# Modulatory Role of Macrophage Migration Inhibitory Factor on Cytokines and Clinical Features of Sarcoidosis

**Morvarid Elahi**
Wayne State University School of Medicine
**Jaya Talreja**
Wayne State University School of Medicine
**Laura L. Koth**
University of California, San Francisco
**Lobelia Samavati** ( ay6003@wayne.edu )
Wayne State University School of Medicine

**Research Article**

**Keywords:** Sarcoidosis, Macrophages, MIF, IFN γ, IL-6, IL-10, IL-18, Bronchoalveolar lavage, cytokines

**Posted Date:** January 10th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1154059/v2

# Abstract

**Background:** Sarcoidosis is a systemic granulomatous disease of unknown etiology with a significant heterogeneity in organ manifestations, severity, and clinical course. Subjects with sarcoidosis share several features such as, non-necrotizing granuloma, hypergammaglobulinemia, increased local and circulating inflammatory cytokines, such as interleukin (IL)-6, IL-18, and interferon gamma (IFN-γ). Macrophage migration inhibitory factor (MIF) is a pluripotent chemokine produced by various cell types. The expression of MIF at sites of inflammation suggests a regulatory role in the function of macrophages. The objective of this study was to investigate the role of MIF in the serum and bronchoalveolar lavage (BAL) fluid of sarcoidosis patients in association with clinical features and other cytokines.

**Methods:** Sera and BALs of sarcoidosis patients (n=55) were collected at the time of diagnosis and patients were followed longitudinally for 3 years. Additionally, fifteen healthy controls participated in the study. The medical records of all patients including, demographics, radiography stages, pulmonary function tests, and organ involvements were recorded. The levels of MIF, IL-18, IL-10, IL-6, IFN-γ and lysozyme in serum and BAL samples were measured by ELISA. Statistical analyses were performed using SPSS software.

**Results:** Serum MIF had a remarkable positive correlation with IL-10 and IFN-γ but had a negative correlation with serum IgG levels. Importantly, longitudinal follow-up showed a positive correlation between MIF and % predicted diffusion capacity (%DLCO) at 3-year. Serum IL-18 had a significant positive correlation with serum lysozyme, but a negative correlation with % predicted total lung capacity at 3-year follow up. We identified two groups of sarcoidosis subjects with distinct clinical and cytokine features. A group with prominent extrapulmonary involvement, and low serum MIF, IL-10 and IFN-γ levels and a group with elevated serum MIF, IL-10 and IFN-γ levels. Moreover, we found a negative correlation between BAL IL-18 and BAL MIF in sarcoidosis subjects.

**Conclusions:** Patients with low serum MIF, IL-10 and IFN-γ levels has severe and mostly extrapulmonary sarcoidosis with elevated lysozyme and IL-18 levels. Our work provides understanding of phenotypic diversity in association with heterogeneity in cytokine landscape in sarcoidosis.


# Background

Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine produced by various cell types, including macrophages, neutrophils, lymphocytes, endothelial and epithelial cells. Additionally, MIF is released by the anterior pituitary and adrenal gland and functions as a hormone[1, 2]. Initially, MIF was identified as lymphokines released by lymphocytes to inhibit the migration of macrophages and acquired its name owing to this function. MIF interacts with its membrane receptor, CD74, in association with CD44 to modulate macrophage function [3]. MIF acts as a proinflammatory cytokine but also has chaperon like functions and exhibits a thiol-protein oxidoreductase activity[4]. MIF acts also as a non-

cognate ligand for the chemokine receptors, CXCR2, CXCR4, and CXCR7[2, 5]. Therefore, MIF has a regulatory role for both innate and adaptive immune systems. MIF has a dichotomous action on immunity; MIF can act as a pro-inflammatory factor in response to stressors to potentiate of inflammatory signaling [6]. Others reported a protective role of MIF in the immunity against pathogens [1, 7-9]. For instance, MIF-deficient mice exhibit higher burden of mycobacterial tuberculosis bacteria and succumb to earlier death [10].

Sarcoidosis is a systemic granulomatosis disease of unknown etiology with variable clinical presentation and prognosis [11, 12]. The granulomatous inflammation in sarcoidosis has been linked to enhanced T helper (Th1) function, B cells as well as macrophage activation [12, 13]. Hypergammaglobulinemia is a frequent finding in sarcoidosis that may suggest active humoral immunity to unknown antigen(s). Studies indicate that B cells and increased IgG levels may play a role in the development of anergy or autoimmunity [14]. Because MIF acts in crosstalk between T and B lymphocytes, monocytes and macrophages[15], investigating the role of MIF as a potential biomarker in sarcoidosis may prove to be beneficial.

In this work, we investigated the role of MIF protein levels as well as several important cytokines in association with sarcoidosis clinical features. Specifically, we compared levels of MIF in the lung compartment (BAL) and peripheral compartment (serum) to sarcoidosis clinical phenotypes. Furthermore, we investigated if MIF levels were correlated with the levels of lysozyme, IL-10, IL-18, and interferon gamma (IFN-γ). We found that there were two distinct classes of sarcoidosis patients: 1) a group with high levels of MIF, and 2) a group with low MIF levels. The high MIF group was associated with elevated IL-10 and IFN-γ, whereas the low MIF group was associated with elevated levels of lysozyme and IL-18 and increased level of IgG.

## Materials And Methods

# Study Design:

The Committee for Investigations Involving Human Subjects at Wayne State University approved the protocol (IRB# 019111M1E) for obtaining alveolar macrophages by bronchoalveolar lavage (BAL) and blood by phlebotomy from control subjects and patients with sarcoidosis. Sarcoidosis diagnosis was based on the ATS/ERS/WASOG statement [16]. The enrollment criteria were: (i) histologic demonstration of non-caseating granulomas with compatible clinical/radiographic features consistent with sarcoidosis, and (ii) exclusion of diseases capable of producing a similar clinical picture, such as fungus, mycobacteria. Exclusion criteria: (i) receiving immune suppressive medication (corticosteroid and/or other immune modulatory agents), (ii) had positive microbial culture in routine laboratory examinations or viral infection; or (iii) had known hepatitis or HIV infections or any immune suppressive condition. The criteria for enrollment in the control group were: (i) absence of chronic respiratory diseases (ii) absence of HIV or hepatitis infection. A total of 55 patients with sarcoidosis and 15 healthy controls participated in

this study. The medical records of all patients were reviewed, and demographics, radiography stages, pulmonary function tests, and organ involvements were recorded as previously described[11].

### Pulmonary Function Test

PFTs were performed in patients following American Thoracic Society guidelines in a licensed laboratory[17]. All spirometry studies were completed using a calibrated pneumotachograph and lung volumes were measured in a whole-body plethysmograph (Jaeger Spirometry and Sensor Medics Vmax 22; VIASYS Respiratory Care, Inc., Yorba Linda, CA, USA) according to guideline [18]. The initial PFT's were performed at the first attendance in the clinic. The PFTs completed in 3 years later were considered as the follow-up PFTs.

### BAL collection

BAL was collected during bronchoscopy after administration of local anesthesia and before tissue biopsies, as previously described [11-13]. Briefly, a total of 200 mL of sterile saline solution was injected via fiberoptic bronchoscopy; the BAL fluid was retrieved, measured, and centrifuged. Samples were aliquoted and stored in -80º C freezer until use.

*Enzyme- Linked Immunosorbent Assay (ELISA*): The levels of MIF, IL-18, IL-10, IL-6 and IFN-γ lysozyme were measured by ELISA according to the manufacturer's instructions (ELISA Duo Kits; R&D Systems, Minneapolis, MN) as previously described [13].

### Measurement of Immunoglobulin

Immunoglobulin measurement and subclassification performed in the diagnostic laboratory at Detroit Medical Center according to the standard procedure. Data were extracted from electronic medical records.

### Statistical Analyses

Statistical analyses were performed using SPSS software, version 27.0 (SPSS Inc., Chicago, IL). ELISA results were expressed as mean ± SEM. For all analyses, two-tailed p values of less than 0.05 were considered to be significant. A Pearson correlation test was applied to identify any association between the cytokines and mediators found to be altered in sarcoidosis patients, as compared with healthy controls. A p value of <0.05 was considered statistically significant.

# Results

**Serum MIF, IL-6, IL-18 and IFN-γ levels in sarcoidosis patients.** We enrolled 70 subjects in this study comprising 55 sarcoidosis patients and 15 heathy subjects. Diagnosis of sarcoidosis was established based on American Thoracic Society criteria and exclusion of infection by examining samples for bacterial and viral infection[16]. All sarcoidosis subjects were ambulatory patients and sera were

collected during first clinical encounter, when they were on no prior treatment. Clinical characteristics of the study subjects are demonstrated in Table 1.

Table 1
Subject Demographics

| Characteristic | Patient Subjects n=55 | Control Subjects n=15 | p-value |
|---|---|---|---|
| **Age, Years (mean ± SD)** | 48 ± 11 | 40 ± 10 | 0.47 |
| **Sex (female)** | 34 (61) | 10 (66) | 0.91 |
| **BMI, $kg.m^{-2}$ (mean ± SD)** | 30 ± 7 | 27 ± 5 | 0.38 |
| **Race (n, %)** | | | |
| African - American | 49 (89) | 12 (80) | 0.52 |
| White | 3 (5) | 3(20) | 0.31 |
| Other | 3 (5) | 0 | 0.08 |
| **Smoker (PY)(mean ± SD)** | 5 ± 8 | 0 | **0.00** |
| **Organ Involvements (n, %)** | | | |
| Pulmonary Involvement | 54 (98) | NA | – |
| Extra Pulmonary Involvement | 42 (76) | NA | – |
| Skin | 28 (50) | NA | – |
| Eye | 24 (43) | NA | – |
| Heart | 5 (9) | NA | – |
| CNS [a] | 4 (7) | NA | – |
| Other | 20 (36) | NA | – |
| **Initial Chest Radiograph Stages (n, %)** | | | |
| Stage 0 | 2 (3) | NA | – |
| Stage 1 | 9 (16) | NA | – |
| Stage 2 | 31 (56) | NA | – |
| Stage 3 | 6 (10) | NA | – |
| Stage 4 | 4 (7) | NA | – |
| **Initial PFT [b], Mean (IQR [c])** | | | |
| FVC [d] (% predicted) | 86 (44 - 123) | NA | – |
| FEV1 [e] (% predicted) | 80 (27 - 121) | NA | – |

| Characteristic | Patient Subjects n=55 | Control Subjects n=15 | p-value |
|---|---|---|---|
| FEV1/FVC (% predicted) | 74 (36 - 89) | NA | – |
| TLC [f] (% predicted) | 80 (37 - 116) | NA | – |
| DL,CO [g] (% predicted) | 65 (27 - 99) | NA | – |

[a] Central nerve system, [b] Pulmonary function test, [c] Interquartile range, [d] Forced vital capacity, [e] Forced expiratory volume, [f] Total lung capacity, [g] Diffusing capacity of the lung for carbon monoxide

Production of cytokines is a well-regulated host defense mechanism against pathogens that is modulated by cellular cues and the cytokine milieu. To understand the regulation of group of cytokines implicated in sarcoidosis, we assessed the levels of IL-6, IL-18, IFN-γ and MIF in the serum samples of sarcoidosis patients (n=55) and healthy controls (n=15) via ELISA. IL-6 is a pro-inflammatory cytokine and has been suggested to play a role in the pathogenesis of sarcoidosis [19]. IL-18 belongs to IL-1 family of cytokines produced by monocytes and macrophages that has been shown to be increased in sarcoidosis patients [20, 21]. Among 55 sarcoidosis subjects, only 10 patients show measurable IL-6 levels, and there was no significant difference (p= 0.47) in the levels of IL-6 between patients and healthy controls (Figure 1A). In contrast, IL-18 was detectable in the sera from all participants (Figure 1B). Sarcoidosis patients expressed significantly higher levels of IL-18 (mean± SD: 274±202 pg/mL) as compared to healthy controls (53±69 pg/mL). IFN-γ levels were also higher (71±145 pg/mL) in sarcoidosis as compared to the healthy group (18±40 pg/mL) (Figure 1C). Similarly, we assessed the MIF in the serum of our study subjects. Surprisingly, all healthy subjects had detectable MIF levels (237±219 pg/mL). The Figure 1D shows that the sarcoidosis patients have significantly higher levels of MIF. The mean values of MIF levels in sarcoidosis patients were 519±809 pg/mL. As the Box plot indicates, there were a wide variation among sarcoidosis patients in regard to MIF levels (Fig. 1D). In fact, MIF was not measurable in a large number of patients or if it was detected, the levels were below the MIF mean values of healthy subjects. In contrast, some patients exhibited very high MIF levels. To further gain insight into the potential clinical value of MIF, we classified our sarcoidosis cohort based on MIF values into two groups and performed subgroup analysis. The mean serum MIF-value in the healthy group (237pg/mL), therefore, this value was selected as the cut off value. The serum MIF values of less than 237pg/mL were classified as low serum MIF-group, whereas MIF higher than 237pg/mL were classified as high MIF-group (Figure 2A). First, we analyzed the characteristics of these two groups in terms of clinical features and other clinical biomarkers. Clinical characteristics of patients stratified by low and high serum MIF levels are provided in Table 2. We found that the low serum MIF group was characterized by greater multiorgan involvements including, cardiac (p=0.05) and CNS (p=0.09) as compared to the high MIF group. Interestingly, patients with extensive skin disease exhibited elevated serum MIF levels (high MIF group).

Table 2
Clinical Characteristics Based on Serum MIF Level

| Clinical Characteristic | Low MIF Serum n=32(58%) | High MIF Serum n=23(42%) | p-value |
|---|---|---|---|
| **Smoker (PY)(mean ± SD)** | 4 ± 8 | 2.5 ± 4 | 0.32 |
| **Serum Indicator (mean ± SD)** | | | |
| MIF [a] (pg/mL) | 90 ± 81 | 1218 ± 1058 | **0.001** |
| Interferon-γ (pg/mL) | 23 ± 21 | 135 ± 36 | **0.01** |
| Interleukin-10 (pg/mL) | 0 | 833 ± 1494 | **0.03** |
| Interleukin-18 (pg/mL) | 264 ± 206 | 290 ± 200 | 0.90 |
| Lysozyme (pg/mL) | 25 ± 1 | 21 ± 1 | **0.03** |
| CRP [b] (mg/L) | 22 ± 10 | 9 ± 2 | 0.22 |
| IgG [c] (mg/dL) | 1613 ± 575 | 999 ± 468 | **0.03** |
| **Organ Involvements (n, %)** | | | |
| Pulmonary Involvement | 32 (100) | 22 (95) | 0.20 |
| Extra Pulmonary Involvement | 26 (81) | 12 (52) | **0.05** |
| Skin | 18 (56) | 10 (43) | 0.30 |
| Eye | 15 (46) | 9 (39) | 0.71 |
| Heart | 5 (15) | 0 | **0.05** |
| CNS [d] | 4 (12) | 0 | 0.09 |
| Other | 12 (37) | 8 (34) | 0.47 |
| **Initial PFT [e] (mean, IQR [f])** | | | |
| FVC [g] (% predicted) | 81 (60 - 105) | 82 (44 - 102) | 0.70 |
| FEV1 [h] (% predicted) | 73 (27 - 106) | 72 (44 - 98) | 0.91 |
| FEV1/FVC (% predicted) | 67 (36 - 85) | 71 (43 - 88) | 0.24 |
| TLC [K] (% predicted) | 81 (54 - 96) | 79 (37 - 102) | 0.98 |
| DLCO [s] (% predicted) | 57 (50 - 71) | 64 (34 - 97) | 0.40 |
| **Follow Up PFT (mean, IQR)** | | | |

| Clinical Characteristic | Low MIF Serum n=32(58%) | High MIF Serum n=23(42%) | p-value |
|---|---|---|---|
| FVC (% predicted) | 92 (37 - 123) | 95 (51 - 134) | 0.72 |
| FEV1 (% predicted) | 82 (35 - 112) | 82 (31 - 135) | 0.95 |
| FEV1/FVC (% predicted) | 74 (36 - 86) | 67 (39 - 80) | 0.11 |
| TLC (% predicted) | 82 (45 - 114) | 86 (73 - 107) | 0.55 |
| DLCO (% predicted) | 62 (31 - 94) | 71 (37 - 103) | 0.16 |

[a] Macrophage inhibitory factor, [b] C-Reactive protein, [c] Immunoglobulin G, [d] Central nerve system, [e] Pulmonary function test, [f] Interquartile range, [g] Forced vital capacity, [h] Forced expiratory volume, [K] Total lung capacity, [s] Diffusing capacity of lung for carbon monoxide

**Sarcoidosis patients with low MIF levels exhibit elevated lysozymes but decreased IFN-γ and IL-10**. MIF has important immunomodulatory functions, both protective and detrimental roles, in the innate and adaptive immune system[2, 8, 15]. To understand the relationship between low and high levels of MIF with levels of cytokines and other parameters, we examined levels of CRP, lysozyme stratified based on MIF levels. We observed a trend in the low MIF group to have an elevated CRP (Figure 2B), but the difference did not reach statistical significance. Assessment of serum lysozyme is routinely used for evaluation of sarcoidosis [22, 23] and it is considered to be a marker for macrophage and monocytic activation [24]. Similarly, hypergammaglobulinemia is a feature of sarcoidosis and elevated levels of IgG has been reported [25]. Classification based on MIF values showed that patients with low MIF levels exhibit higher lysozyme levels (p=0.03) (Figure 2C) and elevated IgG levels (p=0.03) (Figure 2D). IFN-γ is a pleotropic cytokine modulating both the innate and adaptive immune response against pathogens and plays a role in sarcoidosis [26]. We found that higher IFN-γ levels were almost exclusively observed in the high MIF group compared to the low MIF group (p=0.01) (Figure 2E). Next, we examined the levels of IL-10 stratified by MIF levels. IL-10 has potent anti-inflammatory activities by suppressing the granuloma formation [27]. Figure 2F shows a significant difference (p=0.03) between two MIF group in terms of IL-10 levels.

**Correlation of serum cytokines, lysozyme and IgG.** To gain additional insights in the pathophysiological roles of measured mediators and their correlation, we performed a two-tailed Pearson correlation (Table 3). We found no significant correlation between serum MIF and IL-18. In contrast, there were a significant correlation between MIF and IFN-γ (r=0.57; p=0.001) (Figure 3A). Surprisingly, we found that MIF values highly correlates with IL-10 values (r=0.82; p value< 0.001) (Figure 2B). Similarly, we determined the correlation between serum immunoglobulin G (IgG) and MIF and found that serum MIF negatively correlates (r=-0.55, p=0.01) with IgG levels (Figure 3C). Serum lysozyme significantly positively correlated to IL-18 (r=0.59, p=0.001) but negatively to IFN-γ (r=-0.3; p=0.03) (Figure 3D and 3E). Although serum lysozyme and MIF correlated negatively but did not reach statistical significance (p=0.16). There was a significant correlation between IFN-γ and IL-10 (r=0.57; p<0.001).

Table 3
Serum Cytokines and Biomarkers Correlations

| | | MIF | IFN-γ | IL-10 | IL-18 | Lysozyme | CRP | IgG |
|---|---|---|---|---|---|---|---|---|
| **MIF** | Correlation | 1 | **0.57**** | **0.82**** | 0.05 | -0.20 | -0.17 | **-0.55**** |
| | Sig. | | **0.001** | **<0.001** | 0.71 | 0.16 | 0.31 | **0.01** |
| **IFN-γ** | Correlation | **0.57**** | 1 | **0.57**** | -0.21 | **-0.30*** | -0.14 | -0.43 |
| | Sig. | **0.001** | | **<0.001** | 0.15 | **0.03** | 0.39 | 0.07 |
| **IL-10** | Correlation | **0.82**** | **0.57**** | 1 | -0.08 | -0.21 | 0.35 | **-0.62*** |
| | Sig. | **<0.001** | **<0.001** | | 0.60 | 0.21 | 0.23 | **0.03** |
| **IL-18** | Correlation | 0.05 | -0.21 | -0.08 | 1 | **0.59**** | **0.40*** | 0.40 |
| | Sig. | 0.71 | 0.15 | 0.60 | | **0.001** | **0.01** | 0.07 |
| **Lysozyme** | Correlation | -0.20 | **-0.30*** | -0.21 | **0.59**** | 1 | **0.32*** | 0.14 |
| | Sig. | 0.16 | **0.03** | 0.21 | **0.001** | | **0.05** | 0.54 |
| **CRP** | Correlation | -0.17 | -0.14 | 0.35 | **0.40*** | **0.32*** | 1 | 0.30 |
| | Sig. | 0.31 | 0.39 | 0.23 | **0.01** | **0.05** | | 0.23 |
| **IgG** | Correlation | **-0.55**** | -0.43 | **-0.62*** | 0.40 | 0.14 | 0.30 | 1 |
| | Sig. | **0.01** | 0.07 | **0.03** | 0.07 | 0.54 | 0.23 | |

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

**Correlation of serum MIF and IL-18 with %TLC and %DLCO.** Longitudinal measurements of lung function, such as % predicted total lung capacity (%TLC) and diffusion capacity of carbon monoxide (%DLCO) provide invaluable information about the progression of pulmonary sarcoidosis [28]. We evaluated the relationship of cytokine levels with PFT values: 1) PFTs at the time of sarcoidosis diagnosis and 2) PFTs obtained after 3 years follow-up. We found no correlation between the cytokines and first PFT values. Next, we evaluated Pearson correlation using %TLC values after 3 years follow up. Figure 4A shows that there is a positive, but not significant association between MIF and %TLC (r=0.14, p=0.45). However, MIF shows a significant correlation with %DLCO (r=0.4, p=0.02) (Figure 4B). There was a negative correlation of IL-18 with %TLC (r=-0.36, p=0.03) (Figure 4C). In contrast, IL-18 value tended to have negative association with %DLCO (r=-0.12) but lack the statistical significance (p=0.48) (Figure 4D). None of other markers, including lysozyme, CRP showed any correlation with PFT values.

**MIF, IL-18, IFN-γ, IL-6 and IL-10 levels in BAL of sarcoidosis patients.** Sarcoidosis is a systemic disease with prominent lung involvement, but the degree of lung involvement and its progression varies among

patients. It has been recognized that serum cytokine level may not mirror the cytokine values of the lungs or other tissues [29]. Next, we asked if these cytokines can be measured in BAL samples. All BAL and serum samples were collected, when the first diagnosis of sarcoidosis was established, and none were receiving any immune modulatory agents. The levels of MIF, IL-18, IFN-γ, IL-6 and IL-10 were measured in the BAL samples. About two third of patients had a detectable MIF in their BAL-samples with the mean value of 822±901 pg/mL. Half of patients had a detectable IL-18 with the mean value of and 17±21 pg/mL. IFN-γ was measurable in only one BAL sample (534 pg/mL). Similarly, IL-6 was detectable only in three BAL samples with the mean value of 3.2 pg/mL. In contrast, none of patients expressed IL-10 in their BALs. Figure 5A shows the MIF values in BALs corresponding to their serum MIF values. The average of BAL MIF levels is significantly greater than the serum MIF level ($p<0.001$) (Figure 4A). In contrast, the average of serum IL-18 levels was significantly greater than the BAL IL-18 values ($p<0.001$) (Figure 4B). We found a negative correlation between MIF and IL-18 levels in BAL samples ($r=-0.35$, $p=0.02$) (Figure 4C).

# Discussion

The immunopathogenesis of sarcoidosis remains poorly understood. Evidence suggests that exposure to unknown antigen(s) or environmental factors may lead to activation of macrophages, T and B cells [12, 13, 25, 29]. Hypergammaglobulinemia is a frequent finding in sarcoidosis suggesting active humoral immunity [14, 25]. Although some sarcoidosis clinical features, such as erythema nodosum, are associated with better prognosis [30]. However, it is not clear which cytokine pattern is associated with better clinical outcome. Therefore, cytokine immunophenotyping in association with clinical phenotype may provide some insight in the immunological characteristics and it could improve therapeutic targeting.

Here, we found that there was significant heterogeneity among sarcoidosis patients with respect to serum cytokine profile, including IFN-γ, IL-18, IL-10 and MIF. Serum IL-18 levels were measurable in almost all sarcoidosis patients. In contrast, IL-6 was measurable only in 18% of sarcoidosis patients. There were significant correlations between IL-18 and lysozyme levels (Table 3). Moreover, we found a significant negative correlation of IL-18 with % predicted TLC in follow up. We also measured cytokine levels in BALs obtained from the same patients at the time of diagnosis. In 2/3 of patients, MIF was measurable in the BAL samples. IL-18 was measurable in approximately half of the patients. We found a negative correlation between BAL IL-18 and MIF levels. Our data corroborates with previous data showing detectable IL-18 BAL levels of sarcoidosis subjects [20]. There was no significant correlation between BAL values and serum levels of MIF and IL-18.

MIF is a pleiotropic protein mediating its action through binding to receptor CD7 regulating innate and adoptive immunity[1, 15, 31]. The role of MIF in inflammatory disorders is diverse and it appears to be context dependent. Early investigations demonstrated that MIF plays a role in many forms of inflammatory diseases[15]. However, recent studies highlighted a protective role of MIF against oxidative stress-mediated DNA damage as well as various infection[1, 7, 8, 10]. Based on recent experimental

evidence, MIF may play a beneficial role by protecting from tissue senescence and damage in the lungs[32, 33]. The role of MIF in sarcoidosis is poorly elucidated. We investigated if MIF levels can explain, at least in part, the phenotypic diversity in sarcoidosis patients. We identified a group of sarcoidosis patients with lower MIF levels, whereas another group of patients exhibited significantly higher serum MIF levels. Interestingly, subjects with low MIF levels tended to have more systemic and extra-pulmonary involvements and B symptoms such as weight loss, fatigue, night sweat, elevated lysozyme and IgG levels but lower IFN-γ levels. Here, we found a significant correlation between the MIF and IFN-γ ($r=0.57$; $p=0.001$) and IL-10 ($r=0.82$; $p<0.001$) levels. There was a significant negative correlation between serum MIF and IgG levels ($r=-0.55$). In contrast, patients with high MIF levels had higher serum IFN-γ and IL-10 levels. Our data indicate that elevated MIF levels correlate with improved follow-up % predicted DLCO. Previous reports have indicated that MIF and activation of CD74 are important in reducing tissue injury and promoting tissue repair [1, 32, 34]. Increased IFN-γ have been reported in sarcoidosis patients [13, 26]. IFN-γ could have protective and/or detrimental effects in the course of sarcoidosis [26]. IFN-γ orchestrates numerous protective functions in wound healing and repair [35]. IFN-γ has immunomodulatory effects through enhancing antigen processing and presentation, increasing leukocyte trafficking, boosting anti-microbial functions, and affecting cellular proliferation and apoptosis [35, 36]. One previous study showed that elevated serum IFN-γ in sarcoidosis patients is associated with better lung function [26]. As MIF highly correlates with serum IFN-γ and IL-10 values, it is not clear whether the protective effects of MIF are direct or through IFN-γ and IL-10. It appears that IFN-γ and MIF can mutually regulate each other and synergistically modulate cell effector function [31, 37]. Recent studies identified a critical role of MIF in pathogen clearance in Th1 mediated (mycobacterial tuberculosis)[10] and Th2 mediated parasitic infection [31]. Interestingly, it has been shown that coactivation of IL-10 and IFN-γ downregulates the activation of antigen presenting cells and controls the pro-inflammatory responses via induction of regulatory T-cells (Treg) [38, 39]. It has been shown that MIF promotes AKT, ERK activation and alveolar repair by stimulating alveolar epithelial cell proliferation [33]. ERK activation is mechanistically linked to upregulation of IL-10 in dendritic cells and Treg [40]. It is possible that MIF enhanced ERK activation, promotes IL-10 production. Because previously, we have shown that activation of p38 MAPK but not ERK play a critical role in sarcoidosis inflammatory responses[11, 12]. The MIF effect on ERK upregulation may in turn interferes with the activation of p38 MAPK. This notion is supported by Fallica, et.al. experimental data that MIF downregulates ASK mediated p38 activation in response to cigarette smoke[41]. As our current data in sarcoidosis indicate that MIF levels are positively associated with IFN-γ and IL-10, but negatively associated with IgG level. We speculate that elevated MIF improves antigen clearance and suppresses B cell function. Our results suggest that MIF and IFN-γ synergistically enhance IL-10 production that further modulates the antigen clearance and suppresses B-cell responses. Further experimental studies need to elucidate the MIF role in IL-10 and IFN-γ as well as B cell function in sarcoidosis.

Conclusions: We identified two subgroups of sarcoidosis patients: one group with higher MIF as compared to healthy controls and another group with undetectable levels of MIF. The last subgroup has severe and mostly extrapulmonary sarcoidosis with elevated lysozyme and IL-18 levels. Here, we advance

our knowledge by identifying MIF as a systemic modulator of cytokine spectrum and clinical phenotype in sarcoidosis.

# Abbreviations

**MIF: Macrophage migration inhibitory factor**

**IL: Interleukin**

**IFN-γ: Interferon gamma**

**Ig: Immunoglobulin**

**ELISA: Enzyme- Linked Immunosorbent Assay**

**BAL: Bronchoalveolar lavage**

**CRP: C-reactive protein**

**DLCO: Diffusion capacity of carbon monoxide**

**TLC: Total lung capacity**

**PFT: Pulmonary Function Test**

**ERK: Extracellular signal-regulated kinase**

**MAPK: Mitogen-activated protein kinase**

# Declarations

**Ethics approval and consent to participate:** Written informed consent was obtained from all participants, and the study was approved by the Wayne State University, Institutional Review Board (IRB). The IRB number of the study is 019111M1E.

**Consent for publication:** Not applicable.

**Competing financial interests:** None of the authors of this manuscript had any financial relationship with a commercial company.

**Funding:** This work was supported by a grant R01HL113508 (LS).

**Author contributions:** ME conducted the analysis, collection of samples and the clinical data and drafted the manuscript. JT contributed to writing the manuscript. LK contributed to writing the manuscript. LS

conceived and designed the study, participated in all areas of the research and oversaw data analysis and writing of the manuscript.

# References


1. Farr L, Ghosh S, Moonah S. Role of MIF Cytokine/CD74 receptor pathway in protecting against injury and promoting repair. *Frontiers in Immunology* 2020: 11.
2. Günther S, Fagone P, Jalce G, Atanasov AG, Guignabert C, Nicoletti F. Role of MIF and D-DT in immune-inflammatory, autoimmune, and chronic respiratory diseases: From pathogenic factors to therapeutic targets. *Drug discovery today* 2019: 24(2): 428-439.
3. Sanchez-Niño MD, Sanz AB, Ihalmo P, Lassila M, Holthofer H, Mezzano S, Aros C, Groop P-H, Saleem MA, Mathieson PW. The MIF receptor CD74 in diabetic podocyte injury. *Journal of the American Society of Nephrology* 2009: 20(2): 353-362.
4. Kleemann R, Kapurniotu A, Frank RW, Gessner A, Mischke R, Flieger O, Jüttner S, Brunner H, Bernhagen J. Disulfide analysis reveals a role for macrophage migration inhibitory factor (MIF) as thiol-protein oxidoreductase. *Journal of molecular biology* 1998: 280(1): 85-102.
5. Tillmann S, Bernhagen JD, Noels H. Arrest functions of the MIF ligand/receptor axes in atherogenesis. *Frontiers in immunology* 2013: 4: 115.
6. Ningyan G, Xu Y, Hongfei S, Jingjing C, Min C. The role of macrophage migration inhibitory factor in mast cell-stimulated fibroblast proliferation and collagen production. *PLoS One* 2015: 10(3): e0122482.
7. Koebernick H, Grode L, David JR, Rohde W, Rolph MS, Mittrücker H-W, Kaufmann SH. Macrophage migration inhibitory factor (MIF) plays a pivotal role in immunity against Salmonella typhimurium. *Proceedings of the National Academy of Sciences* 2002: 99(21): 13681-13686.
8. Stoppe C, Averdunk L, Goetzenich A, Soppert J, Marlier A, Kraemer S, Vieten J, Coburn M, Kowark A, Kim B-S. The protective role of macrophage migration inhibitory factor in acute kidney injury after cardiac surgery. *Science translational medicine* 2018: 10(441).
9. Roger T, Schneider A, Weier M, Sweep FC, Le Roy D, Bernhagen J, Calandra T, Giannoni E. High expression levels of macrophage migration inhibitory factor sustain the innate immune responses of neonates. *Proceedings of the National Academy of Sciences* 2016: 113(8): E997-E1005.
10. Das R, Koo M-S, Kim BH, Jacob ST, Subbian S, Yao J, Leng L, Levy R, Murchison C, Burman WJ. Macrophage migration inhibitory factor (MIF) is a critical mediator of the innate immune response to Mycobacterium tuberculosis. *Proceedings of the National Academy of Sciences* 2013: 110(32): E2997-E3006.
11. Rastogi R, Du W, Ju D, Pirockinaite G, Liu Y, Nunez G, Samavati L. Dysregulation of p38 and MKP-1 in response to NOD1/TLR4 stimulation in sarcoid bronchoalveolar cells. *American journal of respiratory and critical care medicine* 2011: 183(4): 500-510.

12. Talreja J, Talwar H, Ahmad N, Rastogi R, Samavati L. Dual Inhibition of Rip2 and IRAK1/4 Regulates IL-1beta and IL-6 in Sarcoidosis Alveolar Macrophages and Peripheral Blood Mononuclear Cells. *J Immunol* 2016: 197(4): 1368-1378.
13. Talreja J, Talwar H, Bauerfeld C, Grossman LI, Zhang K, Tranchida P, Samavati L. HIF-1alpha regulates IL-1beta and IL-17 in sarcoidosis. *Elife* 2019: 8.
14. Tobón GJ, Izquierdo JH, Cañas CA. B lymphocytes: development, tolerance, and their role in autoimmunity—focus on systemic lupus erythematosus. *Autoimmune diseases* 2013: 2013.
15. Calandra T, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. *Nature reviews immunology* 2003: 3(10): 791-800.
16. 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(4): 735-737.
17. Bourbonnais JM, Samavati L. Clinical predictors of pulmonary hypertension in sarcoidosis. *Eur Respir J* 2008: 32(2): 296-302.
18. Culver BH, Graham BL, Coates AL, Wanger J, Berry CE, Clarke PK, Hallstrand TS, Hankinson JL, Kaminsky DA, MacIntyre NR. Recommendations for a standardized pulmonary function report. An official American Thoracic Society technical statement. *American journal of respiratory and critical care medicine* 2017: 196(11): 1463-1472.
19. Bihl MP, Laule-Kilian K, Bubendorf L, Rutherford RM, Baty F, Kehren J, Eryüksel E, Staedtler F, Yang JQ, Goulet S. Progressive pulmonary sarcoidosis--a fibroproliferative process potentially triggered by EGR-1 and IL-6. *Sarcoidosis, vasculitis, and diffuse lung diseases: official journal of WASOG* 2006: 23(1): 38-50.
20. Shigehara K, Shijubo N, Ohmichi M, Yamada G, Takahashi R, Okamura H, Kurimoto M, Hiraga Y, Tatsuno T, Abe S. Increased levels of interleukin-18 in patients with pulmonary sarcoidosis. *American journal of respiratory and critical care medicine* 2000: 162(5): 1979-1982.
21. Kieszko R, Krawczyk P, Jankowska O, Chocholska S, Król A, Milanowski J. The clinical significance of interleukin 18 assessment in sarcoidosis patients. *Respiratory medicine* 2007: 101(4): 722-728.
22. Tomita H, Sato S, Matsuda R, Sugiura Y, Kawaguchi H, Niimi T, Yoshida S, Morishita M. Serum lysozyme levels and clinical features of sarcoidosis. *Lung* 1999: 177(3): 161-167.
23. Miyoshi S, Hamada H, Kadowaki T, Hamaguchi N, Ito R, Irifune K, Higaki J. Comparative evaluation of serum markers in pulmonary sarcoidosis. *Chest* 2010: 137(6): 1391-1397.
24. Keshav S, Chung P, Milon G, Gordon S. Lysozyme is an inducible marker of macrophage activation in murine tissues as demonstrated by in situ hybridization. *Journal of Experimental Medicine* 1991: 174(5): 1049-1058.
25. Belhomme N, Jouneau S, Bouzillé G, Decaux O, Lederlin M, Guillot S, Perlat A, Jego P. Role of serum immunoglobulins for predicting sarcoidosis outcome: A cohort study. *PLoS One* 2018: 13(4): e0193122.

26. Prior C, Haslam PL. Increased levels of serum interferon-gamma in pulmonary sarcoidosis and relationship with response to corticosteroid therapy. *The American review of respiratory disease* 1991: 143(1): 53-60.
27. Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. *Critical Reviews™ in Immunology* 2012: 32(1).
28. Spagnolo P, Rossi G, Trisolini R, Sverzellati N, Baughman RP, Wells AU. Pulmonary sarcoidosis. *The Lancet Respiratory Medicine* 2018: 6(5): 389-402.
29. Muller-Quernheim J. Sarcoidosis: immunopathogenetic concepts and their clinical application. *European Respiratory Journal* 1998: 12(3): 716-738.
30. Muller-Quernheim J, Schurmann M, Hofmann S, Gaede KI, Fischer A, Prasse A, Zissel G, Schreiber S. Genetics of Sarcoidosis. *Clin Chest Med* 2008: 29(3): 391-414.
31. Filbey KJ, Varyani F, Harcus Y, Hewitson JP, Smyth DJ, McSorley HJ, Ivens A, Nylén S, Rottenberg M, Löser S. Macrophage migration inhibitory factor (MIF) is essential for type 2 effector cell immunity to an intestinal helminth parasite. *Frontiers in immunology* 2019: 10: 2375.
32. Florez-Sampedro L, Soto-Gamez A, Poelarends GJ, Melgert BN. The role of MIF in chronic lung diseases: looking beyond inflammation. *American Journal of Physiology-Lung Cellular and Molecular Physiology* 2020: 318(6): L1183-L1197.
33. Marsh LM, Cakarova L, Kwapiszewska G, von Wulffen W, Herold S, Seeger W, Lohmeyer J. Surface expression of CD74 by type II alveolar epithelial cells: a potential mechanism for macrophage migration inhibitory factor-induced epithelial repair. *American Journal of Physiology-Lung Cellular and Molecular Physiology* 2009: 296(3): L442-L452.
34. Habibie H. D-dopachrome tautomerase contributes to lung epithelial repair via atypical chemokine receptor 3-dependent Akt signaling. *Elsevier* 2021.
35. Lin F-C, Young HA. The talented interferon-gamma. 2013.
36. Barrat FJ, Crow MK, Ivashkiv LB. Interferon target-gene expression and epigenomic signatures in health and disease. *Nat Immunol* 2019: 20(12): 1574-1583.
37. Ruiz-Rosado JdD, Olguín JE, Juárez-Avelar I, Saavedra R, Terrazas LI, Robledo-Avila FH, Vazquez-Mendoza A, Fernández J, Satoskar AR, Partida-Sánchez S. MIF promotes classical activation and conversion of inflammatory Ly6Chigh monocytes into TipDCs during murine toxoplasmosis. *Mediators of inflammation* 2016: 2016.
38. Yanagawa Y, Iwabuchi K, Onoé K. Co-operative action of interleukin-10 and interferon-γ to regulate dendritic cell functions. *Immunology* 2009: 127(3): 345-353.
39. Wood KJ, Sawitzki B. Interferon γ: a crucial role in the function of induced regulatory T cells in vivo. *Trends in immunology* 2006: 27(4): 183-187.
40. Qian C, Jiang X, An H, Yu Y, Guo Z, Liu S, Xu H, Cao X. TLR agonists promote ERK-mediated preferential IL-10 production of regulatory dendritic cells (diffDCs), leading to NK-cell activation. *Blood* 2006: 108(7): 2307-2315.

41. Fallica J, Varela L, Johnston L, Kim B, Serebreni L, Wang L, Damarla M, Kolb TM, Hassoun PM, Damico R. Macrophage Migration Inhibitory Factor: A Novel Inhibitor of Apoptosis Signal-Regulating Kinase 1–p38–Xanthine Oxidoreductase–Dependent Cigarette Smoke–Induced Apoptosis. *American journal of respiratory cell and molecular biology* 2016: 54(4): 504-514.

# Figures

**Figure 1**

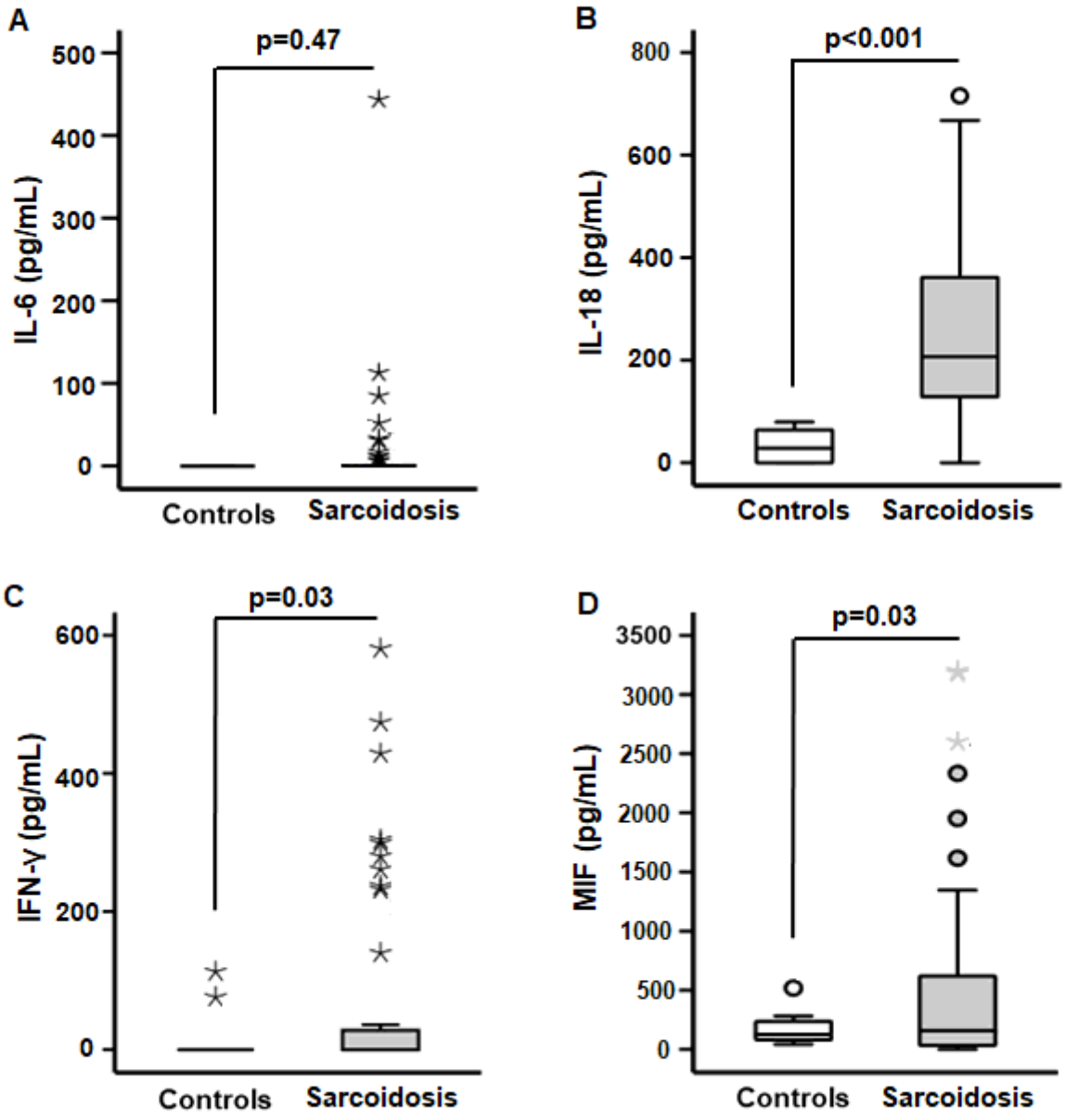



Figure 1

**Comparison of levels of serum cytokines and biomarkers in sarcoidosis patients and healthy controls.** There was no significant difference in levels of IL-6 between sarcoidosis patients and healthy controls **(A)**. Sarcoidosis patients displayed significant higher levels of serum IL-18 (p<0.001) **(B)**, IFN-γ (p=0.03) **(C)** and MIF (p=0.03) **(D)** as compared to healthy controls.

Figure 2

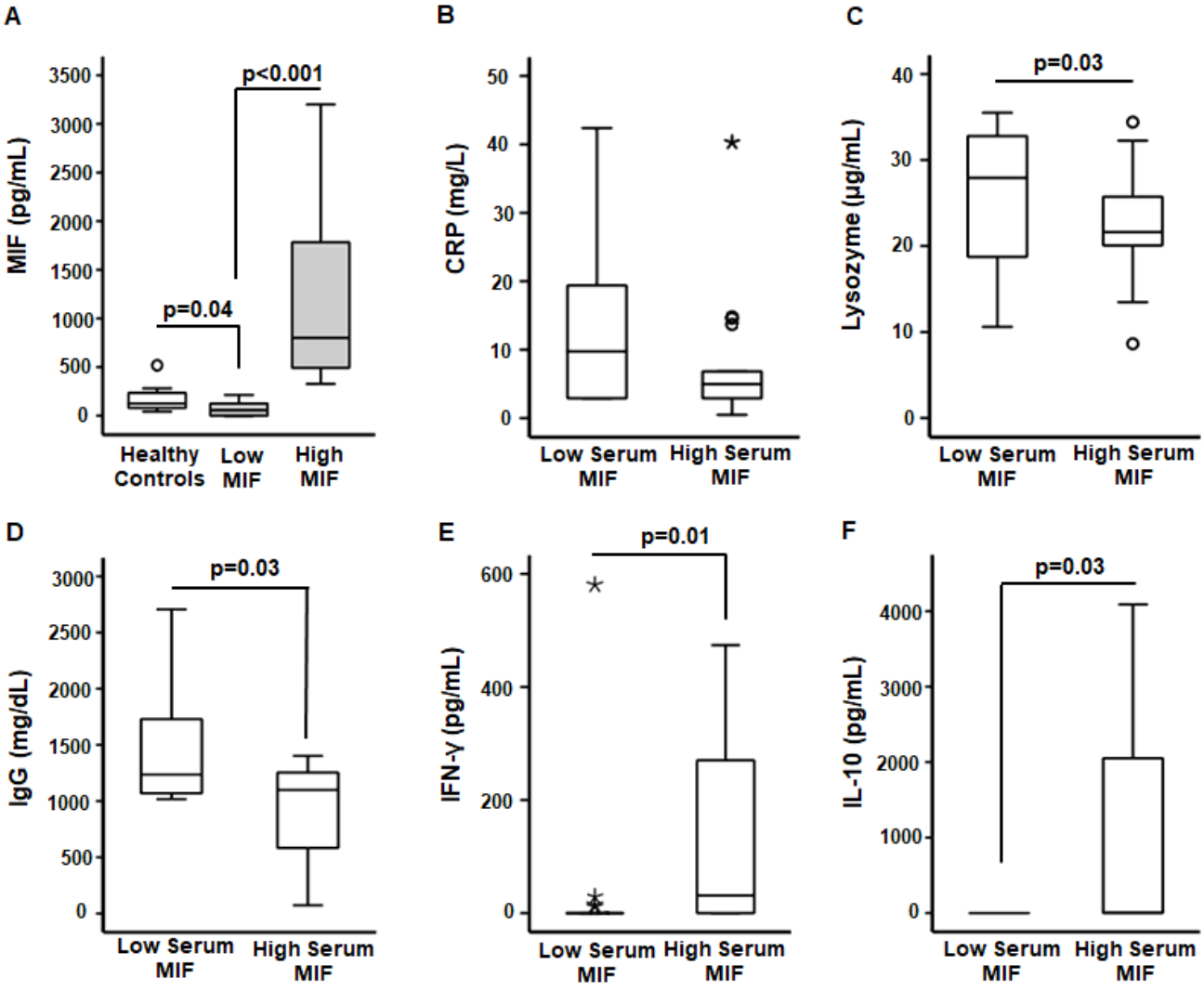
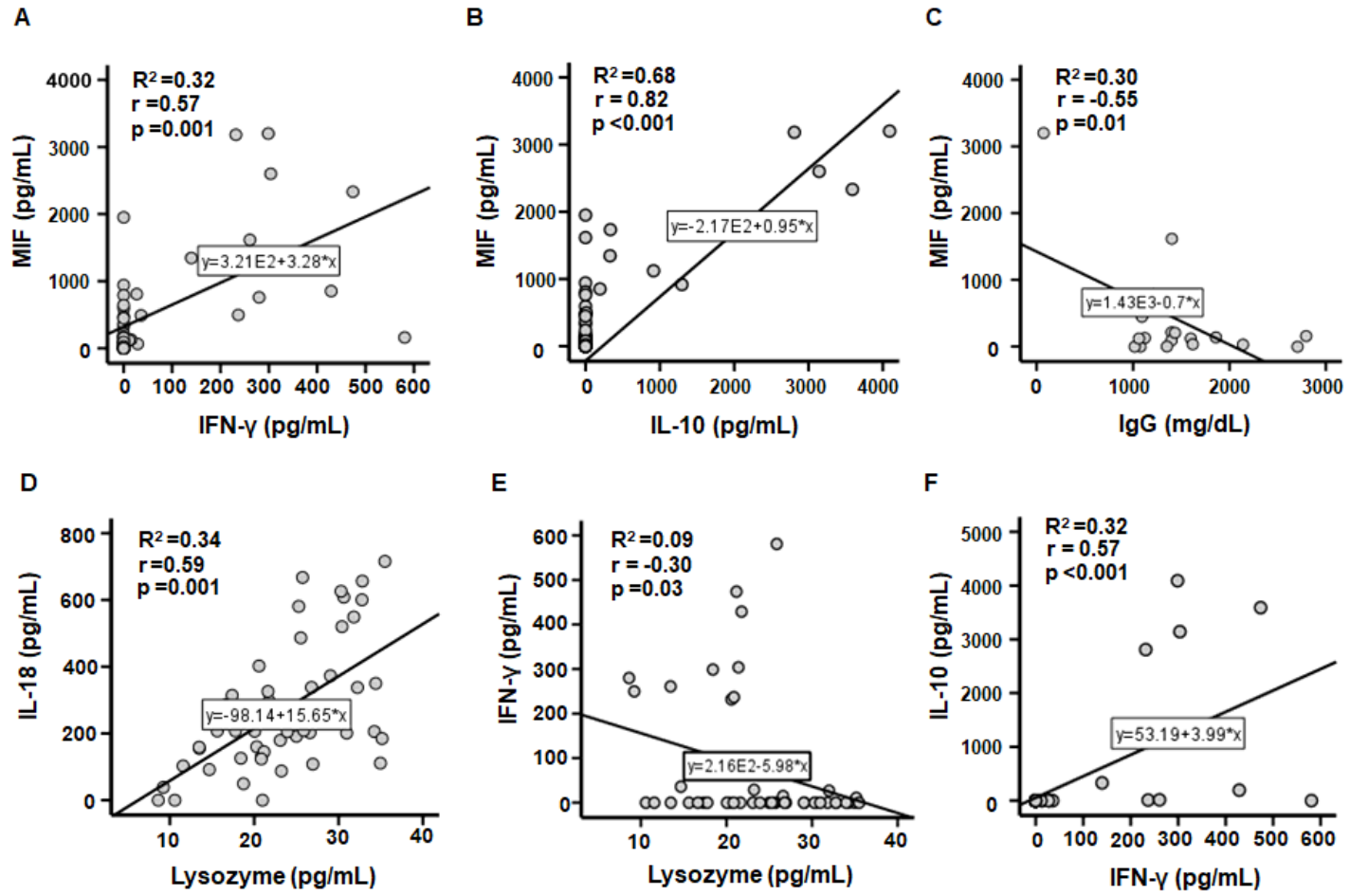



Figure 2

**Classification of sarcoid patients based on serum MIF levels.** The sarcoid patients with serum MIF values of less than 237pg/mL were classified as low serum MIF-group, whereas MIF higher than 237pg/mL were classified as high MIF-group **(A)**. There was no significant difference in levels of CRP between low serum MIF and high serum MIF groups **(B)**. Low serum MIF-group exhibited significant higher levels of serum lysozyme (p=0.03) **(C)** and higher levels of serum IgG (p=0.03) **(D)** as compared to high serum MIF-group. High serum MIF-group exhibited significantly higher (p=0.01) levels of serum IFN-γ **(E)** and higher levels of serum IL-10 (p=0.0903) **(F)** as compared to low serum MIF-group.

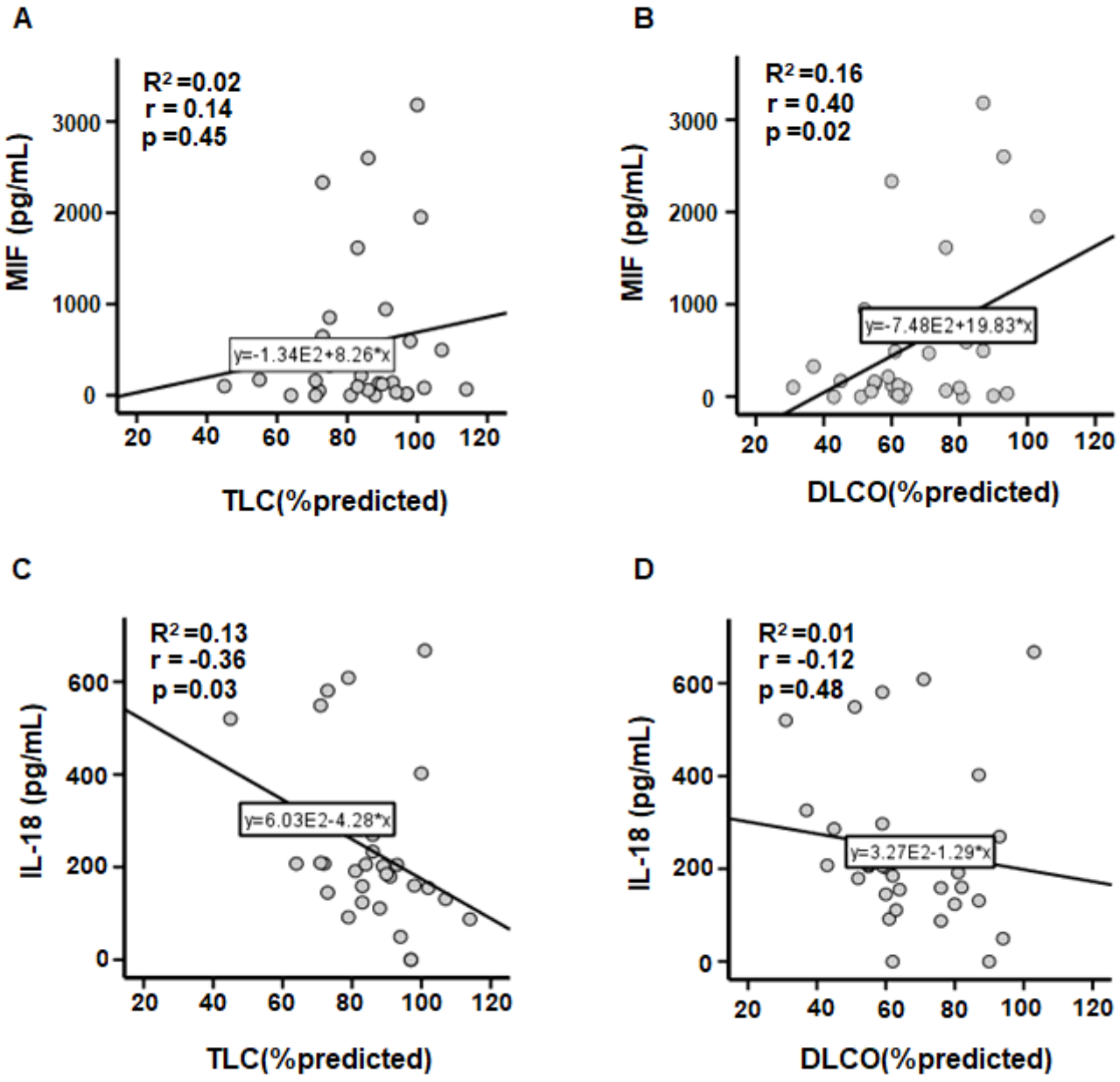
Figure 3




## Figure 3

**Correlation of serum cytokines and biomarkers in sarcoid patients.** Serum levels of MIF displayed significant positive correlations with serum levels of IFN-γ **(A)** and IL-10 **(B)**. Serum levels of MIF displayed a significant negative correlation with serum levels of IgG **(C)**. Serum lysozyme levels exhibited a significant positive correlation with serum levels of IL-18 **(D)** while it exhibited a significant negative correlation with serum levels of IFN-γ **(E)**. Serum levels of IFN-γ and IL-10 displayed a significant positive correlation **(F)**.

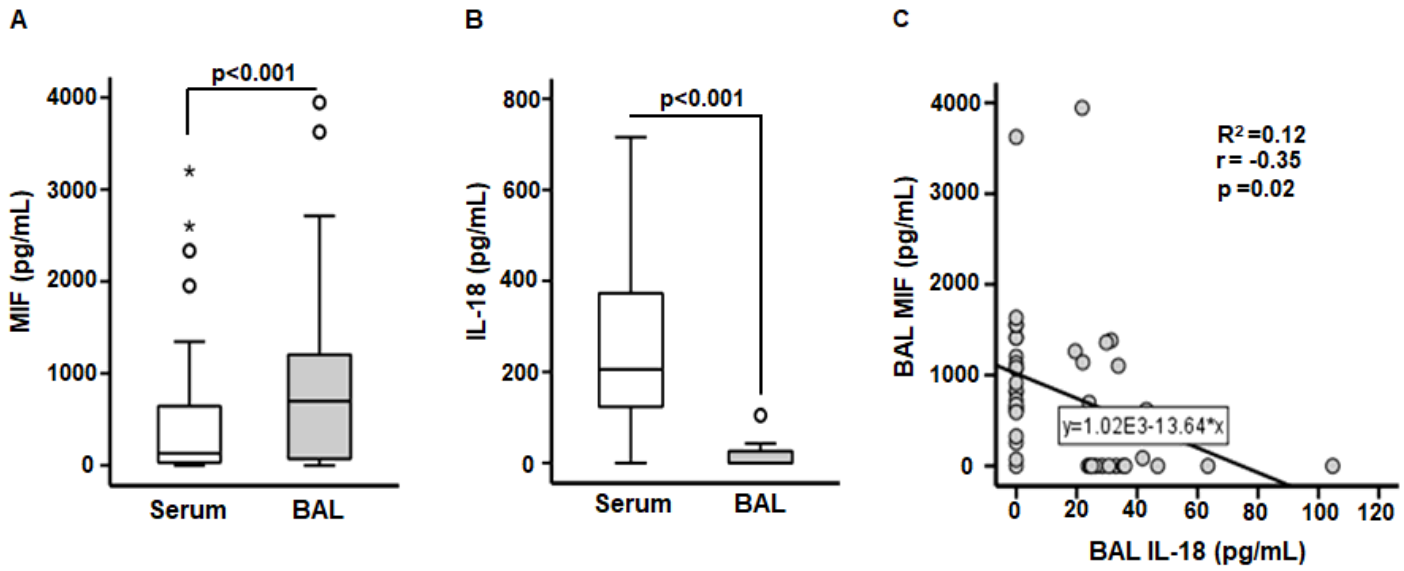
Figure 4




## Figure 4

**Correlation of serum cytokines and follow up PFT values in sarcoid patients.** Serum levels of MIF displayed negative association with %predicted TLC. **(A)**. MIF displayed significant positive correlations with %predicted DLCO after 3 years **(B)**. IL-18 serum levels displayed significant negative correlations with %predicted TLC in 3 years follow up **(C)** and negative association with %predicted DLCO but not significant **(D)**.

Figure 5




## Figure 5

**Comparison and correlation of MIF and IL-18 levels in serum and BAL of sarcoid patients.** MIF BAL samples displayed significant higher levels as compared to MIF serum (p<0.001) **(A)**. Serum IL-18 level was significantly higher (p<0.001) as compared to BAL IL-18 (B). There was a significant negative correlation between the levels of BAL IL-18 and BAL MIF (C).