CD14 levels in a cohort of 74 sarcoidosis patients and compared these to a cohort of 85 healthy controls. We further sought to identify the presence of correlations between clinical phenotype, specific genotypes (TT, CT, CC) and soluble CD14 levels.

## Materials and methods

### Study population

Patients were recruited from a 12-year database (1995–2006), of patients diagnosed with Sarcoidosis by the pathology service at the Hadassah Medical Center Jerusalem, Israel and from patients attending a large outpatient pulmonary clinic in Jerusalem. Charts of patients with a biopsy from lung, lymph nodes or skin compatible with sarcoidosis (non-caseating granulomata), were evaluated. Patients with a compatible clinical picture as determined by symptoms, laboratory abnormalities and/or imaging and without another identified cause of granulomatous disease were included in the initial cohort. Clinical and demographic information was gathered from a patient questionnaire, hospital medical records and a treating physician questionnaire. Where we found discrepancies between the data reported by the patient and that found in physician's questionnaires or in medical records, we used the latter. Blood samples were obtained from patients for DNA analysis. A cohort of healthy subjects with no significant medical background, and no regular medications was recruited as a control group ($n = 85$). All participants signed an Informed Consent Form and the study was approved by the institutional Ethics Committee.

### Assesement of disease extent and severity

Duration of symptoms prior to diagnosis was evaluated from patient reports and documented medical records. Cases in which duration of symptoms could not be accurately evaluated were excluded from analysis. Determination of which organ systems were involved in the disease was evaluated based on patient records and physician questionnaires.

The severity assessment of sarcoidosis was done using the modified Sarcoidosis Activity (SAC) Score.[21] This score assesses disease severity using the following equation:

$$\begin{aligned}\text{Score} = {} & 11.46 + 3.9C + 2.56N + 1.56(IS) \\ & -0.051(\text{FVC \% predicted}) + 1.75(AA) \\ & -0.054(FEV1/FVC)\end{aligned}$$

where C = cardiac involvement (0/1), N = Neurologic Involvement, IS = non-steroid immunosuppression (0/1), AA = African American.

### Evaluation of sCD14 levels

Plasma was separated from the blood samples obtained, and immediately frozen at $-20\,^{\circ}C$. sCD14 levels in the plasma were measured using the commercially available CD14 DuoSet ELISA development Kit, according to the protocol supplied by the manufacturer (R&D Systems, Minneapolis, MN).

### Genotype analysis

Genomic DNA was extracted from anticoagulated whole blood collected in EDTA from patients and controls. Isolation of DNA was done by phenol–chloroform extraction and alcohol precipitation as previously described.[22]

The CD14 $-159C \rightarrow T$ polymorphism was assessed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP), as previously described.[23] PCR amplification was performed using the following primers:

Forward: 5'GCCTCTGACAGTTTATGTAATC3'.

Reverse: 5'GTGCCAACAGATGAGGTTCAC3'. (Sigma, Israel).

The PCR product was then digested by the restriction enzyme AVAII (New England Biolabs, Ipswich, MA) in 37 °C for 10 h, resolved by electrophoresis on 1.8% Agarose Gel (SeaKem LE Agarose, Cambrex) and stained with Ethidium Bromide (Sigma, Israel). The presence of the T allele creates a restriction site for AVAII, yielding a 144-bp and 353-bp bands, whereas the C allele, which remains uncut, yields a single 497-bp band.

### Lung function tests

For this analysis, we used only lung function tests (LFT) that were performed at the time of diagnosis when patients were on no treatment. Patients that did not have available LFT at the time of diagnosis were excluded from this analysis. Spirometry was performed using a pneumotachograph-based system. The reference equations and corrections used for the measurements of spirometry, lung volumes and DLCO were those published by the European Community for Coal and Steel (ECCS).[24] No ethnic correction factors are used for our patients.

### Statistical analysis

Differences in demographic parameters in the study groups, the frequencies of the polymorphic genotypes and clinical differences between them were analyzed using the proper $\chi^2$-test (either $2 \times 2$ or $2 \times 3$ according to the question tested). The difference between sCD14 levels in controls vs. Sarcoidosis patients, and the comparisons of continuous parameters (i.e. lung function tests) comparing the CC genotype to the non-CC genotype were analyzed using Student's *t*-test. To evaluate the level of sCD14 in the different genotypes, we used ANOVA with appropriate post hoc testing. $p < 0.05$ was considered to be statistically significant.

## Results

### Study population and patient characteristics

A total of 350 sarcoidosis patients were identified. Of these, seventy-four patients together with 85 control subjects were recruited for this study. 32.4% of the patients and 42.8% of the controls were males, 77% of the patients and 82.4% of the controls were of Jewish origin (both differences - NS). Remaining subjects in both groups were of Arab origin.

Table 1 presents the clinical data of the sarcoidosis patients. The average age at diagnosis was $52.7 \pm 11.5$ years. Most patients in this cohort (82%) were diagnosed by endobronchial and/or transbronchial biopsies performed via fiberoptic bronchoscopy. In only one patient a surgical lung biopsy was required for diagnosis. The most common symptoms at presentation were cough (60%) and dyspnea (66%) and 77% of the patients had at-least one of these two respiratory symptoms. An additional 12% of patients, who did not have respiratory symptoms, were found to have pulmonary involvement on biopsy or imaging studies. Thoracic lymph nodes were enlarged in 85% of the patients, as noted by imaging. Pulmonary parenchymal involvement was noted in 85% of the patients. Interstitial markings in the lung parenchyma were reported in 47%, and nodular patterns in 47%. The majority of the patients (61%) had the Scadding radiographic score of 2. About half the patients required treatment with systemic corticosteroids during the course of their disease and 17.6% received additional treatment. 8.1% of patients were treated with methotrexate.

### CD14 genotypes in sarcoidosis patients and healthy subjects

Fig. 1 shows the frequency of the different genotypes of CD14 in the sarcoidosis patients and the healthy controls. The distribution of the genotypes was not significantly different between patients with sarcoidosis and controls. However, we noted a trend toward more of the sarcoidosis patients being homozygous for the T allele (genotype TT, 29.7% vs. 20% in controls), and less being homozygous for the CC allele (Genotype CC, 23% vs. 32%).

### sCD14 serum levels

Mean serum levels of sCD14 were $1.9 \pm 0.96$ μg/ml in the sarcoidosis patients, and $1.31 \pm 0.77$ μg/ml in the healthy control group ($p = 0.001$) (Fig. 2). We noted a trend that in the sarcoidosis patients with sCD14 > 1.9 (mean level), the percentage of patients with the CC genotype was 29.4% (10/34) compared to 17.5% only (7/40) in the patients with sCD14 levels below that level ($p = 0.09$). We did not find clear correlation between any specific genotype and sCD14 serum levels in either the patients (Fig. 2b) or healthy control subjects (data not shown). In order to factor in the effect of corticosteroid treatment at the time of sCD14 evaluation, we compared the level of sCD14 between patients that were not treated at all ($n = 24$), and patients that were treated with corticosteroids at any time ($n = 37$). The level of sCD14 was $1.87 \pm 0.15$ without treatment, and $1.82 \pm 0.18$ with corticosteroids (NS). The level of sCD14 in sarcoidosis patients that were never treated was significantly higher than in healthy controls ($p < 0.05$).

### CD14 alleles and clinical phenotype

In order to evaluate the influence of CD14 genotypes, we compared the clinical characteristics of the patients according to different genotypes. Our first impression was that the patients with the CC phenotype had milder disease. This premise was supported by assessing the severity of the disease using the SAC score.[21] As can be seen in Fig. 3, the mean score in the CC homozygous group (patients lacking the T allele, 17/74, 23% of patients) was $2.19 \pm 0.24$. This was significantly different than the mean score in the TT homozygous group ($3.44 \pm 0.31$, $p < 0.05$), and from the mean score in the TC heterozygous group ($3.30 \pm 0.39$, $p < 0.05$). There was no significant difference between the TC and the TT groups. Furthermore, similar to

**Table 1** Demographic and clinical characteristics of the sarcoidosis patients.

| | | % of patients (*n*) |
|---|---|---|
| Age at diagnosis (years) | | 52.7 ± 11.5 |
| Gender | Male | 32.4 (24) |
| | Female | 67.6 (50) |
| Symptoms | Dyspnea | 59.5 (44) |
| | Cough | 66.2 (49) |
| | Any respiratory symptom | 77 (57) |
| | Fever | 16.2 (12) |
| | Weight loss | 16.2 (12) |
| | Arthralgia/arthritis | 25.7 (19) |
| | Ocular symptoms | 4.1 (3) |
| | Skin | 13.5 (10) |
| | Asymptomatic | 14.9 (11) |
| | Others | 12.2 (9) |
| Duration of symptoms prior to diagnosis ($n = 54$) | <1 m | 15 (8) |
| | 1—3 m | 38.9 (21) |
| | 4—6 m | 3.7 (2) |
| | 7—12 m | 20.4 (11) |
| | >12 m | 22.2 (12) |
| Pathological involvement shown (granulomata) | Lung | 83.8 (62) |
| | Thoracic LN | 4.1 (3) |
| | Extra Thoracic LN | 4.1 (3) |
| | Liver | 2.7 (2) |
| | Bone marrow | 1.4 (1) |
| | Skin | 2.7 (2) |
| | Others | 1.4 (1) |
| Extent of disease (Objective and/or subjective) | Pulmonary | 89.2 (66) |
| | Thoracic LN | 87.8 (65) |
| | Extra-thoracic LN | 23 (17) |
| | Parenchymal Disease | 20.3 (15) |
| | Ocular | 14.9 (11) |
| | Joints | 27 (20) |
| | Hypercalcemia/hypercalciuria | 5.4 (4) |
| | Skin | 14.9 (11) |
| | neurologic | 2.7 (2) |
| | Cardiac | 2.7 (2) |
| | Others | 2.7 (2) |
| Radiology findings | Thoracic LN | 85.1 (63) |
| | Interstitial markings | 47.3(35) |
| | Nodular opacities | 47.3 (35) |
| | Cervical/axillary/supra-clavicular LN | 6.8 (5) |
| | Retroperitoneal/mesenteric LN | 10.8 (8) |
| | Hepato-splenic involvement | 20.3 (15) |
| | Unknown | 2.7 (2) |
| Scadding chest radiographic class | 0 | 2.7 (2) |
| | 1 | 13.5 (10) |
| | 2 | 68.9 (51) |
| | 3 | 8.1 (6) |
| | 4 | 6.8 (5) |
| Pulmonary function tests (% of predicted ± SD) | Total lung capacity (TLC) | 92.9 ± 14.5 |
| | Functional residual capacity (FRC) | 92.8 ± 18 |
| | Vital capacity (VC) | 92.4 ± 20.6 |
| | Residual volume (RV) | 106.4 ± 26.3 |
| | Forced expiratory volume 1.0 s (FEV1) | 85.5 ± 21.6 |
| | Forced vital capacity (FVC) | 89 ± 19.7 |
| | FEV1/FVC | 79.1 ± 10.3 |
| | DLCO | 80.4 ± 18.5 |
| Treatment (tx) | Methotrexate | 8.11 (6) |
| | Ever Required Steroids | 48.6 (36) |
| | Treated — not with steroids/cytotoxics | 9.5 (7) |
| | No Tx or unknown | 33.8 (25) |

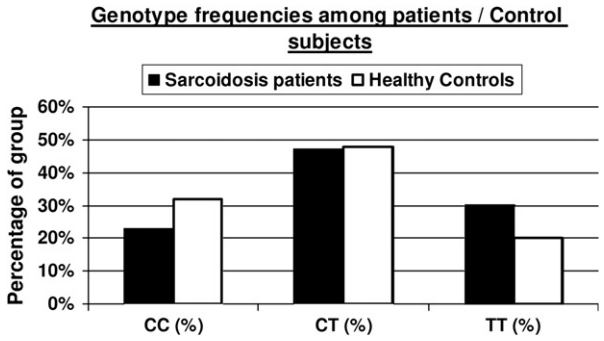


**Figure 1** Polymorphism in CD14 in patients and healthy controls — polymorphism of the CD14 promoter site 159C → T was assessed in 74 sarcoidosis patients and 85 healthy controls by polymerase chain reaction-restriction Fragment Length Polymorphism (PCR-RFLP). The percentage of patients and controls with each of the three possible combinations (CC, CT and TT) was calculated, and the prevalence of each genotype was compared in patients and controls.

the trend in our data, it has been shown that the presence of the T allele (e.g. TC and TT) is associated with increased susceptibility for developing sarcoidosis,[18] and developing Crohn's disease,[13–15] which has some pathophysiological similarities to sarcoidosis. Based on these findings, we evaluated the clinical differences between the CC patient group and the rest of the patients. The SAC score in all non-CC genotypes was $3.39 \pm 0.24$, significantly higher than in the patients with the CC genotype ($p < 0.01$). There was a greater predominance of females (82.4% vs. 63.2%, $p = 0.04$) and the age at diagnosis was significantly higher in the CC group ($57.8 \pm 8.3$ vs. $51.1 \pm 12.9$, $p = 0.006$). CC patients were less likely to present with acute disease. From the patients in whom relevant information prior to diagnosis could be found, 0/15 of CC patients were diagnosed within one month of onset of symptoms vs. 8/39 (21%) in the other genotypes ($p = 0.049$). Pulmonary function tests (PFTs) at diagnosis were significantly better in the CC patients. The greatest difference was found in forced vital capacity ($100.6 \pm 21.6$% of predicted in the CC patients vs. $85.6 \pm 18.1$ % in the other patients: ($p < 0.04$)). Significant differences between CC and other patients were also observed for TLC, and FEV1. The percentage of patients with an abnormality in pulmonary function tests (either Total Lung capacity (TLC) < 80%, Forced Vital Capacity (FVC) < 80% or Forced Expiratory Volume in 1 sec. (FEV1) < 70%) was significantly lower in CC patients (17.6%) as compared to the other patients (42.6%). The major differences between the patients carrying the allele T and those who don't are summarized in Table 2. When comparing patients with the TT genotype to "non-TT" patients, the only significant finding was a lower incidence of ocular involvement with the TT genotype (4.5%) vs. the other patients (19.2%) ($p = 0.001$).

## Discussion

Sarcoidosis is an inflammatory disease in which an aberrant immune response directed against an unknown antigen or infection in a genetically susceptible host is the most likely etiological mechanism of disease.[3,4] In the current study, we found an association between genotype at the polymorphic site of the CD14 proximal promoter at the −159 site, and both disease prevalence and clinical phenotype in an Israeli cohort of sarcoidosis patients. We also found that serum levels of soluble CD14 were significantly higher in patients compared to controls.

The demographic characteristics of our cohort were similar to a previous report of sarcoidosis in Israel in terms of age and gender distribution with a female to male ratio of 2:1.[25] Most of the patients in our cohort were diagnosed by flexible bronchoscopy and lung biopsy, and in the vast majority of cases, the lungs and thoracic lymph nodes were involved in the disease, a finding similar to previous studies.[1,26] As in the previous report from Israel,[25] about half of our patients required treatment with systemic corticosteroids during the course of their disease, a higher rate than reported in other parts of the world.[26,27]

Although the disease entity termed "sarcoidosis" has been known to modern medicine for a very long period of time, little is known about the etiology and pathogenesis of the disease.[2] The pathophysiology of sarcoidosis and that of Crohn's disease share several similarities such as in the significant role for the Th1 like immune response in both diseases.[3,7] A major factor leading to Th1 differentiation is exposure to bacterial pathogens, and specifically, the Gram-negative endotoxin lipopolysaccharide (LPS).[8]

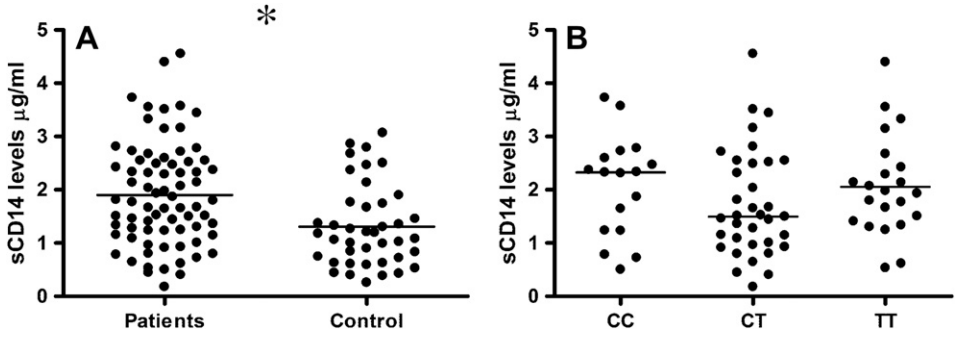


**Figure 2** Soluble CD14 levels —the level of soluble CD14 in the plasma of sarcoidosis patients and healthy controls was evaluated by ELISA. Panel A compares patients with healthy controls (*$p < 0.001$). Panel B shows the different genotypic groups in the Polymorphic CD14 promoter site 159C → T (CC, CT and TT) in patients with sarcoidosis. Each dot represents an individual patient.

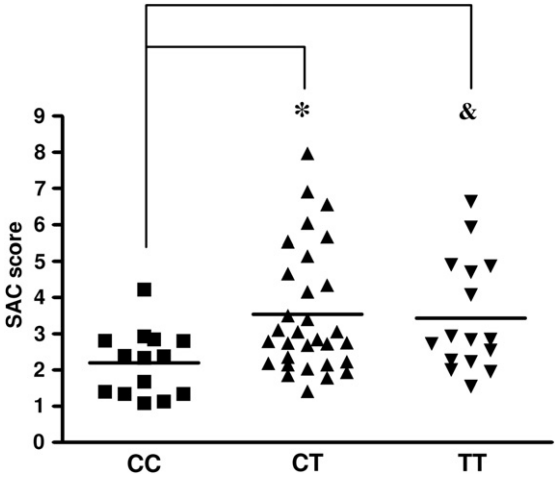


**Figure 3** Polymorphism in CD14 and the SAC score — patients were evaluated for disease severity using the modified sarcoidosis activity (SAC) score. This figure shows the individual scores of the different genotypic groups in the Polymorphic CD14 promoter site 159C → T (CC, CT and TT). Each dot represents an individual patient. (*$p < 0.05$, and $p = 0.07$).

Bacterial pathogens have been implicated in the pathogenesis of Crohn's Disease.[28] An etiological role for bacterial infection in sarcoidosis have been suggested, and several studies have found correlations between sarcoidosis and exposure to different bacteria, including common Gram-negative bacteria such as Moraxella catarhalis and Hemophilus influenza,[19] Mycobacteria,[29] Ricketssial species and propionibacteria.[29,30]

CD14 is the myeloid receptor for LPS and mediates the interaction between the immune system and Gram-negative bacteria, leading to the Th1 like immune response.[8,9] The CD14 −159 C/T polymorphism in the gene promoter has been shown to be relevant in several disease states including atopic diseases and asthma,[11,31] Kawasaki[23] and Crohn's disease.[13–15] Only one recent study, performed in a Greek population, found a correlation between polymorphism at the −159 site and the incidence of sarcoidosis. Similar to the trend we noted, the authors found that the TT genotype is more frequent in sarcoidosis patients compared to healthy controls but they found no correlation between the specific genotypes and disease phenotype.[18] Our results, therefore supports the relevance of the specific CD14 genotype to the prevalence of sarcoidosis, and further show that this genotype affects the clinical phenotype of the disease.

Previous studies of CD14 in sarcoidosis patients have evaluated the presence of the soluble protein in serum and bronchoalveolar fluid in the patients, and the expression of the membrane bound protein on the surface of monocytes derived from these patients.[16,17] In two previously published small cohorts, it has been shown that sCD14 serum levels in sarcoidosis patients are higher than in control subjects.[16,17] Striz et al. showed increased levels of sCD14 in thirteen patients with active disease and not in another nine patients with inactive sarcoidosis.[16] In our study, we did not stratify patients according to disease activity, or based on the use of immunosuppressant medications at the time the blood was drawn. However, we did find significantly higher levels of sCD14 in sarcoidosis patients compared to controls in a much larger patient cohort.

**Table 2** Sarcoidosis patients with CC genotype in the −159C/T polymorphic site of the CD14 gene compared to patients carrying the T allele (TC or TT).

| | | CC genotype % (*n*) | All other patients % (*n*) | *p*-value |
|---|---|---|---|---|
| Age at diagnosis (years) | | 57.8 ± 8.3 | 51.1 ± 12.9 | 0.006 |
| Gender | Male | 17.6% (3) | 36.8% (21) | 0.04 |
| | Female | 82.4% (14) | 63.2% (36) | |
| Symptoms prior to diagnosis < 1 month | | 0 | 22% (8) | 0.048 |
| Skin involvement | | 29.4% (5) | 10.5% (6) | 0.006 |
| Radiological findings | Interstitial markings | 68.7% (11) | 42.1% (24) | 0.02 |
| | Retroperitoneal/mesenteric LN | 0 | 14.5% (8) | 0.09 |
| Scadding chest radiographic class | 0 | 6. 2% (1) | 1.8% (1) | NS |
| | 1 | 0 | 15.8% (9) | |
| | 2 | 81.3% (13) | 66.6% (38) | |
| | 3 | 12.5% (2) | 7% (4) | |
| | 4 | 0 | 8.8 %(5) | |
| Pulmonary function tests at diagnosis (% of predicted ± SD) | Total lung capacity (TLC) | 98.9 ± 14.6 | 91.3 ± 14.2 | 0.04 |
| | Vital capacity (VC) | 104.7 ± 19.8 | 88.5 ± 19.7 | 0.02 |
| | Residual volume (RV) | 102.2 ± 25.1 | 107.3 ± 26.2 | NS |
| | Forced expiratory volume 1.0 s (FEV1) | 95.8 ± 21 | 82.5 ± 20.9 | 0.04 |
| | Forced vital capacity (FVC) | 100.6 ± 21.6 | 85.6 ± 18.1 | 0.04 |
| | DLCO | 82.1 ± 19.6 | 79.8 ± 18.5 | NS |
| Abnormalities in pulmonary function tests | FVC < 80% | 12.5 % (2) | 24.3 % (17) | 0.07 |
| | TLC < 80% | 6.7 % (1) | 26.1 % (12) | 0.09 |
| | FEV1 < 70% | 5.9 % (1) | 9.3 % (5) | NS |
| | Either 1 of the 3 above | 17.6 % (3) | 42.6 % (23) | 0.001 |

Furthermore, we found no difference in the level of sCD14 in sarcoidosis patients that were never treated with corticosteroids compared to those that were treated with corticosteroids at any time (including at the time of blood draw). This result further supports our assessment that corticosteroids do not significantly change the level of sCD14 in sarcoidosis patients. sCD14 could be an acute phase protein that can be seen with active inflammation and may not be specific to sarcoidosis. Parallel evaluation of other acute phase reactants (i.e. CRP or IL-6) would be of value to help clarify whether the increase in sCD14 can be attributed directly to the granulomatous process rather than to non-specific inflammation. Interestingly, the use of corticosteroids has been actually shown to reduce the level of sCD14.[32] The lack of adjustment to therapy may have lead to an underestimation of the magnitude of the difference in sCD14 between sarcoidosis patients and healthy controls. Our data suggest the possibility that there may be a cutoff for sCD14 to distinguish between patients and control subjects, which may be of value in the diagnosis of Sarcoidosis. This possibility should be further evaluated by assessing sCD14 levels in patients with other similar diseases such as tuberculosis and idiopathic pulmonary fibrosis (IPF), and also by stratifying the patients based on activity and the use of medications.

The presence of the T allele in the −159 polymorphism site in the CD14 gene promoter has been previously suggested to be related to higher levels of sCD14.[11,12] In our study, we did not find a clear relationship between any specific genotype and serum levels in both the patients and control groups. It is possible that we could not show a clear association between the genotype and sCD14 levels due to an insufficient size of the cohort as well as due to the ethnic variation in our study group. Another factor could be the fact that we did not match for disease activity or the medications taken at the time of trial recruitment and blood draw. It is possible however, that polymorphism in the CD14 gene does not affect the level of soluble CD14 but does have an effect on the membrane bound form of CD14.

Attempts to correlate genotype with disease phenotype in our patient cohort suggest that differences may exist. We found that patients with a CC genotype may have a milder more indolent form of disease, however such analyses may be confounded by differences in ethnic and geographical variables and much larger multinational analyses are required to definitively evaluate genotype−phenotype correlations.

In conclusion, our study provides several lines of evidence connecting CD14 and sarcoidosis and suggests a role of CD14 in the pathogenesis of the disease. CD14 has an important function in antigen presentation to the cell.[12] It is possible that gene polymorphism affects the process of antigen presentation and immune function in sarcoidosis, although further mechanistic studies are needed in order to establish the specific role of CD14 in the etiology and pathogenesis of sarcoidosis.

## Conflict of interest

The authors have no conflict of interest.

## References


1. Costabel U, Hunninghake GW. ATS/ERS/WASOG statement on sarcoidosis. Sarcoidosis Statement Committee. American Thoracic Society. European Respiratory Society. World Association for Sarcoidosis and Other Granulomatous Disorders. *Eur Respir J* 1999;**14**:735−7.
2. Rybicki BA, Iannuzzi MC, Frederick MM, Thompson BW, Rossman MD, Bresnitz EA, Terrin ML, Moller DR, Barnard J, Baughman RP, DePalo L, Hunninghake G, Johns C, Judson MA, Knatterud GL, McLennan G, Newman LS, Rabin DL, Rose C, Teirstein AS, Weinberger SE, Yeager H, Cherniack R, the ARG. Familial aggregation of sarcoidosis. A case−control etiologic study of sarcoidosis (ACCESS). *Am J Respir Crit Care Med* 2001; **164**:2085−91.
3. Martin II WJ, Iannuzzi MC, Gail DB, Peavy HH. Future directions in sarcoidosis research: summary of an NHLBI working group. *Am J Respir Crit Care Med* 2004;**170**:567−71.
4. Schurmann M, Reichel P, Muller-Myhsok B, Schlaak MAX, Muller-Quernheim J, Schwinger E. Results from a genome-wide search for predisposing genes in sarcoidosis. *Am J Respir Crit Care Med* 2001;**164**:840−6.
5. Pandey JP, Frederick M. TNF-[alpha], IL1-[beta], and immunoglobulin (GM and KM) gene polymorphisms in sarcoidosis. *Human Immunol* 2002;**63**:485−91.
6. Iannuzzi MC, Maliarik MJ, Poisson LM, Rybicki BA. Sarcoidosis susceptibility and resistance HLA-DQB1 alleles in African Americans. *Am J Respir Crit Care Med* 2003;**167**:1225−31.
7. Knoell KA, Hendrix Jr JD, Stoler MH, Patterson JW, Montes CM. Absence of human herpesvirus 8 in sarcoidosis and crohn disease granulomas. *Arch Dermatol* 2005;**141**:909−10.
8. Guha M, Mackman N. LPS induction of gene expression in human monocytes. *Cell Signal* 2001;**13**:85−94.
9. Jacque B, Stephan K, Smirnova I, Kim B, Gilling D, Poltorak A. Mice expressing high levels of soluble CD14 retain LPS in the circulation and are resistant to LPS-induced lethality. *Eur J Immunol* 2006;**36**:3007−16.
10. Tapping R, Tobias P. Soluble CD14-mediated cellular responses to lipopolysaccharide. *Chem Immunol* 2000;**74**:108−21.
11. Baldini M, Carla Lohman I, Halonen M, Erickson RP, Holt PG, Martinez FD. A Polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol* 1999;**20**:976−83.
12. Leung T-F, Tang NLS, Wong GWK, Fok T-F. CD14 and toll-like receptors: potential contribution of genetic factors and mechanisms to inflammation and allergy. *Curr Drug Targets − Inflamm Allergy* 2005;**4**:169−75.
13. Klein W, Tromm A, Griga T, Folwaczny C, Hocke M, Eitner K, Marx M, Duerig N, Epplen JT. Interaction of polymorphisms in the CARD15 and CD14 genes in patients with Crohn disease. *Scand J Gastroenterol* 2003;**38**:834.
14. Gazouli M, Mantzaris G, Kotsinas A, Zacharatos P, Papalambros E, Archimandritis A, Ikonomopoulos J, Gorgoulis V. Association between polymorphisms in the Toll-like receptor 4, CD14, and CARD15/NOD2 and inflammatory bowel disease in the Greek population. *World J Gastroenterol* 2005;**11**:681−5.
15. Klein W, Tromm A, Griga T, Fricke H, Folwaczny C, Hocke M, Eitner K, Marx M, Duerig N, Epplen JT. A polymorphism in the CD14 gene is associated with Crohn disease. *Scand J Gastroenterol* 2002;**37**:189−91.
16. Striz I, Zheng l, Wang Y, Pokorna H, Bauer P, Costabel U. Soluble CD14 is increased in bronchoalveolar lavage of active sarcoidosis and correlates with alveolar macrophage membrane-bound CD14. *Am J Respir Crit Care Med* 1995;**151**: 544−7.

17. Pforte A, Schiessler A, Gais P, Beer B, Ehlers M, Schutt C, Ziegler-Heitbrock H. Expression of CD14 correlates with lung function impairment in pulmonary sarcoidosis. *Chest* 1994;**105**:349—54.
18. Gazouli M, Koundourakis A, Ikonomopoulos J, Gialafos EJ, Rapti A, Gorgoulis VG, Kittas C. CARD15/NOD2, CD14, and toll-like receptor 4 gene polymorphisms in Greek patients with sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2006;**23**:23—9.
19. Kelly DM, Greene CM, Meachery G, O'Mahony M, Gallagher PM, Taggart CC, O'Neill SJ, McElvaney NG. Endotoxin up-regulates interleukin-18: potential role for gram-negative colonization in sarcoidosis. *Am J Respir Crit Care Med* 2005;**172**:1299—307.
20. Temple SEL, Cheong KY, Almeida CM, Price P, Waterer GW. Polymorphisms in lymphotoxin alpha and CD14 genes influence TNF[alpha] production induced by gram-positive and gram-negative bacteria. *Genes Immun* 2003;**4**:283—8.
21. Wasfi YS, Rose CS, Murphy JR, Silveira LJ, Grutters JC, Inoue Y, Judson MA, Maier LA. A new tool to assess sarcoidosis severity. *Chest* 2006;**129**:1234—45.
22. Kochl S, Niederstatter H, Parson W. DNA extraction and quantitation of forensic samples using the phenol-chloroform method and real-time PCR. *Methods Mol Biol* 2005;**297**:13—30.
23. Nishimura S, Zaitsu M, Hara M, Yokota G, Watanabe M, Ueda Y, Imayoshi M, Ishii E, Tasaki H, Hamasaki Y. A polymorphism in the promoter of the CD14 gene (CD14/-159) is associated with the development of coronary artery lesions in patients with Kawasaki disease. *J Pediatrics* 2003;**143**:357—62.
24. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests, European community for steel and coal. Official statement of the European respiratory society. *Eur Respir J Suppl* 1993;**16**:5—40.
25. Yigla M, Badarna-Abu-Ria N, Tov N, Ravell-Weiller D, Rubin A-HE. Sarcoidosis in northern Israel; clinical characteristics of 120 patients. *Sarcoidosis Vasc Diffuse Lung Dis* 2002;**19**:220—6.
26. Baughman RP, Teirstein AS, Judson MA, Rossman MD, Yeager HJ, Bresnitz EA, Depalo L, Hunninghake G, Iannuzzi MC, Johns CJ, McLennan G, Moller DR, Newman LS, Rabin DL, Rose C, Rybicki B, Weinberger SE, Terrin ML, Knatterud GL, Cherniak R, Group, A.C.C.E.S.O.S.R.. Clinical characteristics of patients in a case control study of sarcoidosis. *Am J Respir Crit Care Med*. 2001;**164**:1885—9.
27. Chappell A, Cheung W, Hutchings H. Sarcoidosis: a long-term follow up study. *Sarcoidosis Vasc Diffuse Lung Dis* 2000;**17**: 167—73.
28. Sartor RB. Microbial influences in inflammatory bowel diseases. *Gastroenterology* 2008;**134**:577—94.
29. Ishige I, Usui Y, Takemura T, Eishi Y. Quantitative PCR of mycobacterial and propionibacterial DNA in lymph nodes of Japanese patients with sarcoidosis. *Lancet* 1999;**354**:120—3.
30. Nilsson K, Pyen;hlson C, Lukinius A, Eriksson L, Nilsson L, Lindquist O. Presence of *Rickettsia helvetica* in granulomatous tissue from patients with sarcoidosis. *J Infect Dis* 2002;**185**: 1128—38.
31. Martin AC, Laing IA, Khoo S-K, Zhang G, Rueter K, Teoh L, Taheri S, Hayden CM, Geelhoed GC, Goldblatt J, LeSouef PN. Acute asthma in children: relationships among CD14 and CC16 genotypes, plasma levels, and severity. *Am J Respir Crit Care Med* 2006;**173**:617—22.
32. Nockher WA, Scherberich JE. Expression and release of the monocyte lipopolysaccharide receptor antigen CD14 are suppressed by glucocorticoids in vivo and in vitro. *J Immunol* 1997; **158**:1345—52.