Circulatory TGF-β1 is significantly higher in early stage of pulmonary sarcoidosis

Mehdi Mirsaeidi1, Hesham R. Omar2, Andrew Calzadilla1, Ahmad El Khatib3, Philip Whitney4, Nadera Sweiss5, Daniel Culver6, Michael Campos1, Robert Baughman7, Roberto Machado2

1Division of Pulmonary, Critical Care, Sleep and Allergy, Department of Medicine, University of Miami, FL, USA; 2Internal Medicine Department, Mercy Medical Center, Iowa, USA; 3Division of Pulmonary, Critical Care, Sleep and Allergy, Department of Medicine, University of Illinois at Chicago, IL, USA; 4Department of Pulmonary, Allergy, and Critical Care Medicine, Cleveland Clinic, Cleveland, OH, USA; 5Division of Pulmonary, Critical Care, Sleep and Allergy, Department of Medicine, University of Cincinnati, OH, USA

Abstract. Introduction: The pathogenesis of pulmonary fibrosis in sarcoidosis is not known. We hypothesized that higher levels of circulatory growth factors are present in early stages of pulmonary sarcoidosis and may be associated with pulmonary fibrosis. Methods: Age and sex-matched subjects with sarcoidosis stage 0-1 (n=18), stage 4-5 (n=13) and healthy controls (n=5) had their serum TGF-β1, FGF, and VEGF levels measured as well as their gene expressions determined in peripheral blood mononuclear cells. Results: TGF-β1 levels were significantly higher in patients with stage 0-1 sarcoidosis compared with normal healthy control patients (25,488 vs. 13,800 pg/ml, P=0.05). Patients with sarcoidosis stage 4 had a 1.3-fold higher peripheral blood mononuclear cells (PBMC) gene expression of TGF-β1 compared with subjects at stage 0-1 (P= 0.041). The serum levels of FGF, and VEGF had a trend towards higher levels in sarcoidosis subjects compared to normal controls. Conclusion: These results suggest that cell growth factors levels are high in early stages of sarcoidosis. These findings should be validated in larger studies. (Sarcoidosis Vasculit. Diffuse Lung Dis 2018; 35: 213-217)

Key words: sarcoidosis, growth factor, TGF-β1, FGF, and VEGF

Introduction

Sarcoidosis is a granulomatous disease that involves the lungs in more than 90% of the cases (1). The incidence of sarcoidosis differs broadly throughout the world due to environmental and genetic factors. We and others reported that pulmonary fibrosis occurs in 10-20% of affected individuals and is the most common cause of sarcoidosis-related mortality (2). The pathogenesis of pulmonary fibrosis in sarcoidosis is not known. It is theorized that certain mediators like transforming growth factor (TGF-β1), Platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF) have a role in its pathogenesis (3). If confirmed, it opens the possibility of treatment with medications that antagonize these growth factor receptors to prevent further fibrosis formation.

We hypothesized that subjects with early stage pulmonary sarcoidosis (Stages 0-1) will have higher levels of PDGF, FGFs and VEGF proteins and higher gene expression in peripheral blood mononuclear cells (PBMC) compared with fibrotic pulmonary sarcoidosis patients and healthy controls.
Methods

Patient population

This is an IRB-approved retrospective study of adult subjects >18 years at the University of Illinois in Chicago diagnosed with sarcoidosis according to the European Respiratory Society (ERS), American Thoracic Society (ATS) and World Association of Sarcoidosis and other Granulomatous Disorders (WASOG) criteria (4) between January 2010 and January 2015. The study included three groups of patients (all between 18-70 years): 1st group comprised 18 subjects with confirmed sarcoidosis stage 0 or 1, 2nd group comprised 13 subjects with confirmed sarcoidosis stage 4 or 5 and 3rd group comprised 5 healthy controls (without history of sarcoidosis or pulmonary fibrosis). All subjects were African American and matched for sex and age. All participants signed an informed consent. Sarcoidosis staging from 0-4 was based on pattern of chest radiographic findings. Exclusion criteria included patients who were taking full-dose anticoagulant therapy or high-dose antiplatelet therapy at screening, patients with non-cutaneous malignancy treated in the past two years or if hemoglobin was <7gm/dL.

Measurement of growth factors and gene expression

The concentration of serum TGF-β1, VEGF, acidic fibroblast growth factor (aFGF), bFGF and their genes expression in PBMC were measured and compared across the three groups. Subjects who satisfied the inclusion and exclusion criteria for the study were subjected to blood draw during routine laboratory blood tests. Serum was isolated and stored for PDGF, bFGF, and VEGF, TGF-β1 measurements via ELISA as previously described (5, 6). RNA was extracted from blood mononuclear cells for each sample. Growth factor genes expression was evaluated utilizing Affymetrix GeneChip based global expression profiling in the University of Illinois Genomic Center (7). Peripheral blood mononuclear cells were compared amongst 2 groups of subjects with sarcoidosis stage 0-1 and those with confirmed sarcoidosis stage 4.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) and compared using Student t-test and ANOVA (with Tukey’s post-hoc test). Mean comparison was used for continues variables. Regression model was used to establish cause-and-effect relationships between low DLCO and circulatory growth factors levels.

A P-value ≤0.05 was considered statistically significant. All statistical significance was assessed using a 2-sided P values. Data were analyzed using IBM SPSS 22.0 statistical software (IBM SPSS Version 21.0. Armonk, NY) and GraphPad Prism.

Results

Compared with patients with sarcoidosis stages 0-1, those with sarcoidosis stage 4 had lower body mass index (P=0.014), higher frequency of cough (P=0.013), sputum production (P=0.019), and dyspnea (P=0.034), and lower mean FVC (P=0.043), FEV1 (P=0.03) and DLCO (P=0.002) by univariate analysis. There was no significant difference on medical therapy between two sarcoidosis groups. Table 1 compares baseline demographics, clinical and laboratory characteristics, and pulmonary function tests among patient with sarcoidosis stages 0-1 versus stage 4.

We found a statistically significant increase in the average serum levels of TGF-β1 in subjects with stage 0-1 sarcoidosis as compared with the normal healthy controls (25,488 vs. 13800 pg/ml, P=0.05). The average VEGF serum levels in stages 0-1 was non-significantly increased when compared to the normal healthy patient as well (9483 vs. 328 pg/ml, P=0.375). Figure 1 illustrates the mean levels of growth factors among the three study groups. We have also found that the PBMC gene expression levels of TGF-β1 in African American subjects with stage 4 pulmonary sarcoidosis were 1.30-fold higher compared with stage 0-1 (P= 0.041) (table 2). Table 2 illustrates the gene expression levels of growth factors in PBMC among the sarcoidosis subjects. We were not able to find any growth factor as an independent variable associated with low DLCO In the regression model (data not shown).
Growth factors in sarcoidosis

**Discussion**

TGF-β superfamily is a large group of proteins which are active in cell growth, differentiation signals, and regulation of extracellular matrix production (8). TGF-β is produced from different cell lines including macrophages, T-cells, bronchial epithelial cells and type II alveolar epithelial cells. TGF-β induces differentiation of fibroblasts and epithelial cells to myofibroblast-like cells. TGF-β also exhibits pro- and anti-inflammatory properties, and its increased production is associated with fibrotic diseases. Zissel and co-workers found that lung cells of sarcoidosis subjects with persistent active disease produced significantly lower levels of TGF-β compared with those whose disease spontaneously regressed within 6 months (9). Interestingly, in our study patients with Stage 4 sarcoidosis had a mean circulatory TGF-β level of 21,977 pg/mL that was lower than subjects with non-fibrotic stage 0-1 (25,488 pg/mL). Lower circulatory TGF-β in the fibrotic group supports previous study findings and suggests that low circulatory TGF-β may be a potential biomarker for a poorer prognosis in pulmonary sarcoidosis. The higher circulatory TGF-β levels in subjects with sarcoidosis compared with normal healthy subjects is expected due to the inflammatory nature of disease. The TGF-β mechanism of effect in pulmonary sarcoidosis still remained unclear and required further investigation.

Our study showed a non-significant trend towards increased values of circulatory VEGF for patients that had sarcoidosis when compared with normal patients. Sekiya et al., evaluated the relationship of serum VEGF with the clinical status of sarcoidosis (10). They measured the concentrations of VEGF in serum of 33 subjects with confirmed sarcoidosis and

**Table 1. Comparison of baseline demographics, clinical and laboratory characteristics, and pulmonary function tests among patient with sarcoidosis stages 0-1 versus stage 4**

|                                | Sarcoidosis Stage 0-1 (N=18) | Sarcoidosis Stage 4 (N=13) | P-value |
|--------------------------------|-------------------------------|----------------------------|---------|
| Age (year, m±SD)               | 50.9±10.8                     | 51.2±8.4                   | 0.952   |
| Male sex % (n)                 | 16.7% (3/18)                  | 7.7% (1/13)                | 0.462   |
| BMI (Kg/m2, m±SD)              | 37.8±7.3                     | 32.2±15.5                  | 0.014   |
| Sarcoidosis duration (year, m±SD) | 9.3±6.9                    | 12.2±7.6                   | 0.236   |
| Extrapulmonary sarcoidosis % (n) | 64.7% (11/17)          | 61.5% (8/13)               | 0.858   |
| Hypertension % (n)             | 72.2% (13/18)                | 33.8% (7/13)               | 0.291   |
| DM % (n)                       | 27.8% (5/18)                 | 7.7% (1/13)                | 0.162   |
| Cough % (n)                    | 50% (9/18)                   | 92.3% (12/13)              | 0.013   |
| Sputum production % (n)        | 25% (4/16)                   | 75% (6/8)                  | 0.019   |
| Dyspnea % (n)                  | 43.8% (7/16)                 | 83.3% (10/12)              | 0.034   |
| Fatigue % (n)                  | 27.8% (5/18)                 | 53.8% (7/13)               | 0.141   |
| FVC volume (L, m±SD)           | 2.7±0.5                      | 2.2±0.5                    | 0.043   |
| FVC % (m±SD)                   | 84.4±34.3                    | 79.2±19.9                  | 0.386   |
| FEV1 volume (L, m±SD)          | 2.2±0.5                      | 1.6±0.4                    | 0.03    |
| FEV1 % (m±SD)                  | 91.1±21.6                    | 69.8±23.5                  | 0.047   |
| VC volume (L, m±SD)            | 2.75±0.5                     | 2.43±0.4                   | 0.277   |
| VC % (m±SD)                    | 100.5±18                     | 86.9±18.3                  | 0.277   |
| TLC volume (L, m±SD)           | 4.29±0.8                     | 4.1±0.5                    | 0.248   |
| TLC % (m±SD)                   | 89.8±15                      | 80.3±11.5                  | 0.178   |
| RV (L, m±SD)                   | 103.3±18.7                   | 99.1±16.2                  | 0.565   |
| DLCO vol (ml/min/mmHg, m±SD)   | 17.6±2.4                     | 11.1±3.3                   | 0.002   |
| DLCO % (m±SD)                  | 74.6±11.5                    | 60±13.8                    | 0.058   |
| ESR (mm/hr, m±SD)              | 54.1±47.5                    | 57.8±35.6                  | 0.382   |
| CRP (mg/L, m±SD)               | 3.14±4.6                     | 2.1±1.2                    | 0.947   |
| ACE (U/L, m±SD)                | 52±43.7                      | 73±35.3                    | 0.237   |
| Oral steroid % (n)             | 82.4% (14/17)                | 92.3% (12/13)              | 0.427   |
| DMARD % (n)                    | 50% (9/18)                   | 54.5% (6/11)               | 0.812   |
| Methotrexate % (n)             | 41.2% (7/17)                 | 33.3% (3/9)                | 0.696   |
| Leflunomide % (n)              | 11.1% (2/18)                 | 18.2% (2/11)               | 0.592   |
| Hydroxychloroquine % (n)       | 11.1% (2/18)                 | 27.3% (3/11)               | 0.264   |

BMI: body mass index, DM: Diabetes Mellitus, FVC: forced vital capacity, FEV: forced expiratory volume, VC: vital capacity, TLC: total lung capacity, RV: residual volume, DLCO: diffusion capacity of lungs for carbon monoxide, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, ACE: angiotensin converting enzyme, DMARD: disease modifying anti-rheumatic drugs
investigated VEGF values with extension of disease, and prognosis. They found that serum VEGF was higher among subjects who required treatment with steroids when compared to those with spontaneous remission (P<0.05). It was also found that patients with pulmonary sarcoidosis alone had lower values of average VEGF when compared to those with pulmonary and extrapulmonary sarcoidosis. They concluded that serum VGEF might be a novel marker for prognosis in sarcoidosis.
Antoniou et al. compared VEGF and its receptor (fms-like tyrosine kinase 1, Flt-1) levels in BAL of subjects with idiopathic pulmonary fibrosis (IPF) and pulmonary sarcoidosis (11). They enrolled 3 groups of subjects in the study, 18 subjects with IPF, 16 subjects with sarcoidosis and 10 healthy volunteers. They found significant increases of VEGF gene expression in IPF comparing to pulmonary sarcoidosis (P=0.02) but no statistically significant difference was found in VEGF protein levels between sarcoidosis and IPF (mean levels 154 pg/mL versus 344 pg/mL, p=0.2). Comparing to normal healthy subjects, VEGF gene expression in BAL cells of sarcoidosis subjects was higher. In addition, there was no significant difference on the levels of VEGF receptor gene expression in BAL fluid of sarcoidosis compare to IPF or healthy subjects (p=0.4). This study did not address the sarcoidosis stages and fibrotic sarcoidosis.

Our study is limited in number of samples in each group and only showed a non-significant trend towards increased values of circulatory FGFs for patients with sarcoidosis when compared with normal patients. Recently, Sexton and coworkers investigated FGF-23 serum levels in 39 subjects with acute sarcoidosis (12). They found that serum level of FGF-23 was more than 9.9 pg/mL in 15% of sarcoidosis subjects; those had higher serum calcium (P=0.007) and lower serum iPTH (P<0.001). FGF-23 was below the level of detection in the majority of subjects. The role of FGF in the sarcoidosis needs more investigation.

Conclusion

Our preliminary data confirms that cell growth factors levels are higher in sarcoidosis subjects particularly those in early stages in comparison to normal healthy subjects. Our findings suggest that circulatory levels of serum growth factors may be novel markers for prognosis in sarcoidosis, although, they should be validated in a larger sample size. The protein and gene expression of cell growth factors in BAL cells (alveolar macrophages and fibroblasts) should be investigated to address local activity of growth factors. This approach will improve our risk stratification of sarcoidosis subjects and probably find novel therapeutic targets.

References

1. Mirsaedi M, Machado RF, Schraufnagel D, Sween NJ, Baughman RF. Racial difference in sarcoidosis mortality in the United States. Chest 2015; 147(2): 438-49. doi: 10.1378/chest.14-1120. PubMed PMID: 25188873; PubMed Central PMCID: PMCPMC4314818.
2. Mirsaedi M, Banoei MM, Nienow CK, Abassi T, Hakim A, Schraufnagel D, et al. Plasma metabolic profile in fibrosing pulmonary sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 2016; 33(1): 29-39. PubMed PMID: 27055833.
3. Ando M, Miyazaki E, Ito T, Hiroshige S, Nureki SI, Ueno T, et al. Significance of serum vascular endothelial growth factor level in patients with idiopathic pulmonary fibrosis. Lung 2010; 188(3): 247-52. doi: 10.1007/s00408-009-9223-x. PubMed PMID: 20666538.
4. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. American journal of respiratory and critical care medicine 1999; 160(2): 736-55. doi: 10.1164/ajrccm.160.2.atv-49. PubMed PMID: 10430755.
5. Peterson JE, Zurakowski D, Italiano JE, Jr., Michel LV, Connors S, Orenick M, et al. VEGF, PF4 and PDGF are elevated in platelets of colorectal cancer patients. Angiogenesis 2012; 15(2): 265-73. doi: 10.1007/s10456-012-9259-z. PubMed PMID: 22402885.
6. Peterson JE, Zurakowski D, Italiano JE, Jr., Michel LV, Fox L, Klemp GL, et al. Normal ranges of angiogenesis regulatory proteins in human platelets. American journal of hematology 2010; 85(7): 487-93. doi: 10.1002/ajh.21732. PubMed PMID: 20575035.
7. Sahu SC, Zheng J, Younck JJ, Sprando RL, Gao X. Toxicogenomic responses of human liver HepG2 cells to silver nanoparticles. J Appl Toxicol 2015; 35(10): 1160-8. doi: 10.1002/jat.3170. PubMed PMID: 26014281.
8. Khalil N, O’Connor RN, Ururu HW, Warren PW, Flanders KC, Kemp A, et al. Increased production and immunohistochemical localization of transforming growth factor-beta in idiopathic pulmonary fibrosis. American journal of respiratory and cell molecular biology. 1991;5(2):155-62. PubMed PMID: 1892646.
9. Zissel G, Holomolka J, Schlaak J, Schlaak M, Muller-Quernheim J. Anti-inflammatory cytokine release by alveolar macrophages in pulmonary sarcoidosis. American journal of respiratory and critical care medicine 1996; 154(3 Pt 1): 713-9. doi: 10.1164/ajrccm.154.3.8810610. PubMed PMID: 8810610.
10. Sekiya M, Owada A, Miura K, Takahashi S, Fukuchi Y. Serum vascular endothelial growth factor as a possible prognostic indicator in sarcoidosis. Lung. 2003;181(5):259-65. PubMed PMID: 14705769.
11. Antoniou KM, Soufla G, Prokouda A, Margaritopoulos G, Choula-ki C, Lymbouridou R, et al. Different activity of the biological axis VEGF-Flt-1 (fms-like tyrosine kinase 1) and CXC chemokines between pulmonary sarcoidosis and idiopathic pulmonary fibrosis: a bronchoalveolar lavage study. Clin Dev Immunol 2009; 2009: 537929. doi: 10.1155/2009/537929. PubMed PMID: 20169144; PubMed Central PMCID: PMCPMC2821758.
12. Sexton DJ, O’Reilly MW, Geoghegan P, Kinsella SM, Moran PJ, O’Regan AW. Serum fibroblast growth factor 23 in acute sarcoidosis and normal kidney function. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG / World Association of Sarcoidosis and Other Granulomatous Disorders 2016; 33(2): 139-42. PubMed PMID: 27537716.