Cytokine Signaling and Matrix Remodeling Pathways Associated with Cardiac Sarcoidosis Disease Activity Defined Using FDG PET Imaging

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Summary

While cardiac imaging has improved the diagnosis and risk assessment for cardiac sarcoidosis (CS), treatment regimens have consisted of generalized heart failure therapies and non-specific anti-inflammatory regimens. The overall goal of this study was to perform high-sensitivity plasma profiling of specific inflammatory pathways in patients with sarcoidosis and with CS.

Specific inflammatory/proteolytic cascades were upregulated in sarcoidosis patients, and certain profiles emerged for CS patients.

Plasma samples were collected from patients with biopsy-confirmed sarcoidosis undergoing F-18 fluorodeoxyglucose positron emission tomography (n = 47) and compared to those of referent control subjects (n = 6). Using a high-sensitivity, automated multiplex array, cytokines, soluble cytokine receptor profiles (an index of cytokine activation), as well as matrix metalloproteinase (MMP), and endogenous MMP inhibitors (TIMPs) were examined.

The plasma tumor necrosis factor (TNF) and soluble TNF receptors sCD30 and sTNFRI were increased using sarcoidosis, and sTNFRII increased in CS patients (n = 18). The soluble interleukin sIL-2R and vascular endothelial growth factor receptors (sVEGFR2 and sVEGFR3) increased to the greatest degree in CS patients. When computed as a function of referent control values, the majority of soluble cytokine receptors increased in both sarcoidosis and CS groups. Plasma MMP-9 levels increased in sarcoidosis but not in the CS subset. Plasma TIMP levels declined in both groups.

The findings from this study were the identification of increased activation of a cluster of soluble cytokine receptors, which augment not only inflammatory cell maturation but also transmigration in patients with sarcoidosis and patients with cardiac involvement.

Key words: Biomarkers, FDG PET/CT, TNFα, IL-2, MMP, TIMP, Proteases, Inflammation

Sarcoidosis is a systemic inflammatory disease characterized by the infiltration of one or more organs with noncaseating granulomas. The lungs are involved in approximately 90% of patients, but multiple organ systems may be affected, including the nervous system, skin, and heart. Although cardiac sarcoidosis (CS) occurs in a minority of patients with sarcoidosis, complications from CS account for approximately one quarter of sarcoidosis deaths. Clinical evaluation is often coupled with multimodality cardiac imaging to evaluate cardiac disease activity and therapeutic response to immunosuppression. Cardiac 18F-fluorodeoxyglucose positron emission tomography (FDG PET) is currently a gold standard imaging method of identifying CS. In practice, FDG PET has emerged as the method of choice for the quantitative and serial monitoring of active cardiac inflammation, in part due to its ability to differentiate a scar from active inflammation. However, recent results have indicated that there is both interpretive uncertainty and suboptimal reproducibility for FDG PET for CS, providing justification for additional/adjunctive approaches to identifying CS disease and severity.

One of the downstream consequences of inflammation is the induction and activation of proteases that cause...
local tissue degradation and remodeling, a key structural event in sarcoidosis and disease progression. Specifically, the matrix metalloproteinases (MMPs) are a family of degradative enzymes, which are induced with inflammation and tissue remodeling processes, and are in turn regulated by endogenous inhibitors, the tissue inhibitors of MMPs (TIMPs). Profiling of pulmonary biopsies and lavage fluid have identified elevated levels of MMPs and lower levels of TIMPs, whereby alveolar macrophages have demonstrated a cytokine-associated increase in MMP-9 and decrease in TIMP-1. However, there have been no systematic studies reporting comprehensive MMP/TIMP plasma profiling in sarcoidosis, and, in particular, patients with CS. Based upon the fact that inflammation and structural remodeling form a cornerstone in the pathogenesis of CS, this study tested the central hypothesis that a specific plasma signature emerges with respect to the inflammatory and proteolytic domains in sarcoidosis patients, and, more importantly, in those patients with active CS.

Methods

Study patients: Following approval by the Yale University Institutional Review Board, 201 consecutive patients referred to a single tertiary care center for cardiac FDG PET were prospectively screened between February 2017 and June 2018, whereby 47 patients had biopsy clinically diagnosed extracardiac sarcoidosis. Clinically diagnosed extracardiac sarcoidosis was defined by a radiographic appearance on either chest CT and/or FDG PET to include typical findings such as symmetric hilar and mediastinal lymphadenopathy (FDG avid on PET), peribronchial nodular thickening in the mid/upper lobes, pulmonary nodules, ground glass opacities, and/or pulmonary fibrosis in classic patterns without alternative explanations. Biopsy confirmation data were available for 32 subjects (28 pulmonary, 1 lacrimal gland and cutaneous lesion, 1 neurological, 1 lacrimal gland and cutaneous lesion, 1 neuro-lymphatic, 1 endomyocardial, and 1 abdominal lymph node and hepatic). Exclusion criteria included low likelihood of CS, the inability to give consent, recent myocardial infarction, and recent cardiac surgery or invasive procedure. In addition, patients were enrolled in this study with what was defined as intermediate risk for CS based on the presence of heart failure of unknown etiology, palpitations, pre-syncpe or syncope, right or left bundle branch block or left anterior fascicular block, abnormal Q waves in ≥ 2 leads, first-degree AV block > 240 ms, second/third-degree AV block, frequent PVCs, sustained or non-sustained VT, systolic dysfunction (left ventricular ejection fraction < 50%), and/or regional wall motion abnormalities. For the purposes of establishing referent control values, age-matched plasma samples (n = 6) were included in this analysis and were obtained from a study cohort described previously. For this referent control cohort, the mean age was 60 ± 2 years, equal gender distribution (3/6 female), 2/6 African-American, and LV ejection fraction by echocardiography within normal limits (66% ± 1%).

PET imaging: Patients were prepared for imaging by following a 24-hour high-fat, low-carbohydrate diet and an overnight (> 12 hour) period of fasting as previously described. The dietary preparation was provided in written instructions, and compliance was reinforced by coaching by clinical staff through appointments and/or telephone calls. Cardiac FDG-Positron Emission Tomography/Computed Tomography (PET/CT) imaging was performed on a GE Discovery ST 690 PET/CT scanner using 3D acquisition. If fasting blood glucose was > 200 mg • dL−1, the test was not performed. Patients were injected with 8-10 mCi of FDG, and images were acquired using a single, cardiac-centered bed position following a 90-minute incubation. Resting ECG-gated dynamic Rubidium-82 (82Rb) PET imaging was performed using 18-30 mCi of 82Rb. Attenuation correction was performed using low-dose CT images (120 kV, 50-150 mA based on BMI) before 82Rb and FDG acquisition sequences.

Fused FDG PET/CT images were analyzed qualitatively and semi-quantitatively displayed on a standardized uptake value (SUV)-based scale using a GE AW workstation as previously described. Visual analysis of FDG uptake above background (blood pool) was interpreted from the fused FDG PET/CT imaging. A quantitative analysis was performed on the fused FDG PET/CT images by measuring the minimal LV blood pool SUV, cardiac SUVmax, cardiac metabolic volume, and cardiac metabolic activity as previously described. Analysis of 82Rb perfusion images was performed using Invia 4DM v.2016, utilizing patient sex-specific normal databases for 82Rb cardiac distribution. Quantitative resting myocardial blood flow was calculated using the Lortie method.

Plasma profiling: Patient plasma was divided into aliquots of ~50 μL and frozen at −70°C. Samples were thawed on ice and subjected to an optimized high-sensitivity, high-throughput multiplex format for several functional pathways tested: human soluble cytokine receptor (HSCRMAG-32K, Milliplex), human cytokine (M 5000007A, Bio-Rad), human MMP (LMPM000, R and D Systems), and human TIMP (LKT0M003, R and D Systems). Each individual analyte tested is provided in Table I. All measurements were performed using an internally validated and calibrated instrument that detects a fluorescent signal utilizing magnetic beads (Magpix, Millipore Sigma). The sensitivity for all assays was in the pg/mL range with an intra-assay coefficient of variations less than 13%. All sample values were computed using either a four-parameter curve or linear curve based on the best fit of the standards provided and the samples tested. The entire sample set of sarcoidosis patients (n = 47) and the referent control set (n = 6) were analyzed in duplicate and in a blinded fashion (HM, KEO, and LAF), and the coded results were not broken until all of the measurements were completed and the results locked in terms of quality control and conversion to absolute values using standardized curves.

Statistical analysis: All data were expressed as the mean and standard error of the mean. For demographic and baseline clinical data, Fisher’s exact test was used for comparison of categorical variables, and unpaired Student’s t-test, for continuous variables. Statistical analyses of baseline variables were performed using Prism version 7.04 (GraphPad Software, La Jolla, California). For the
Table I. Performance Metrics for Biomarker Arrays

| Analytes                                      | Average CV of Standards |
|-----------------------------------------------|-------------------------|
| Soluble Cytokine Receptors                    |                         |
| IFN-γ – Interferon gamma                      | 7.08                    |
| GM-CSF – Granulocyte-macrophage colony-stimulating factor | 5.88                    |
| TNF-α – Tumor necrosis factor alpha           | 5.09                    |
| IL-2 – Interleukin 2                          | 11.09                   |
| IL-4 – Interleukin 4                          | 7.86                    |
| IL-6 – Interleukin 6                          | 11.23                   |
| IL-8 – Interleukin 8                          | 6.44                    |
| IL-10 – Interleukin 10                        | 2.55                    |
| Soluble Cytokines                             |                         |
| sCD30 – Tumor necrosis factor receptor superfamily, member 8 | 7.54                    |
| sEGFR – Epidermal growth factor receptor      | 5.27                    |
| sgp130 – Interleukin 6 signal transducer     | 8.46                    |
| sIL-1RI – Interleukin 1 receptor, type 1      | 5.24                    |
| sIL-1RII – Interleukin 1 receptor, type II    | 3.02                    |
| sIL-2Ra – Interleukin 2 receptor, alpha       | 4.67                    |
| sIL-4R – Interleukin 4 receptor               | 11.92                   |
| sIL-6R – Interleukin 6 receptor               | 8.44                    |
| sRAGE – Advanced glycosylation end-product-specific receptor | 5.82                    |
| sTNFRI – Tumor necrosis factor receptor superfamily, member 1A | 7.30                    |
| sTNFRII – Tumor necrosis factor receptor superfamily, member 1B | 12.19                   |
| sVEGFR1 – Fms-related tyrosine kinase 1       | 9.03                    |
| sVEGFR2 – Kinase insert domain protein receptor | 6.22                    |
| sVEGFR3 – Fms-related tyrosine kinase 4       | 2.76                    |
| sCD30 – Tumor necrosis factor receptor superfamily, member 8 | 7.54                    |
| MMPs and TIMPs                                |                         |
| MMP-1 – Matrix Metalloproteinase 1            | 4.39                    |
| MMP-2 – Matrix Metalloproteinase 2            | 7.19                    |
| MMP-7 – Matrix Metalloproteinase 7            | 8.73                    |
| MMP-9 – Matrix Metalloproteinase 9            | 7.94                    |
| MMP-10 – Matrix Metalloproteinase 10          | 3.33                    |
| TIMP-1 – Tissue inhibitor of metalloproteinases 1 | 1.38                    |
| TIMP-2 – Tissue inhibitor of metalloproteinases 2 | 1.95                    |
| TIMP-3 – Tissue inhibitor of metalloproteinases 3 | 1.80                    |
| TIMP-4 – Tissue inhibitor of metalloproteinases 4 | 2.28                    |

multiplex results, values were placed in a categorical fashion: referent control, cardiac FDG-positive, and cardiac FDG-negative. The Winsorized method of data transformation was used on the raw data values of each group of samples to reduce the effects of outliers. For the raw data values, one-way ANOVA was conducted to compare biomarker concentrations between the groups. Next, the data were computed as a function of change from the referent control group. Then, t-tests were conducted to compare each group to the referent control. All tests were two-tailed, and $P < 0.05$ was considered to indicate a statistically significant difference. IBM SPSS Statistics 25 (IBM, New York, USA) software was used for this analysis.

Results

Baseline characteristics: Table II provides the demographics and baseline characteristics for the patients with biopsy-proven sarcoidosis referred for CS evaluation using FDG PET imaging. Overall, age and gender were not different between all sarcoid-positive patients and those with cardiac FDG-positive PET imaging (cardiac FDG (+)). Of the 47 patients scanned, 18 demonstrated positive cardiac FDG uptake, and 29 were negative. For the purposes of definition and clarity, cardiac uptake of FDG by PET (FDG (+)) was defined as the presence of CS. In the cardiac FDG (+) group, the LV ejection fraction was lower, with a tendency for a greater proportion being to be male, as well as a higher prevalence of coronary artery disease and implantable defibrillators. The use of immunosuppression therapy was equally distributed across groups.

Plasma profiling: The overall summary results for the referent controls, the entire sarcoid patient group, and the subsets of patients with cardiac FDG (+) and cardiac FDG (-) are presented in Table III. As expected, and consistent with previous studies, significant biological variability was encountered with the soluble cytokine and proteolytic profiles. However, significant differences emerged nonetheless in terms of differences in absolute values from the referent control group. The results will be first summarized in terms of these absolute values and then presented as a function of transformed values with respect to referent control values.

Soluble cytokine and cytokine receptor profiles: Tumor necrosis factor alpha (TNFα) levels increased with sarcoidosis, whereby absolute TNF values were significantly increased in patients with cardiac FDG (+). IL-8 and IL-10
remained unchanged from referent normal values. Several of the cytokines were at low to non-detectable levels and were expressed in a categorical fashion, such as IL-2, IL-6, and IL-4 (Table III). The relative detectable levels of these interleukins were not different from referent control values (Figure 1). In terms of the soluble cytokine receptor profiles, significant changes occurred in sarcoidosis patients and in cardiac FDG (+) patients (Table III). The TNF receptor family (sCD30, TNFRI, and TNFRII) was increased approximately 2-fold in patients with sarcoidosis and cardiac FDG (+) patients (Table III). The most notable difference from this analysis was that TIMP-1 levels were reduced in the sarcoidosis and cardiac FDG (+) patients as well. Absolute values for soluble receptors from the IL domain demonstrated a variable pattern, with a notable increase in the soluble IL-2Ra receptor and the soluble advanced glycosylation end-product receptor (sRAGE) in the cardiac FDG (+) group. In addition, the soluble receptors for the vascular endothelial growth factor (VEGF) domain (VEGFR2 and VEGFR3) were increased in the cardiac FDG (+) group. When expressed as a function of referent control values, these changes in soluble receptor profiles with sarcoidosis and cardiac FDG (+) became more evident (Figures 2, 3). These results are illustrated in companion figures because of the scaling factors and for clarity in presentation. From this analysis, the majority of plasma values measured from the soluble cytokine receptor array increased in both the sarcoidosis group as a whole and in patients with cardiac FDG positivity, with the exception of the VEGFRI receptor. Overall, plasma values in the FDG (-) group were similar to those of the composite sarcoidosis group.

**MMP and TIMP profiles:** The absolute values for plasma levels of MMPs and TIMPs are provided in Table III. The most notable difference from this analysis was that TIMP-1 levels were reduced in the sarcoidosis and cardiac FDG (+) groups compared to referent control values. When expressed as a function of referent control values (Figure 4), MMP-10 values were increased in both the sarcoidosis and cardiac FDG (+) groups, whereas MMP-9 levels were increased in the sarcoidosis group with a directionally opposite change in MMP-9 levels in the cardiac FDG (+) group. Relative values for all TIMPs (TIMP-1, -2, -3, and -4) fell in both the sarcoidosis and cardiac FDG (+) groups.

**Discussion**

There remains a paucity of diagnostic screening and management tools for sarcoidosis, specifically those patients with known or suspected cardiac involvement, that is, CS. In the present study, CS was defined as a positive cardiac uptake of FDG using PET imaging (FDG (+)). The new and unique findings of the present study have implications regarding plasma profiling of specific cytokine and proteolytic pathways that may give diagnostic

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**Table II. Patient Demographics and Hemodynamics**

| Demographics                      | Referent Control | Sarcoi + | Cardiac FDG + | Cardiac (-) | P value |
|-----------------------------------|-----------------|---------|---------------|-------------|---------|
| Number of patients (%)            | 6               | 47      | 18 (38)       | 29 (62)     |         |
| Age, mean (SD), years             | 55–65           | 57.6 (11.0) | 57.8 (11.4) | 57.5 (10.7) | 0.890   |
| Men, n (%)                        | 31 (66.0)       | 15 (83.3) | 16 (55.2)     |             | 0.062   |
| Ethnicity, n (%)                  |                 |         |               |             | 0.851   |
| White                             | 33 (70.2)       | 12 (66.7) | 21 (72.4)     |             |         |
| Black                             | 11 (23.4)       | 5 (27.8)  | 6 (20.7)      |             |         |
| Hispanic                          | 3 (6.4)         | 1 (5.6)  | 2 (6.9)       |             |         |
| Other                             | 0 (0.0)         | 0 (0.0)  | 0 (0.0)       |             |         |
| Physical and laboratory findings  |                 |         |               |             |         |
| Systolic BP, mean (SD), mm Hg     | 131.8 (18.7)    | 135.8 (13.9) | 130.1 (20.7) | 0.408   |
| Diastolic BP, mean (SD), mm Hg    | 73.9 (11.1)     | 76.5 (10.2) | 73.7 (11.2)   | 0.615   |
| Heart rate, mean (SD), beats/minute | 62.1 (11.0) | 67.6 (9.1)  | 70.2 (11.9)   | 0.376   |
| Ejection fraction, mean (SD), %   | 46.0 (17.4)     | 37.8 (19.8) | 51.9 (14.4)   | 0.025   |
| Medical history, n (%)            |                 |         |               |             |         |
| CAD                               | 5 (10.6)        | 4 (22.2) | 1 (3.4)       | 0.063   |
| Hypertension                      | 22 (46.8)       | 10 (55.6) | 12 (41.4)     | 0.382   |
| History of Ventricular Tachycardia| 222 (31.8)      | 4 (22.2) | 9 (31.0)      | 0.739   |
| Premature Ventricular Contractions| 24 (51.1)       | 9 (50.0)  | 15 (51.7)     | 0.767   |
| Diabetes                          | 8 (17.0)        | 4 (22.2)  | 4 (13.8)      | 0.692   |
| LBBB                              | 3 (15.9)        | 0 (0.0)  | 3 (12.2)      | 0.276   |
| RBBB                              | 10 (21.3)       | 6 (33.3)  | 4 (13.8)      | 0.150   |
| Any AV Block (CHB, BBB, A/PFB)    | 17 (36.2)       | 8 (44.4)  | 9 (31.0)      | 0.371   |
| Regional wall motion abnormalities, echo | 11 (23.4) | 5 (27.8)  | 6 (20.7)      | 0.734   |
| Prior cardiac MRI                 | 30 (63.8)       | 12 (66.7) | 18 (62.0)     |         |
| Positive for LGE                  | 18 (60.0)       | 7 (58.3)  | 11 (61.1)     | 1.000   |
| ICD                               | 25 (53.2)       | 13 (72.2) | 12 (41.4)     | 0.070   |
| Immunosuppression therapy         | 34 (72.3)       | 14 (77.7) | 20 (68.9)     | 0.511   |

BP indicates blood pressure; SD, standard deviation; LGE, late gadolinium enhancement; CAD, coronary artery disease; AV, atrioventricular; CHB, complete heart block; BBB, bundle branch block; A/PFB, anterior or posterior fascicular block; ICD, implantable cardioverter-defibrillator; and R/LB BB, Right/left bundle branch block.
and mechanistic insight into patients with sarcoidosis, particularly CS. First, soluble cytokine receptor profiles, which are indices of inflammation/cytokine activation, were increased in patients with sarcoidosis when compared to referent control values. An example of inflammation/cytokine receptor activation was the fact that soluble TNF receptors increased by approximately 2-fold in patients with sarcoidosis. Second, in absolute terms, cytokine and growth factor receptor activation pathways that promote inflammatory cell migration (sIL-2Ra, VEGFR2, and VEGFR3) were increased to the greatest degree in CS patients. Third, TIMP levels, which inhibit both cytokine (such as TNF) activation and localized tissue proteolysis, were increased in sarcoidosis, including CS, patients. Overall, these findings provide quantitative evidence that cytokine cascades, which promote localized inflammation and degradation of normal tissue parenchyma, could promote the progression of sarcoidosis, particularly CS.

### Table III. Cytokine, Cytokine Receptors, and MMP/TIMP Plasma Profiles for Referent Control and Sarcoid Patients

| Plasma Cytokine Profiles | Referent Control (n = 6) | Sarcoid Biopsy + (n = 47) | Cardiac FDG + (n = 18) | Cardiac FDG − (n = 29) |
|--------------------------|-------------------------|---------------------------|-----------------------|-----------------------|
| **Cytokine (pg/mL)**     |                         |                           |                       |                       |
| IFN-γ – Interferon gamma | 0.66 ± 0.19             | 0.68 ± 0.08               | 0.69 ± 0.15           | 0.67 ± 0.10           |
| TNF-α – Tumor necrosis factor alpha | 18.53 ± 1.83 | 26.73 ± 3.29              | 33.98 ± 5.27*         | 22.23 ± 1.69          |
| IL-6 – Interleukin 6     | 1.80 ± 0.33             | 2.07 ± 0.24               | 2.42 ± 0.50           | 1.86 ± 0.23           |
| IL-10 – Interleukin 10   | 3.53 ± 0.56             | 3.16 ± 0.19               | 3.10 ± 0.21           | 3.19 ± 0.29           |
| **Percent of Detectable Samples [%] [#]** |                       |                           |                       |                       |
| IL-6 – Interleukin 6     | 0, (0)                  | 11, (5)                   | 6, (1)                | 14, (4)               |
| GM-CSF – Granulocyte-macrophage colony-stimulating factor | 0, (0) | 6, (3) | 0, (0) | 10, (3) |
| IL-2 – Interleukin 2     | 33, (2)                 | 28, (13)                  | 39, (7)               | 21, (6)               |
| IL-4 – Interleukin 4     | 17, (1)                 | 2, (1)                    | 0, (0)                | 3, (1)                |
| **Soluble Cytokine Receptor Profiles** |                       |                           |                       |                       |
| sCD30 – Tumor necrosis factor receptor superfamily, member 8 | 22 ± 1 | 54 ± 6* | 60 ± 11* | 51 ± 6 |
| sEGFR – Epidermal growth factor receptor | 33.917 ± 5.165 | 55.574 ± 4.698 | 58.966 ± 8.868 | 53.469 ± 5.469 |
| sgp130 – Interleukin 6 signal transducer | 124.464 ± 40.657 | 240.684 ± 28.120 | 238.881 ± 48.582 | 241.803 ± 34.868 |
| sIL-1R1 – Interleukin 1 receptor, type I | 162 ± 53 | 373 ± 36* | 371 ± 57 | 375 ± 48* |
| sIL-1R1I – Interleukin 1 receptor, type II | 11.296 ± 3.347 | 22.714 ± 2.939 | 29.804 ± 5.281* | 18.312 ± 2.363 |
| sIL-2RA – Interleukin 2 receptor, alpha | 781 ± 231 | 2.028 ± 299 | 3.080 ± 613* | 1.375 ± 238 |
| sIL-4R – Interleukin 4 receptor | 11.632 ± 5.469 | 22.237 ± 2.952 | 26.600 ± 5.144 | 19.530 ± 3.542 |
| sIL-6R – Interleukin 6 receptor | 43.467 ± 17.180 | 89.141 ± 10.544 | 96.621 ± 16.461 | 84.498 ± 13.852 |
| sRAGE – Advanced glycosylation end product-specific receptor | 403 ± 162 | 939 ± 107 | 1.111 ± 189* | 832 ± 126 |
| sTNFRI – Tumor necrosis factor receptor superfamily, member 1A | 1.061 ± 294 | 2.296 ± 216* | 2.624 ± 328* | 2.093 ± 283 |
| sTNFRII – Tumor necrosis factor receptor superfamily, member 1B | 8.156 ± 1.766 | 15.474 ± 1.598 | 19.596 ± 2.826* | 12.916 ± 1.783 |
| sVEGFR1 – Fms-related tyrosine kinase 1 | 13.319 ± 5.857 | 11.821 ± 1.522 | 13.735 ± 2.627 | 10.633 ± 1.853 |
| sVEGFR2 – Kinase insert domain protein receptor | 9.149 ± 1.298 | 15.168 ± 1.340 | 17.262 ± 2.850* | 13.869 ± 1.250 |
| sVEGFR3 – Fms-related tyrosine kinase 4 | 7.783 ± 3.603 | 28.730 ± 3.994 | 33.943 ± 6.612* | 25.494 ± 5.003 |
| **Plasma MMP and TIMP Profiles** |                       |                           |                       |                       |
| MMP (pg/mL)              |                         |                           |                       |                       |
| MMP 1 – Matrix Metalloproteinase 1 | 4.428 ± 764 | 3.385 ± 462 | 3.267 ± 770 | 3.459 ± 586 |
| MMP 2 – Matrix Metalloproteinase 2 [× 10³] | 303 ± 59 | 324 ± 30 | 312 ± 54 | 332 ± 36 |
| MMP 7 – Matrix Metalloproteinase 7 | 603 ± 170 | 659 ± 38 | 689 ± 65 | 640 ± 48 |
| MMP 9 – Matrix Metalloproteinase 9 [× 10³] | 91 ± 11 | 112 ± 10 | 79 ± 7 | 132 ± 15 |
| MMP 10 – Matrix Metalloproteinase 10 | 549 ± 93 | 805 ± 61 | 863 ± 107 | 770 ± 74 |
| TIMP 1 – Tissue inhibitor of metalloproteinases 1 [× 10³] | 149 ± 11 | 116 ± 6* | 113 ± 12* | 119 ± 6 |
| TIMP 2 – Tissue inhibitor of metalloproteinases 2 [× 10³] | 131 ± 13 | 121 ± 5 | 110 ± 9 | 128 ± 6 |
| TIMP 3 – Tissue inhibitor of metalloproteinases 3 | 17.157 ± 1.447 | 15.488 ± 577 | 14.296 ± 662 | 16.212 ± 815 |
| TIMP 4 – Tissue inhibitor of metalloproteinases 4 | 2.227 ± 226 | 2.024 ± 100 | 1.909 ± 129 | 2.094 ± 140 |

*P < 0.05 versus Referent Control, ^P < 0.05 versus Sarcoid Biopsy +.
FDG imaging and potential integration of biomarker profiling: The presence of focal myocardial FDG uptake together with perfusion defects by resting PET imaging can provide additional prognostic value in CS patients. Moreover, FDG-PET imaging can allow for serial assessment during treatment. In addition, the presence of “extra cardiac” FDG uptake on PET imaging in patients with suspected CS has been found to be diagnostically useful.

Based on those data and others, FDG PET imaging is recommended by consensus statements for the evaluation of CS. Although our group has been an active proponent of FDG PET imaging in CS, we recently raised concerns about the repeatability of FDG PET for serial imaging in CS. Thus, the present study suggests the intriguing possibility that a biomarker panel could be used as an adjunct to achieve additional accuracy for CS evaluation.
Furthermore, biomarker profiles could at least moderate the need for frequent serial imaging for therapeutic monitoring. The present study is the first to directly examine the relationship between a plasma-based biomarker approach and FDG PET imaging in patients with CS.

**Plasma profiling in sarcoidosis and cardiac involvement:** Multiple biomarkers have been proposed and evaluated in small studies of patients with CS. These include TNFα, soluble interleukin-2 receptor, angiotensin converting enzyme, cardiac troponins, BNP, miR-126 and miR-223, and the oxidative stress marker 8-hydroxy-2'-deoxyguanosine (U-8-OHdG). However, there remains a paucity of biomarkers specific for active cardiac involvement, and the development of additional CS-specific clinical markers through larger, more robust studies could facilitate more targeted immunosuppressive therapy for patients at elevated risk of adverse outcomes within the sarcoidosis patient population.

Our findings include the marked elevation in the TIMP-3-TNF signaling axis with a trend toward a greater magnitude in patients with active disease as compared to that of systemic sarcoidosis patients without inflammation by FDG at the time of the blood sample. This is concordant with several prior reports, with the prior studies notably limited to pulmonary disease. TNFα has been implicated in granuloma development and maintenance, and soluble TNF receptors are elevated in both BAL fluid and in the blood in pulmonary sarcoidosis patients. In addition, TNF receptor levels are suppressed by corticosteroid therapy, and, conversely, are further elevated in patients with radiographically progressive disease on follow-up. Finally, there is evidence for anti-TNF therapies in cases where corticosteroid therapy fails to achieve adequate disease control.

A key finding of our study is that of increased IL-2 and VEGF signaling in sarcoidosis patients, which is concordant with the established role of these pathways in promoting localized inflammation. VEGF secretion by T cells and macrophages leads to increased production of macrophage chemoattractant protein 1 (MCP-1) by endothelial cells, which in turn recruits more monocytes and facilitates transmigration by increasing endothelial permeability. Although the role of IL-2 in autoimmunity is complex, it is also a key secreted factor in the ongoing T-cell and macrophage recruitment of localized inflammation. The production of the IL-2 receptor by T cells is tightly regulated at the transcriptional and post-transcriptional levels, and the soluble interleukin-2 receptor has been investigated for clinical utility as a marker of dermatological, pulmonary, and multi-organ system sarcoidosis. The present study’s evidence of increased activity of the IL-2 and VEGF signaling pathways supports their role in granuloma pathophysiology and as markers of disease activity.

A second key finding of this study is that sarcoidosis is accompanied by changes in the expression and activation states of extracellular matrix remodeling enzymes that are detectable in the blood and that have been observed in

**Figure 3.** Soluble cytokine receptor profiles in patients with sarcoidosis and in the cardiac sarcoidosis (CS) cohort. The values shown are the absolute change in plasma levels from referent control values. Increased soluble receptor levels, indicative of cytokine activation, increased for a number of IL, TNF, and VEGF pathways. *P < 0.05 versus absolute change from referent control values. Absolute values and sample sizes are shown in Table III.
myocardial remodeling and heart failure in multiple other etiologies. We explored the postulate that matrix remodeling and fibrosis could serve as potential biomarkers, as cardiac remodeling and fibrosis is an important component of CS progression and prognosis. Imaging markers of active MMPs are in early-stage clinical development for human use, raising the potential for an imaging/biomarker combinatorial strategy focused on MMPs in CS.

Several lines of evidence point toward matrix remodeling in the pathogenesis of sarcoidosis. Biopsy immunohistochemistry from patients with cardiac and pulmonary sarcoidosis reveals an abundance of MMPs and overall paucity of TIMPs with levels of MMPs varying by cell type within granulomas. Some genetic polymorphisms in MMP and TIMP genes alter the genes’ expression levels in the blood of sarcoidosis patients. The proteases MMP-12 and ADAMDEC1 are highly expressed in lung tissue and BAL fluid, and macrophages obtained by BAL produce increased levels of MMP-9 in a p38-dependent and NF-kB-dependent manner, identifying MMP-9 as a downstream target of inflammatory cytokine signaling. Although MMP-10 upregulation has been observed in tuberculosis, the upregulation of MMP-10 in sarcoidosis is a novel finding of the present study. Our data extend these findings to CS and to a patient population defined by imaging as having active disease at the time of blood sample collection.

In the present study, immunosuppression therapy had been instituted in the majority of sarcoidosis patients with no difference in the penetrance of this treatment modality in the CS patient cohort. The use of immunosuppression therapy in CS is highly variable in terms of duration and intensity, and the current study had no exception to this limitation. Irrespective of this variable background immunosuppression therapy, the present study identified robust activation of cytokine pathways such as TNF, IL-2, and VEGF in the sarcoidosis patients, with further elevation of several of these cytokine pathways in CS patients. These findings suggest that immunosuppression therapy may be inadequate in terms of duration and intensity and pose several questions for future study. First, can biomarker profiling coupled with titration of immunosuppression therapy reduce the degree of active disease? Second, can strategic targeting of the TNF, IL, and VEGF axes through more selective immunotherapies, reduce the presence and severity of CS as assessed by methods such as FDG PET imaging?

Study limitations: This is a single-center cohort, which may limit the degree to which these findings may be generalized to centers with differing patient populations or imaging protocols.

Despite the growing use of FDG PET to guide therapeutic decisions in sarcoid management, the absolute reduction in FDG uptake that represents a meaningful clinical reduction in active inflammation is not yet defined.
This study utilized thoracic FDG PET only and thus, did not assess sarcoidosis present outside the single field-of-view scan. This could include extra-thoracic organ involvement that may be out of the field of view in the cardiac imaging window. In addition, despite strict inclusion criteria mandating documentation of systemic sarcoidosis, we did not control for the duration of sarcoidosis and/or other co-morbidities that may have affected the signaling pathways we explored. Finally, this study provides associative data regarding inflammation and proteolytic pathways in terms of sarcoidosis and CS, and a cause-effect relation will require future-targeted interventional strategies.

Conclusions

Using a plasma-based biomarker array, this study identified increased activation of a cluster of soluble cytokine receptors, which can augment inflammatory cell maturation and transmigration in patients with sarcoidosis and CS. Proteolytic profiling indicated that a favorable environment exists for tissue/matrix remodeling that would favor disease progression. Profiling receptor/proteolytic activation occurred in patients with sarcoidosis, particularly with a provided diagnostic value and therapeutic relevance. FDG PET is emerging as the modality of choice for monitoring disease status in CS patients because of its ability to differentiate an active inflammatory infiltrate from a scar. By acquiring plasma at the time of FDG PET imaging, this study identified a biomarker subset specific for patients with active disease. Such biomarkers may augment imaging as decisions made regarding titration of immunosuppressive therapy. The identification of soluble factors unique to active disease may also yield new therapeutic targets.

Disclosure

Conflicts of interest: Dr. Miller is a consultant for GE, Pfizer, Eidos, and Alnylam, unrelated to this study.

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