Residential fungal β-(1,3)-D-glucan exposure is associated with decreased pulmonary function in fibrotic pulmonary sarcoidosis

Emma M Stapleton[1], Nervana Metwali[2], Michael Shlossman[1], Linder Wendt[3], Alejandro A Pezzulo[1], Nabeel Y Hamzeh[1], Alejandro P Comellas[1], Peter S Thorne[2], Alicia K Gerke[1]

[1]Department of Internal Medicine, Division of Pulmonary, Critical Care and Occupational Medicine, University of Iowa, Iowa City, IA, USA; [2]Department of Occupational & Environmental Health, College of Public Health, University of Iowa, Iowa City, IA, USA; [3]Institute for Clinical and Translational Science, University of Iowa, Iowa City, IA, USA

Abstract. *Background and aim:* Sarcoidosis is a multi-system disease frequently affecting the lungs. It is thought to be mediated by gene-environment interaction; for example, epidemiological data show organic aerosol exposure increases risk of pulmonary sarcoidosis. The aim of this study was to assess whether exposure to bioaerosol associates with worse lung disease in patients with pulmonary sarcoidosis. *Methods:* Using an observational, cohort study design, we measured residential exposure to fungal and bacterial cell wall material, β-(1,3)-D-glucan (BDG) and endotoxin, respectively, in healthy control subjects and those with pulmonary sarcoidosis. In the case cohort, we compared bioaerosol concentrations to pulmonary disease severity, assessed by pulmonary function testing, qualitative chest computed tomography (CT), and serum biomarkers. Log-transformed bioaerosol concentrations were compared to lung function and significance and correlation determined by Pearson correlation. *Results:* Homes of subjects with sarcoidosis had higher BDG and endotoxin concentrations than control subjects. Patients with significant pulmonary fibrosis had greater disease severity (Wasfi severity score, visual analogue scale) and reduced pulmonary function compared to those without fibrosis (all *P*<0.01). Residential fungal BDG correlated with declining FVC, only in patients with fibrosis on CT imaging (*P*=0.02). Survey data revealed higher BDG concentrations were found in homes of cat-owners, and the number of houseplants owned correlated with declines in FVC and FEV$_1$ (*P*=0.05 and 0.02, respectively). In patients without fibrosis, eight inflammatory markers correlated with BDG (6CKine/CCL21, IL-9, IL-17F, IL-21, IL-28A, I-309, MIP-1β, TARC), while in those with pulmonary fibrosis, BDG correlated with two inflammatory markers (Eotaxin-3, M-CSF), suggesting immune anergy to inhaled antigens in patients with fibrosis. *Conclusions:* In patients with pulmonary fibrosis, disease severity was correlated with residential exposure to fungal cell wall material, but not gram-negative bacterial cell wall material. These patients may experience immune anergy to inhaled antigens.

Key words: sarcoidosis, pulmonary fibrosis, indoor air, bioaerosol, β-(1,3)-D-glucan

Introduction

Sarcoidosis is a multi-system inflammatory granulomatous syndrome principally affecting the lungs; the disease has no available cure (1). It's exact cause remains unknown, but various triggers of sarcoidosis-like granulomatous response have been proposed, including environmental antigens (e.g., silica, talc, mycobacterial (2–5), and *Cutibacterium* (previously

Propionibacterium) *acnes* (6). Gene-environment interaction is strongly suggested (7), with exposures ranging from inorganic (8), to organic antigens (6,9). Several studies, including the multi-center epidemiological study—A Case Control Etiologic Study of Sarcoidosis (ACCESS)— associate exposure to high humidity, water damage and mold/musty odor with sarcoidosis and pulmonary-specific sarcoidosis (7,10–13). Additionally, previous work found patients with newly diagnosed and active disease had greater residential microorganism biomarker concentrations (non-specific N-acetylhexosaminidase) than controls and those without disease recurrence (14). Lastly, bronchoalveolar lavage (BAL) from patients with Löfgren's syndrome was found to have a T-cell receptor epitope recognized and stimulated by an *Aspergillus nidulans*-specific peptide (15). Therefore, a plausible source of perpetuating antigens are bio-aerosols – airborne particulate matter derived from microbes, plants or animals, including inhalant allergens, pollens, fungal spores, bacteria, and viruses (16). Fungal-derived bioaerosols can vary widely in spore size, culturability, phyla, and branching pattern, but purified polysaccharide fungal cell wall material (β-ᴅ-glucan, BDG) is a common fungal biomarker capable of activating the immune system via pattern recognition receptors. Similarly, exposure to pro-inflammatory gram-negative bacterial endotoxin can be estimated by quantifying lipopolysaccharide cell wall material (17). Despite strong epidemiological data, research efforts to systematically assess personal exposure to proposed aerosolized antigens are lacking (13). We asked whether exposure to bioaerosol associates with worse lung disease in patients with pulmonary sarcoidosis with the hypothesis that exposure to residential bioaerosols differentially affects disease course in subjects with pulmonary fibrosis compared to those without fibrosis. To test this hypothesis, we measured fungal and bacterial bioaerosols in residences of control subjects and those with pulmonary sarcoidosis and compared BDG and endotoxin concentrations to pulmonary function outcomes and serum biomarkers.

Materials and Methods

Cohort

In our observational cohort study, adult patients over the age of 18 with biopsy-proven pulmonary sarcoidosis were enrolled at the University's Interstitial Lung Disease clinic. This study has obtained ethics approval from the IRB review board-01 #IRB00000099. Study protocol followed the University's Institutional Review Board requirements (IRBs: #201709743, 201909726, 202009330, Approved by IRB Chair). Subjects completed informed written consent prior to study participation for all study procedures. Homes were predominately located in eastern Iowa with a few recruits from surrounding states (Illinois and Wisconsin). Control subjects for the residential assessment were recruited via advertising for people living within 30 miles of the same academic medical center, as closely age- and sex-matched as possible. Subjects with sarcoidosis completed a survey interrogating daily habits and exposure sources (N=55, survey included in Table S1).

Pulmonary Function Tests (PFTs)

PFT measurements (pre-bronchodilator percent predicted forced vital capacity, FVC, forced expiratory volume in one second FEV_1, FVC/FEV_1 ratio, and diffusing capacity of carbon monoxide, DLCO) temporally nearest bioaerosol measurement were extracted from the medical records.

Disease severity assessment and quantification

Patient medical records were reviewed to extract demographics and clinical indicators of disease severity, including details of confirmed or probable extrapulmonary organ involvement, disease duration (according to diagnostic guidelines (18), and the use of immunosuppression (IS). This information was used to calculate a Wasfi Severity Score according to the multivariate model derived by Wafsi *et al.* (19), details, see Table S2). The presence or absence of lung fibrosis was qualitatively assessed by a clinical radiologist using clinical chest CT scans and confirmed by expert sarcoidosis physician (AKG). Additionally global disease severity was assessed using a tool applied in various diseases (19,20). Patients were rated for disease severity by visual analog scale (VAS). An expert clinician (AKG) blinded to exposure data marked a 5-inch horizontal line, whereby 0 indicated incidentally discovered asymptomatic disease and 10 denoted severe end-stage organ dysfunctions (e.g., progressive fibrotic lung disease requiring

consideration for transplant). The distance from 0 to the mark (in inches), multiplied by 2 defined a patient's VAS score. We included sarcoidosis health questionnaire (SHQ) data regarding daily, emotional and physical function (21) in those who completed these clinical assessments.

Residential bioaerosol exposure assessment

Residential sampling occurred year-round, commencing in March 2021 and finishing in October 2023. Sampling was carried out in the Midwest, typically in rural and peri-urban locations. Electrostatic dust collectors (EDCs) were placed at approximate breathing zone height using sterile endotoxin-free gloves in the primary household occupied room for 2 weeks (22), and subsequently mailed back to our University. EDC cloths were then removed and stored at -20°C. Field and lab blanks (7% of samples) were collected and analyzed identically to the samples.

EDC β-(1,3)-ᴅ-glucan extraction

β-(1,3)-ᴅ-glucan (BDG) was determined using the Glucatell® assay (Associates of Cape Cod, Inc., East Falmouth, MA, USA) of air samples collected on the EDC cloths, based on a modification of the Limulus Amebocyte Lysate Factor G pathway. Room temperature samples were eluted into LAL reagent water (Lonza RTP, Durham, NC, USA), with vortexing and shaking on an orbital shaker (30 min.) followed by sonication (30 min., 22°C), and further shaking (10 min.). Extracts were then centrifuged (15 min., 600 x g, 4°C) and mixed with NaOH (final 0.3N), then shaken (4°C, 30 min.), centrifuged (15 min., 600 x g), and analyzed. BDG was quantified at 37°C using a multimode microplate reader (Molecular Devices M5) at 550 nm. The reagent does not react to other polysaccharides, including β glucans with different glycosidic linkages and is a well-established β-(1,3)-ᴅ-glucan measurement method (23).

EDC endotoxin extraction

Endotoxin was extracted from EDCs by established kinetic chromogenic Limulus amebocyte lysate assay methodology (24,25), with a minor modification (additional 30 min. sonication of suspended cloths).

Phlebotomy and serum cytokine processing

Venipuncture was performed on consented individuals within approximately one year of residential measurement. Of our case cohort, serum was obtained from 36 individuals (no pulmonary fibrosis, N=22; with pulmonary fibrosis N=14). Serum and plasma separator tubes were centrifuged (10 min., 2000 RPM), deidentified and stored at -80°C. Serum was centrifuged (10 min., 1000g, 4°C) then assayed using the Human Cytokine/Chemokine 71-Plex Discovery Assay® Array and the Human Supplemental Biomarker 10-Plex Discovery Assay® Array (Eve Technologies, Alberta, CA, for details, see Table S3).

Statistical analyses

Population size was determined by power analysis (G*Power version 3.1.9.7) based on a publication of a Slovenian population with sarcoidosis – the study measure units of non-specific NAHA from 4hr. filter measurements (14). Control subject homes had a mean NAHA concentration of 10 U/m^3 (N=30, SD=6) and homes of subjects with newly diagnosed sarcoidosis (N=55, SD=37) was 33.6 U/m^3. Our two-tailed *a priori* *t*-test analysis included α=0.05 and a power of 0.8. We allocated 2 patients:1 control subject, for a total sample size of 48 (32 with sarcoidosis, 16 without). To assure sufficient power, we recruited 84 subjects (59 with sarcoidosis, 25 without). Residential characteristics were assessed using R (v.4.3.3) by comparing raw BDG values across various strata via Kruskal-Wallis rank sum tests and Wilcoxon rank sum tests. Comparisons between subjects with sarcoidosis and control subjects were made using Fisher's exact test for categorical variables and using Welch two sample *T*-tests for continuous variables. Right-skewed data were log-transformed before *T*-tests were implemented to properly meet *T*-test assumptions. The same tests were used to compare characteristics of non-fibrotic and fibrotic patients. Remaining statistical analyses were performed using GraphPad Prism (v.10.2.3). Log-transformed bioaerosol concentrations (BDG, endotoxin) were compared to lung function and significance and correlation determined by Pearson correlation. Trend lines were determined by simple linear regression. Differences in residential bioaerosol levels between those with and without significant pulmonary fibrosis were analyzed by unpaired

T-tests. One-way ANOVA with Tukey's adjustment for multiple comparisons was used to assess the effect of season on bioaerosol exposures. Next, BDG and endotoxin concentrations were log transformed and compared to lung function and log-transformed houseplant number +0.1 by simple linear regression. Serum cytokine data were checked for outliers by ROUT, and definitive outliers removed (Q=0.1%), then their association with log-transformed BDG concentrations was evaluated by Pearson r. Herein we present data with a *P* value ≤0.11 and considered significance at *P*≤0.05.

Results

Demographics and sarcoidosis disease severity

Year-round bioaerosol sampling occurred between March 2021-May 2024. Measurements from 59 homes of subjects with pulmonary sarcoidosis and 25 without sarcoidosis were included. The population with sarcoidosis was older (56.7 vs. 42.2 years, *P*<0.01) compared to that without sarcoidosis. Homes of subjects with sarcoidosis had significantly greater BDG and endotoxin concentrations than control subject homes (BDG geometric mean: 1,320.0 vs. 160.5 ng/m², *P*<0.01; endotoxin geometric mean: 934.8 vs. 263.0 EU/m², *P*<0.01; Table 1). Lab and field blank concentrations were well below sample values (BDG geometric mean=0.03 ng/mL, endotoxin geometric mean= 0.31 EU/mL).

We next evaluated differences in our case cohort between those with sarcoidosis experiencing significant fibrotic pulmonary disease compared to those without pulmonary fibrosis. Subjects with fibrotic pulmonary disease were predominantly male, and disease severity was significantly greater, reflected by higher VAS and Wasfi score (*P*<0.01), and lower lung function (*P*<0.01, all). More males were on IS (60% vs. 41% of cohort, data not shown). There were no significant differences in bioaerosol concentrations between the two groups (Table 2). Subjects with pulmonary fibrosis also tended toward less extrapulmonary organ involvement.

Behavioral and housing characteristics associated with BDG concentrations

To assess bioaerosol sources, residential BDG concentrations were compared to housing and behavioral characteristics in our case cohort (Table S1). Residential BDG concentrations were positively associated with cat ownership and daily cooking, *P*<0.05. Number of days anything is burned in the house tended to positively associate with BDG concentration (*P*=0.09), while use of plastic cooking materials (e.g., non-stick), ionizing air purifiers, and recent construction trended as protective (*P*=0.08, *P*=0.11, *P*=0.09, respectively; Table 3).

Table 1. Demographics and residential bioaerosol concentrations of participants with (N=59) and without (N=25) pulmonary sarcoidosis*. Bold text indicates statistical significance (P≤0.05)

Characteristic	Control subjects N = 25	Sarcoidosis N = 59	*P*
Sex			>0.99
F	15 (60%)[1]	35 (59%)[1]	
M	10 (40%)[1]	24 (41%)[1]	
Age	42.2 (11.5)[2]	56.7 (12.7)[2]	**<0.01**
Race			0.54
Other/Unspecified	6 (25%)[1]	10 (17%)[1]	
White	18 (75%)[1]	49 (83%)[1]	
(Missing)	1	0	
β-D-glucan (ng/m²)	160.5 (6.4)[3]	1,320.0 (8.9)[3]	**<0.01**
Endotoxin (EU/m²)	263.0 (3.9)[3]	934.8 (4.4)[3]	**<0.01**

*1/59 patients diagnosed clinically, and not by biopsy.
[1] n (%).
[2] Mean (Standard Deviation, SD).
[3] Geometric Mean (Geometric SD).

Table 2. Demographics, pulmonary and extrapulmonary disease severity, and residential bioaerosol concentrations of participants with (N=19) and without (N=40) fibrotic pulmonary sarcoidosis, determined by expert assessment of clinical chest CT scans. Bold text indicates statistical significance (P≤0.05)

Characteristic	Non-fibrotic N = 40	Fibrotic N = 19	*P*
Age	56 (13)[2]	58 (13)[2]	0.58[5]
Sex			**0.02[4]**
F	28 (70%)[1]	7 (37%)[1]	
M	12 (30%)[1]	12 (63%)[1]	
Race			>0.99[4]
Other/Unspecified	7 (18%)[1]	3 (16%)[1]	
White	33 (83%)[1]	16 (84%)[1]	
Ever smoker (tobacco)	14 (35%)[1]	6 (32%)[1]	>0.99[4]
Immunosuppression (IS)*			0.56[4]
None	21 (62%)[1]	7 (41%)[1]	
Non-Steroidal	7 (21%)[1]	6 (35%)[1]	
Steroidal	3 (8.8%)[1]	2 (12%)[1]	
Both	3 (8.8%)[1]	2 (12%)[1]	
(Missing)	6	2	
Extrapulmonary involvement**			0.09[4]
No	13 (33%)[1]	11 (58%)[1]	
Yes	27 (67%)[1]	8 (42%)[1]	
VAS	3.46 (2.30)[2]	5.82 (2.23)[2]	**<0.01[5]**
Wasfi (19) Severity Score	7.10 (2.02)[2]	8.75 (1.81)[2]	**<0.01[5]**
SHQ (21)*** daily functioning	4.51 (1.20)[2]	4.45 (0.80)[2]	0.85[5]
(Missing)	12	5	
SHQ emotional functioning	4.74 (0.96)[2]	4.43 (0.66)[2]	0.23[5]
(Missing)	12	5	
SHQ physical functioning	4.38 (1.37)[2]	4.12 (0.78)[2]	0.44[5]
(Missing)	12	5	
FVC (% predicted)	97 (14)[2]	82 (16)[2]	**<0.01[5]**
FEV_1 (% predicted)	96 (16)[2]	70 (19)[2]	**<0.01[5]**
FEV_1/FVC ratio	0.78 (0.07)[2]	0.66 (0.13)[2]	**<0.01[5]**
DLCO (% predicted)	94 (15)[2]	72 (16)[2]	**<0.01[5]**
β-ᴅ-glucan (ng/m^2)	1,136 (8.1)[3]	1,808 (10.9)[3]	0.47[6]
Endotoxin (EU/m^2)	897 (4.7)[3]	1,018 (4.0)[3]	0.75[6]

[1]N (%).
[2]Mean (SD).
[3]Geometric Mean (Geometric SD).
[4]Fisher's exact test.
[5]Welch Two Sample T-test.
[6]Welch Two Sample T-test performed on log-transformed values.
*Immunosuppression (IS) was assessed on participants in whom phlebotomy was performed, N=51. Non-steroidal medications included methotrexate, azathioprine, mycophenolate mofetil, leflunomide, and any biologic (N=1); steroidal medication included prednisone; both non-steroidal and IS also included hydroxychloroquine.
**Extrapulmonary involvement was not considered in participants with non-clinically significant liver function abnormalities.
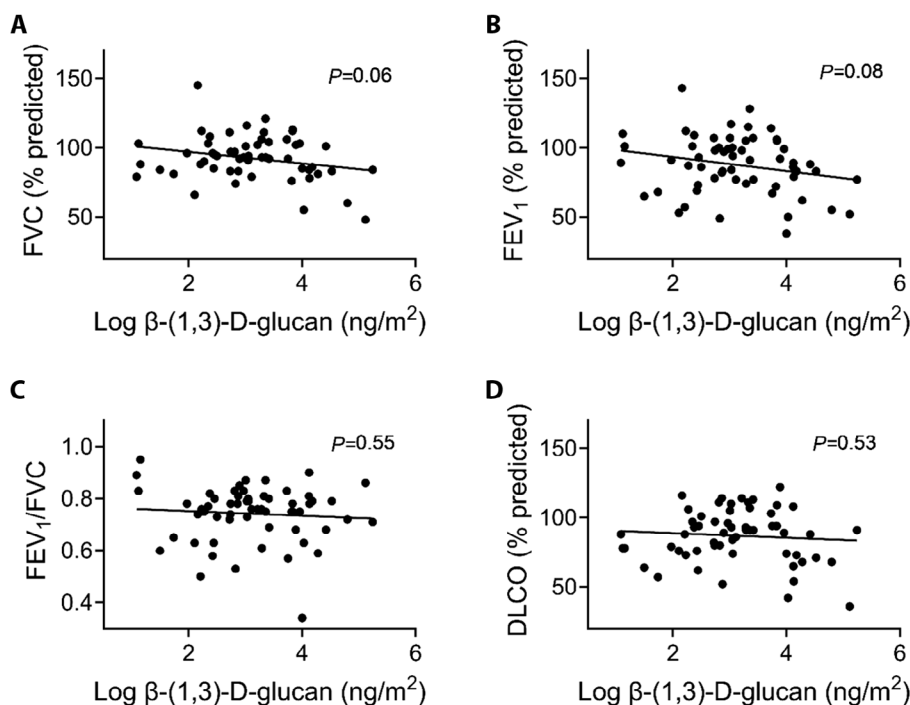***SHQ: Sarcoidosis Health Questionnaire.

Figure 1. Residential β-(1,3)-ᴅ-glucan exposure and lung function in cohort of participants with pulmonary sarcoidosis. **A)** Percent predicted FVC tended to decrease as BDG concentration increased, P=0.06; **B)** Percent predicted FEV_1 tended to decrease as residential BDG concentration increased, P=0.08; **C)** Percent predicted FEV_1/FVC was not related to residential BDG concentrations; P=0.55; **D)** FEV_1/FVC was not related to log-transformed BDG concentrations, P=0.53; N=59.

lung disease and risk/benefit of therapy. The patients not on therapy had either not had significant response to immunosuppression or had been stable for a period of time and immunosuppression was being held while lung function was being monitored.

Discussion

Sarcoidosis pathogenesis has long been thought to be influenced by inhaled environmental antigens, yet direct measurement of residential exposures is lacking. Our study revealed homes of participants with pulmonary sarcoidosis had significantly greater bioaerosol concentrations than those without sarcoidosis. Among our case cohort, pulmonary disease severity was associated with residential fungal BDG concentration. Specifically, residential BDG associated with FVC decline in subjects with pulmonary fibrosis, but not in those without fibrosis. This relationship was not observed with gram-negative bacterial endotoxin concentration. Subjects with

sarcoidosis-related pulmonary fibrosis seem to be more susceptible to residential bioaerosol exposure compared to those with a milder or self-resolving phenotype. These data suggest fungal antigens as potential agents responsible for perpetuating sarcoidosis pulmonary disease severity. Increasing evidence points towards a role for fungal antigens to incite or perpetuate pulmonary disease. For example, the specific glucan analyzed in our study (β-(1,3)-ᴅ-glucan) can increase inflammatory infiltrate into the airway, and prime cells for enhanced inflammatory response to secondary stimuli in a murine model (27). Bioaerosol has also been shown to cause and worsen pulmonary fibrosis (28,29) and in patients with sarcoidosis, peripheral blood mononuclear cell (PBMC) production of IL-12 is increased after stimulation with particulate-BDG (30). In our study, eight cytokines (CCL21, a T-cell chemoattractant, IL-9, IL-17F, IL-21, IL-28A, I-309, MIP-1β, and TARC) significantly correlated with BDG exposure in those with milder disease (no fibrosis), while only
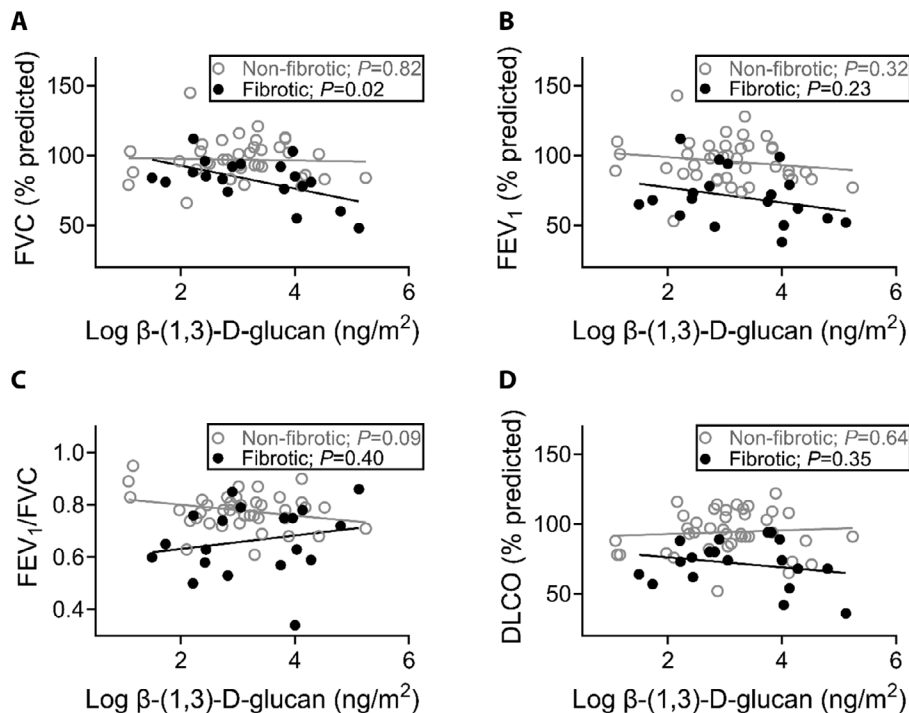
Figure 2. In participants with fibrotic pulmonary sarcoidosis, increased residential exposure to β-(1,3)-D-glucan is correlated with lower FVC. **A)** Percent predicted FVC did not decrease in relation to residential BDG in participants without fibrosis, but did in those with fibrosis (P=0.82 and P=0.02); **B)** Percent predicted FEV$_1$ was not related to log-transformed BDG concentrations in either group (non-fibrotic, P=0.32; fibrotic, P=0.23); **C)** FEV$_1$/FVC tended to be related to residential BDG concentrations in those without fibrosis but was unrelated in those with fibrosis (non-fibrotic, P=0.09; fibrotic, P=0.40); **D)** Percent predicted DLCO was not related to log-transformed BDG concentrations in either group (non-fibrotic, P=0.64; fibrotic, P=0.35); non-fibrotic N=40, fibrotic N=19.

two cytokines (eotaxin-3 and M-CSF) were correlated in those with fibrosis (Table 4). This muted inflammatory response to inhaled antigens in subjects with pulmonary fibrosis might indicate impaired immune surveillance toward exogenous ligands. Impaired regulatory T-cell anergy has been previously reported in sarcoidosis (31). Across the case cohort, serum IL-28A concentrations consistently correlated with residential BDG exposures (Table 4). IL-28A is structurally similar to IL-10 family cytokines, binding with the IL-10 receptor β chain. It is considered a Type III interferon (IFN-λ2) with potent anti-viral and antibacterial activity (32). Type-III interferons protect epithelial barriers, which first encounter incoming pathogens including aerosolized BDGs (33). They can promote Th1 skewing (34), a well-established pathway for sarcoidosis pathogenesis (35). Because IL-28A can be activated by bacterial components, and increases TLR2 expression (36),

BDG binding to pattern recognition receptors likely mediate the increased IL-28A concentrations observed with BDG exposure. Interestingly, Eotaxin-3, a chemokine for eosinophils, consistently correlated with residential BDG exposure across the case cohort independent of disease severity, despite previous work showing increased IFN-λ reduces Th2 cytokines. Sarcoidosis lung histopathology has shown eosinophil adhesion to capillary endothelial cells (37). Eotaxin-3 is characteristic of allergic response and contributes to the fibrotic process; for example, airway eotaxin-3 promotes fibroblast migration (38,39). Of note, fungal *Aspergillus fumigatus* allergen injection is a murine model to study fibrosis of the esophagus, which eotaxin-3 contributes to (39,40). Accordingly, eotaxin-3 response to inhaled fungal allergen might contribute to worsening pathology in sarcoidosis and warrants further study. Eotaxin-3 was one of the two cytokines related to BDG and fibrotic
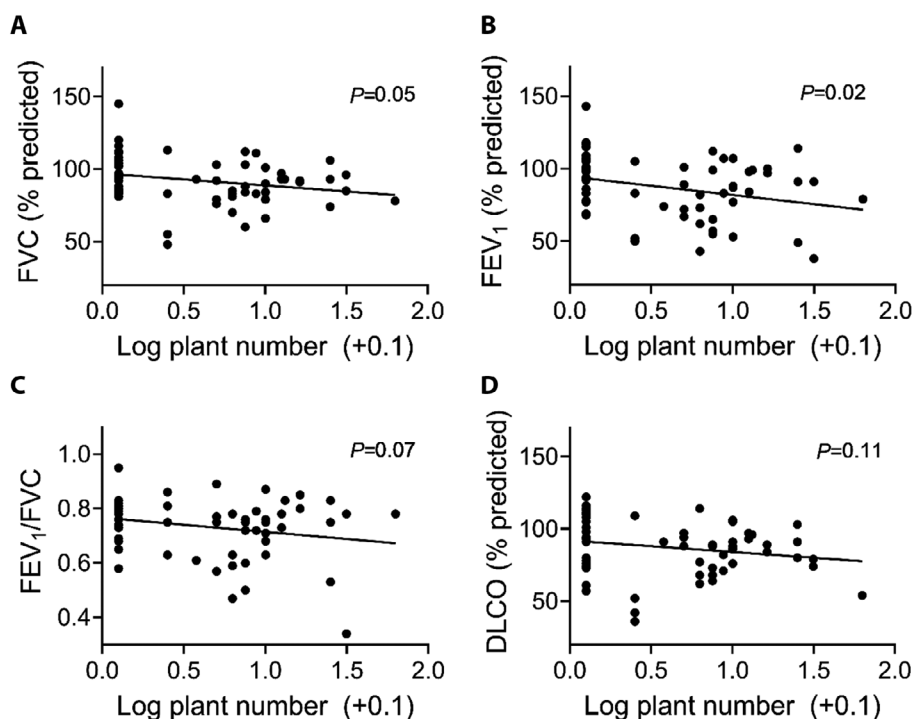
Figure 3. Lung function decline was associated with number of houseplants owned. Houseplant ownership significantly correlated with pulmonary fibrosis. Participant-reported number of houseplants owned were log transformed and compared by simple linear regression to **A)** Percent predicted FVC, P=0.05; **B)** Percent predicted FEV_1, P=0.02; **C)** FEV_1/FVC, P=0.07, and **D)** Percent predicted DLCO, P=0.11.

disease. Its involvement might also suggest imbalanced Th1/Th2 regulation, which has been proposed to be involved in transition towards fibrosis in patients with sarcoidosis (41). Th2 response is known to contribute to the pulmonary fibrotic process (42,43). In fibrotic disease, macrophage colony-stimulating factor (M-CSF) was the most significantly associated with BDG exposure. Altered alveolar macrophage phagocytic pathways have previously been described in sarcoidosis, with downregulation of the oxidative phosphorylation pathway in severe pulmonary disease (44). M-CSF is thought to regulate tissue macrophage and monocytes (45) and can lead to less differentiated monocytes (compared to GM-CSF) (46). It has been reported patients with sarcoidosis have more intermediate monocytes, which are high in MHC-II expression, responsible for binding and presenting antigens to CD4+ T cells (47). MHC-II alleles have previously been shown to interact with mold/musty odor exposure and pulmonary sarcoidosis risk (7). Several sources of bioaerosol and environmental factors affecting disease severity were gleaned by survey assessment. Exposure to bioaerosol frequently increased with activities known to generate combustion aerosol. For example, burning of candles/other tended to correlate with increased BDG concentrations, and daily cooking correlated with increased BDG concentrations (Table 3). Cooking leads to combustion aerosol exposures (48), which can adsorb bioaerosol (49). Cat owners had higher BDG concentrations, likely related to bioaerosol contained in animal hair and skin (50,51). Because BDG correlated with decreased lung function in patients with severe disease, means of decreasing exposure to mold agents might be explored. For example, minimizing exposure to smoke from cooking, cat hair and plants. Survey data also demonstrated a higher proportion of subjects with pulmonary fibrosis owned houseplants, which can be mold reservoirs (26). The number of plants participants owned was significantly correlated with declined FEV_1 and trends for FVC, FEV_1/FVC (Figure 3). These associations may warrant further exploration in future exposure assessment work.

Table 4. Relationship between serum biomarkers and log-transformed residential β-(1,3)-ᴅ-glucan concentrations. *Left*: correlation between serum biomarkers and residential BDG exposure across cohort, N=36; *Middle*: correlation between serum biomarkers and residential BDG exposure in participants without pulmonary fibrosis, N=22; *Right*: correlation between serum biomarkers and residential BDG exposure in participants with pulmonary fibrosis, N=14. Bold text indicates statistical significance (P≤0.05)

Entire cohort	N	Pearson r	P	Non-Fibrotic	N	Pearson r	P	Fibrotic	N	Pearson R	P
CCL21	**32**	**0.46**	**<0.01**	**CCL21**	**19**	**0.48**	**0.04**	sICAM-1	12	-0.50	0.10
Eotaxin-3	**29**	**0.51**	**<0.01**	Eotaxin-3	16	0.47	0.06	**Eotaxin-3**	**13**	**0.56**	**0.04**
G-CSF	**32**	**0.42**	**0.02**	G-CSF	20	0.38	0.10	**M-CSF**	**13**	**0.62**	**0.02**
IL-9	**31**	**0.37**	**0.04**	**IL-9**	**19**	**0.67**	**<0.01**	BCA-1	13	-0.50	0.08
IL-15	**33**	**0.37**	**0.04**	IL-15	19	0.41	0.08	IL-16	13	0.51	0.08
IL-17F	32	0.30	0.10	**IL-17F**	**19**	**0.46**	**0.05**	IL17E/IL-25	12	0.50	0.10
IL-21	**27**	**0.41**	**0.03**	**IL-21**	**15**	**0.62**	**0.01**	IL-18	14	0.45	0.11
IL-28A	**30**	**0.47**	**<0.01**	**IL-28A**	**19**	**0.46**	**0.05**	IL-28A	11	0.56	0.07
I-309	**34**	**-0.41**	**0.01**	**I-309**	**22**	**-0.47**	**0.03**				
FLT-3L	**34**	**0.36**	**0.04**	FLT-3L	20	0.43	0.06				
LIF	29	0.32	0.09	LIF	17	0.41	0.10				
MIP-1β	36	-0.31	0.06	**MIP-1β**	**22**	**-0.43**	**0.05**				
TARC	**36**	**-0.38**	**0.02**	**TARC**	**22**	**-0.58**	**<0.01**				
IFN-α2	32	0.31	0.08	IL-23	15	0.44	0.10				
M-CSF	**32**	**0.43**	**0.01**								
MCP-4	33	0.31	0.08								
SCF	30	0.33	0.08								

Data were assessed for outliers (ROUT) and definitive outliers (Q=0.1%) removed prior to Pearson correlation. *P*-values ≤0.11 reported; bold text indicates significance, determined at *P*≤0.05.

Study strengths

Ours is the first to directly measure specific bacterial and mold residential bioaerosol in sarcoidosis; our study revealed a novel relationship between residential BDG and sarcoidosis pulmonary severity.

Study limitations

Participants were responsible for EDC deployment, and although we contacted participants to confirm adherence, we cannot exclude the possibility that some participants incorrectly followed protocol instructions. Second, sampling occurred throughout the year, which could skew results (52). However, bioaerosol concentrations did not vary based on sampling season (p>0.91), and within-home temporal variability can be minimal (53). Third, although we have strong association with lung function and houseplant number/other home features, this does not confirm causality. Fourth, our study solely measured bioaerosol. Metals, such as aluminum have been implicated in sarcoidosis (54). Homes in the same geographical area have previously been characterized and aluminum was the most highly enriched metal (55). Interaction between metal and mold exposure in homes should be considered in future work. Fifth, ionizing air purifiers, occasionally used in this study, can generate ozone − a potential confounder for PFT results. Conversely, ionizers effectively reduce residential combustion aerosol (56), and their use tended to correlate with a 10-fold reduction in BDG concentrations. Sixth, biomarker collection did not always occur simultaneously with residential measurements, and patients were on various therapies. To overcome these weaknesses, we excluded cytokine outliers and values below the assay's limit of detection and removed definitive outliers (ROUT, Q=0.1%) and compared these to log-transformed bioaerosol concentrations. Further, rates of IS use were similar between phenotypes. Exclusion criteria may have resulted in underestimation of results,

e.g., extremely high, or low concentrations of specific biomarkers being overlooked, yet they provide more confidence in accuracy of findings. Last, our findings are from a relatively small heterogenous cohort; however, power was determined *a priori*, and we oversampled to accommodate residential, demographic, and phenotypic variability. Future work should include a more diverse cohort to confirm findings.

Conclusion

Bioaerosol concentrations were greater in homes of subjects with sarcoidosis than control subjects. In our case cohort, lung function of subjects with fibrotic pulmonary disease associated with residential fungal BDG. Th1 and Th2 cytokines correlated with BDG exposure, but far fewer inflammatory molecules correlated with exposure in those with pulmonary fibrosis, potentially suggestive of T-cell exhaustion. Further investigation of bioaerosol sources is warranted, as interventions can be designed and tested to see if disease course is modifiable by environmental remediation.

Abbreviations

BDG: β-D-glucan
DLCO: Diffusing capacity of carbon monoxide
FEV_1: Forced expiratory volume in one second
FEV_1/FVC: The ratio of FEV_1 over forced vital capacity
FVC: Forced vital capacity
IL: Interleukin
MHC-II: Major Histocompatibility Complex
PBMC: Peripheral blood mononuclear cell
SHQ: Sarcoidosis Health Questionnaire
Wasfi Severity Score: A previously established method of evaluating sarcoidosis disease severity
VAS: Visual Analogue Scale

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article. The authors declare no competing financial interests in relation to the work described.

Ethical Approval: This study has obtained ethics approval from the IRB review board-01 review board #IRB00000099. Study protocol followed the University's Institutional Review Board requirements (IRBs: #201709743, 201909726, 202009330, Approved by IRB Chair). Subjects completed informed written consent prior to study participation for all study procedures.

References

1. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N Engl J Med. 2007 Nov 22;357(21):2153–65. doi: 10.1056/NEJMra071714.
2. Üzmezoğlu B, imşek C, Gülgösteren S, Gebeşoğlu B, Sarı G, Çelik D. Sarcoidosis in iron-steel industry: mini case series. Sarcoidosis Vasc Diffuse Lung Dis Off J WASOG. 2017;34(4):365–72. doi: 10.36141/svdld.v34i4.6185.
3. Chen ES, Moller DR. Etiologies of Sarcoidosis. Clin Rev Allergy Immunol. 2015 Aug;49(1):6–18. doi: 10.1007/s12016-015-8481-z.
4. Müller-Quernheim J, Prasse A, Zissel G. Pathogenesis of sarcoidosis. Presse Medicale Paris Fr 1983. 2012 Jun;41(6 Pt 2):e275-287. doi: 10.1016/j.lpm.2012.03.018.
5. Saltini C, Pallante M, Puxeddu E, et al. M. avium binding to HLA-DR expressed alleles in silico: a model of phenotypic susceptibility to sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis Off J WASOG. 2008 Dec;25(2):100–16. PMID: 19382529.
6. Judson MA. Environmental Risk Factors for Sarcoidosis. Front Immunol. 2020;11:1340. doi: 10.3389/fimmu.2020.01340.
7. Rossman MD, Thompson B, Frederick M, et al. HLA and environmental interactions in sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis Off J WASOG. 2008 Dec;25(2):125–32. PMID: 19382531.
8. Beijer E, Kraaijvanger R, Roodenburg C, Grutters JC, Meek B, Veltkamp M. Simultaneous testing of immunological sensitization to multiple antigens in sarcoidosis reveals an association with inorganic antigens specifically related to a fibrotic phenotype. Clin Exp Immunol. 2021 Jan;203(1):115–24. doi: 10.1111/cei.13519.
9. Barnard J, Rose C, Newman L, et al. Job and industry classifications associated with sarcoidosis in A Case-Control Etiologic Study of Sarcoidosis (ACCESS). J Occup Environ Med. 2005 Mar;47(3):226–34. doi: 10.1097/01.jom.0000155711.88781.91.
10. Newman LS, Rose CS, Bresnitz EA, et al. A case control etiologic study of sarcoidosis: environmental and occupational risk factors. Am J Respir Crit Care Med. 2004 Dec 15;170(12):1324–30. doi: 10.1164/rccm.200402-249OC.
11. Kucera GP, Rybicki BA, Kirkey KL, et al. Occupational risk factors for sarcoidosis in African-American siblings. Chest. 2003 May;123(5): 1527–35. doi: 10.1378/chest.123.5.1527.
12. Laney AS, Cragin LA, Blevins LZ, et al. Sarcoidosis, asthma, and asthma-like symptoms among occupants of a historically water-damaged office building. Indoor Air. 2009 Feb;19(1):83–90. doi: 10.1111/j.1600-0668.2008.00564.x.
13. Huntley CC, Patel K, Mughal AZ, et al. Airborne occupational exposures associated with pulmonary sarcoidosis: a systematic review and meta-analysis. Occup Environ Med. 2023 Oct;80(10):580–9. doi: 10.1136/oemed-2022-108632.
14. Terčelj M, Salobir B, Harlander M, Rylander R. Fungal exposure in homes of patients with sarcoidosis - an environmental exposure study. Environ Health Glob Access Sci Source. 2011 Jan 20;10(1):8. PMID: 21251285.
15. Greaves SA, Ravindran A, Santos RG, et al. CD4+ T cells in the lungs of acute sarcoidosis patients recognize an Aspergillus nidulans

epitope. J Exp Med. 2021 Oct 4;218(10):e20210785. doi: 10.1084/jem.20210785.

16. Douwes J, Thorne P, Pearce N, Heederik D. Bioaerosol health effects and exposure assessment: progress and prospects. Ann Occup Hyg. 2003 Apr;47(3):187–200. doi: 10.1093/annhyg/meg032.

17. Thorne PS. Environmental endotoxin exposure and asthma. J Allergy Clin Immunol. 2021 Jul;148(1):61–3. doi: 10.1016/j.jaci.2021.05.004.

18. Crouser ED, Maier LA, Wilson KC, et al. Diagnosis and Detection of Sarcoidosis. An Official American Thoracic Society Clinical Practice Guideline. Am J Respir Crit Care Med. 2020 Apr 15;201(8):e26–51. doi: 10.1164/rccm.202002-0251ST.

19. Wasfi YS, Rose CS, Murphy JR, et al. A new tool to assess sarcoidosis severity. Chest. 2006 May;129(5):1234–45. doi: 10.1378/chest.129.5.1234.

20. Schanberg LE, Anthony KK, Gil KM, Lefebvre JC, Kredich DW, Macharoni LM. Family pain history predicts child health status in children with chronic rheumatic disease. Pediatrics. 2001 Sep;108(3):E47. doi: 10.1542/peds.108.3.e47.

21. Cox CE, Donohue JF, Brown CD, Kataria YP, Judson MA. The Sarcoidosis Health Questionnaire: a new measure of health-related quality of life. Am J Respir Crit Care Med. 2003 Aug 1;168(3):323–9. doi: 10.1164/rccm.200211-1343OC.

22. Kilburg-Basnyat B, Metwali N, Thorne PS. Effect of deployment time on endotoxin and allergen exposure assessment using electrostatic dust collectors. Ann Occup Hyg. 2015 Jan;59(1):104–15. doi: 10.1093/annhyg/meu063.

23. Aketagawa J, Tanaka S, Tamura H, Shibata Y, Saitô H. Activation of limulus coagulation factor G by several (1-->3)-beta-D-glucans: comparison of the potency of glucans with identical degree of polymerization but different conformations. J Biochem (Tokyo). 1993 Jun;113(6):683–6. doi: 10.1093/oxfordjournals.jbchem.a124103.

24. Kilburg-Basnyat B, Metwali N, Thorne PS. Performance of electrostatic dust collectors (EDCs) for endotoxin assessment in homes: Effect of mailing, placement, heating, and electrostatic charge. J Occup Environ Hyg. 2016;13(2):85–93. doi: 10.1080/15459624.2015.1078468.

25. Hoppe Parr KA, Hađina S, Kilburg-Basnyat B, et al. Modification of sample processing for the Limulus amebocyte lysate assay enhances detection of inflammogenic endotoxin in intact bacteria and organic dust. Innate Immun. 2017 Apr;23(3):307–18. doi: 10.1177/1753425917694084.

26. Kwon JH, Choi O, Kim J. First Report of Kalanchoe Leaf Scorch Caused by Stemphylium xanthosomatis in Korea. Plant Dis. 2012 Feb;96(2):292. doi: 10.1094/PDIS-05-11-0403.

27. Metwali N, Stapleton EM, Hadina S, Thorne PS. Exposure to structurally unique β-d-glucans differentially affects inflammatory responses in male mouse lungs. Physiol Rep. 2024 Jun;12(12):e16115. doi: 10.14814/phy2.16115.

28. Rankin J, Kobayashi M, Barbee RA, Dickie HA. AGRICULTURAL DUSTS AND DIFFUSE PULMONARY FIBROSIS. Arch Environ Health. 1965 Feb;10:278–82. doi: 10.1080/00039896.1965.10663996.

29. Cesta MF, Ryman-Rasmussen JP, Wallace DG, et al. Bacterial lipopolysaccharide enhances PDGF signaling and pulmonary fibrosis in rats exposed to carbon nanotubes. Am J Respir Cell Mol Biol. 2010 Aug;43(2):142–51. doi: 10.1165/rcmb.2009-0113OC.

30. Terčelj M, Stopinšek S, Ihan A, et al. In vitro and in vivo reactivity to fungal cell wall agents in sarcoidosis. Clin Exp Immunol. 2011 Oct;166(1):87–93. doi: 10.1111/j.1365-2249.2011.04456.x.

31. Miyara M, Amoura Z, Parizot C, et al. The immune paradox of sarcoidosis and regulatory T cells. J Exp Med. 2006 Feb 20;203(2):359–70. doi: 10.1084/jem.20050648.

32. Witte K, Witte E, Sabat R, Wolk K. IL-28A, IL-28B, and IL-29: promising cytokines with type I interferon-like properties. Cytokine Growth Factor Rev. 2010 Aug;21(4):237–51. doi: 10.1016/j.cytogfr.2010.04.002.

33. Lazear HM, Schoggins JW, Diamond MS. Shared and Distinct Functions of Type I and Type III Interferons. Immunity. 2019 Apr 16;50(4):907–23. doi: 10.1016/j.immuni.2019.03.025.

34. Egli A, Santer DM, O'Shea D, et al. IL-28B is a key regulator of B- and T-cell vaccine responses against influenza. PLoS Pathog. 2014 Dec;10(12):e1004556. doi: 10.1371/journal.ppat.1004556.

35. Moller DR. Cells and cytokines involved in the pathogenesis of sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis Off J WASOG. 1999 Mar;16(1):24–31. PMID: 10207939.

36. Wolk K, Witte K, Witte E, et al. Maturing dendritic cells are an important source of IL-29 and IL-20 that may cooperatively increase the innate immunity of keratinocytes. J Leukoc Biol. 2008 May;83(5):1181–93. doi: 10.1189/jlb.0807525.

37. Mochizuki I, Kubo K, Hond T. Widespread heavy damage of capillary endothelial cells in the pathogenesis of sarcoidosis--Evidence by monoclonal von Willebrand factor immunohistochemistry in the bronchus and lung of patients with sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis Off J WASOG. 2014 Oct 20;31(3):182–90. PMID: 25363217.

38. Kohan M, Puxeddu I, Reich R, Levi-Schaffer F, Berkman N. Eotaxin-2/CCL24 and eotaxin-3/CCL26 exert differential profibrogenic effects on human lung fibroblasts. Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol. 2010 Jan;104(1):66–72. doi: 10.1016/j.anai.2009.11.003.

39. Collison AM, Sokulsky LA, Sherrill JD, et al. TNF-related apoptosis-inducing ligand (TRAIL) regulates midline-1, thymic stromal lymphopoietin, inflammation, and remodeling in experimental eosinophilic esophagitis. J Allergy Clin Immunol. 2015 Oct;136(4):971–82. doi: 10.1016/j.jaci.2015.03.031.

40. Clayton F, Peterson K. Eosinophilic Esophagitis: Pathophysiology and Definition. Gastrointest Endosc Clin N Am. 2018 Jan;28(1):1–14. doi: 10.1016/j.giec.2017.07.011.

41. Asif H, Ribeiro Neto M, Culver D. Pulmonary fibrosis in sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis Off J WASOG. 2023 Sep 13;40(3):e2023027. doi: 10.36141/svdld.v40i3.14830.

42. Hancock A, Armstrong L, Gama R, Millar A. Production of interleukin 13 by alveolar macrophages from normal and fibrotic lung. Am J Respir Cell Mol Biol. 1998 Jan;18(1):60–5. doi: 10.1165/ajrcmb.18.1.2627.

43. Kikuchi N, Ishii Y, Morishima Y, et al. Nrf2 protects against pulmonary fibrosis by regulating the lung oxidant level and Th1/Th2 balance. Respir Res. 2010 Mar 18;11(1):31. doi: 10.1186/1465-9921-11-31.

44. Kjellin H, Silva E, Branca RM, et al. Alterations in the membrane-associated proteome fraction of alveolar macrophages in sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis Off J WASOG. 2016 Mar 29;33(1):17–28. PMID: 27055832.

45. Ushach I, Zlotnik A. Biological role of granulocyte macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) on cells of the myeloid lineage. J Leukoc Biol. 2016 Sep;100(3):481–9. doi: 10.1189/jlb.3RU0316-144R.

46. Rodriguez RM, Suarez-Alvarez B, Lavín JL, et al. Signal Integration and Transcriptional Regulation of the Inflammatory Response Mediated by the GM-/M-CSF Signaling Axis in Human Monocytes. Cell Rep. 2019 Oct 22;29(4):860-872.e5. doi: 10.1016/j.celrep.2019.09.035.

47. Hofer TP, Zawada AM, Frankenberger M, et al. slan-defined subsets of CD16-positive monocytes: impact of granulomatous inflammation and M-CSF receptor mutation. Blood. 2015 Dec 10;126(24):2601–10. doi: 10.1182/blood-2015-06-651331.

48. Nazaroff WW. The particles around us. Indoor Air. 2018 Mar;28(2):215–7. doi: 10.1111/ina.12444.

49. Liu H, Zhang X, Zhang H, et al. Effect of air pollution on the total bacteria and pathogenic bacteria in different sizes of particulate matter. Environ Pollut Barking Essex 1987. 2018 Feb;233:483–93. doi: 10.1016/j.envpol.2017.10.070.

50. Buma R, Maeda T, Kamei M, Kourai H. Pathogenic bacteria carried by companion animals and their susceptibility to antibacterial agents. Biocontrol Sci. 2006 Mar;11(1):1–9. doi: 10.4265/bio.11.1.

51. Kim KH, Kabir E, Jahan SA. Airborne bioaerosols and their impact on human health. J Environ Sci China. 2018 May;67:23–35. doi: 10.1016/j.jes.2017.08.027.

52. LeBouf R, Yesse L, Rossner A. Seasonal and diurnal variability in airborne mold from an indoor residential environment in northern New York. J Air Waste Manag Assoc 1995. 2008 May;58(5):684–92. doi: 10.3155/1047-3289.58.5.684.

53. Crawford C, Reponen T, Lee T, et al. Temporal and spatial variation of indoor and outdoor airborne fungal spores, pollen, and (1→3)-β-d-glucan. Aerobiologia. 2009 Sep;25(3):147–58. doi: 10.1007/s10453-009-9120-z.

54. Banoei MM, Iupe I, Bazaz RD, et al. Metabolomic and metallomic profile differences between Veterans and Civilians with Pulmonary Sarcoidosis. Sci Rep. 2019 Dec 20;9(1):19584. doi: 10.1038/s41598-019-56174-8.

55. Parker GJ, Ong CH, Manges RB, et al. A Novel Method of Collecting and Chemically Characterizing Milligram Quantities of Indoor Airborne Particulate Matter. Aerosol Air Qual Res. 2019;19(11):2387–95. doi: 10.4209/aaqr.2019.04.0182.

56. Stapleton EM, Simmering JE, Manges RB, et al. Continuous in-home PM2.5 concentrations of smokers with and without a history of respiratory exacerbations in Iowa, during and after an air purifier intervention. J Expo Sci Environ Epidemiol. 2020 Sep;30(5):778–84. doi: 10.1038/s41370-020-0235-1.

Annex

Table S1. Survey questions

#	Question
1	Do you have carpet or uncarpeted floors?
2	How many hours a week is your home cleaned?
3	What is the primary method of cleaning?
4	What type of oven is used in your residence?
5	What type of stove is used?
6	How often do you or someone else cook?
7	Do you have an exhaust fan over the stove/oven?
	If yes, is it vented to the outside, or is air recirculated?
	If yes, how often is the exhaust fan utilized?
8	What kind of materials do you cook with?
9	How many days a week are smoking products smoked in the house?
10	How many times a day are smoking products used in your home?
11	How many days a week are candles, incense, etc., burned in the house?
	What product do you use?
12	Do you use air conditioning at home?
	If yes, what type of air conditioning does your residence have?
	If window units, how many?
	If other, please specify
13	How often was the air conditioning used this past July?
14	How is your home heated?
	If forced air, is there a filter?
	How often do you change the filter?
15	What is the secondary heating source?
	If forced air, is there a filter?
	How often do you change the filter?
16	What is the primary fuel-type used at residence?
17	What is the secondary fuel-type used at residence?
18	Do you have a garage attached to your home?
19	In summer, how many hours a day do you keep the windows open?
	In summer, how many days a week do you keep the windows open?
20	In winter, how many hours a day do you keep the windows open?
21	In winter, how many days a week do you keep the windows open?

#	Question
22	Do you use an additional air purifier/air cleaner?
	If yes, is it stand-alone/portable, central, or both?
	If stand-alone/portable, is it an ionizer, filter-based, or both?
	How often do you use this?
23	Are the walls painted with lead-based paint?
24	How many pets live in the house?
25	What kind of pets are they?
26	Do you have house plants?
	If yes, how many?
27	Has there been recent construction or renovation inside the house?
28	What is the rough square footage of the home?
29	Do you spend >4 weeks a year living at a secondary address?
	If yes, where is your secondary address?
30	Does your building or basement have water damage?
31	Do you smell mold?
32	Do you see mold?
33	On what level of the building do you live?
34	On what level of the building do you sleep?
35	Have you moved homes over the past 5 years?
	If yes, describe the date and location of each

Table S2. Disease Severity Assessment and Quantification

Criteria	Definition
Non-steroidal immunosuppression (IS)	Currently treated with, or prescribed, non-steroidal IS within one year of enrollment.
IS medications	Mycophenolate mofetil, methotrexate, leflunomide, azathioprine, TNF-α inhibitors, rituximab.
Excluded treatments	Hydroxychloroquine monotherapy (due to decreased potency and lack of efficacy for pulmonary sarcoidosis).
Wasfi severity score	$11.47 + 3.51(\text{cardiac}) + 2.27(\text{neurologic}) + 1.41(\text{non-steroidal immunosuppression}) - 0.003(\text{DLCO\%}) - 0.047(\text{FEV}_1/\text{FVC}) + 1.23(\text{Black}) - 0.027(\text{FEV}_1\%) + 0.51(\text{skin})$

Table S3. Phlebotomy and Serum Cytokine Processing

48-Plex Panel	sCD40L, EGF, Eotaxin, FGF-2, FLT-3 Ligand, Fractalkine, G-CSF, GM-CSF, GROα, IFN-α2, IFN-γ, IL-1α, IL-1β, IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-17A, IL-17E/IL-25, IL-17F, IL-18, IL-22, IL-27, IP-10, MCP-1, MCP-3, M-CSF, MDC, MIG/CXCL9, MIP-1α, MIP-1β, PDGF-AA, PDGF-AB/BB, RANTES, TGFα, TNF-α, TNF-β, VEGF-A
23-Plex Panel	6CKine/CCL21, BCA-1, CTACK, ENA-78, Eotaxin-2, Eotaxin-3, I-309, IL-16, IL-20, IL-21, IL-23, IL-28A, IL-33, LIF, MCP-2, MCP-4, MIP-1δ, SCF, SDF-1α+β, TARC, TPO, TRAIL, TSLP
10-Plex Supplemental Panel	BDNF, Cathepsin D, MPO, NCAM, PAI-1(total), PDGF-AA, PDGF-AB/BB, RANTES, sICAM-1, sVCAM-1
Assay Sensitivities	71-plex panel: 0.14–55.8 pg/mL; 10-plex panel: 0.22–200 pg/mL. Individual analyte sensitivity values are available in the MilliporeSigma MILLIPLEX® MAP protocol.

Luminex xMAP technology was used for multiplexed quantification of 71 human cytokines, chemokines, and growth factors, using the Luminex™ 200 system (Luminex, Austin, TX, USA) by Eve Technologies Corp. (Calgary, Alberta). Two kits from Eve Technologies' Human Cytokine 71-Plex Discovery Assay® were used: one 48-plex and one 23-plex (MilliporeSigma, Burlington, MA, USA). The assay followed the manufacturer's protocol.
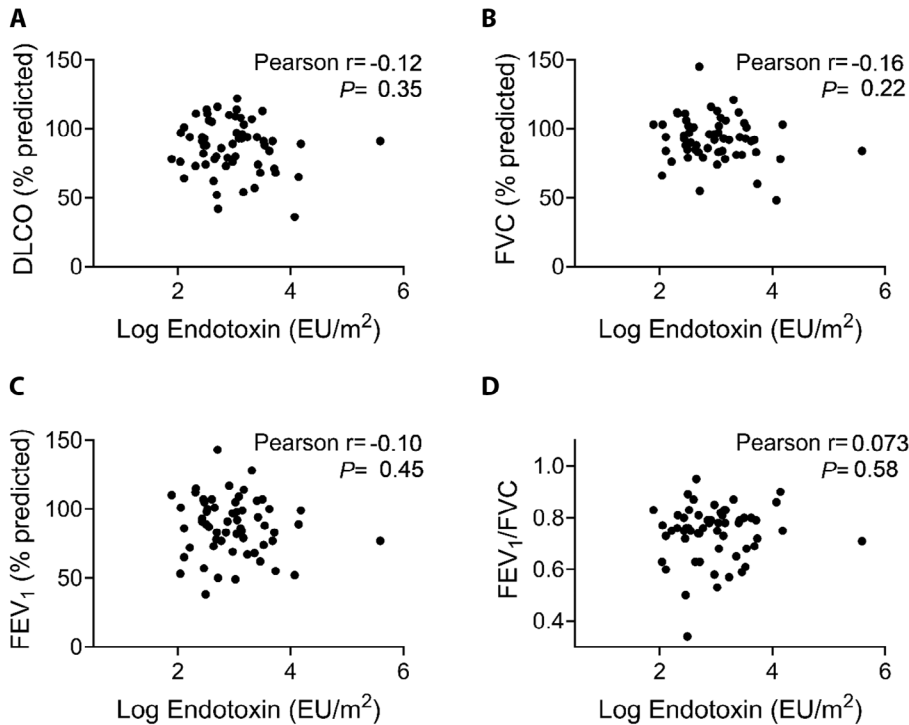

Figure S1. A) Percent predicted DLCO was not related to log-transformed endotoxin concentrations, P=0.35; **B)** Percent predicted FVC was not related to log-transformed endotoxin concentrations, P=0.22; **C)** Percent predicted FEV_1 was not related to log-transformed endotoxin concentrations, P=0.45; **D)** FEV_1/FVC was not related to log-transformed endotoxin concentrations, P=0.58; N=59.
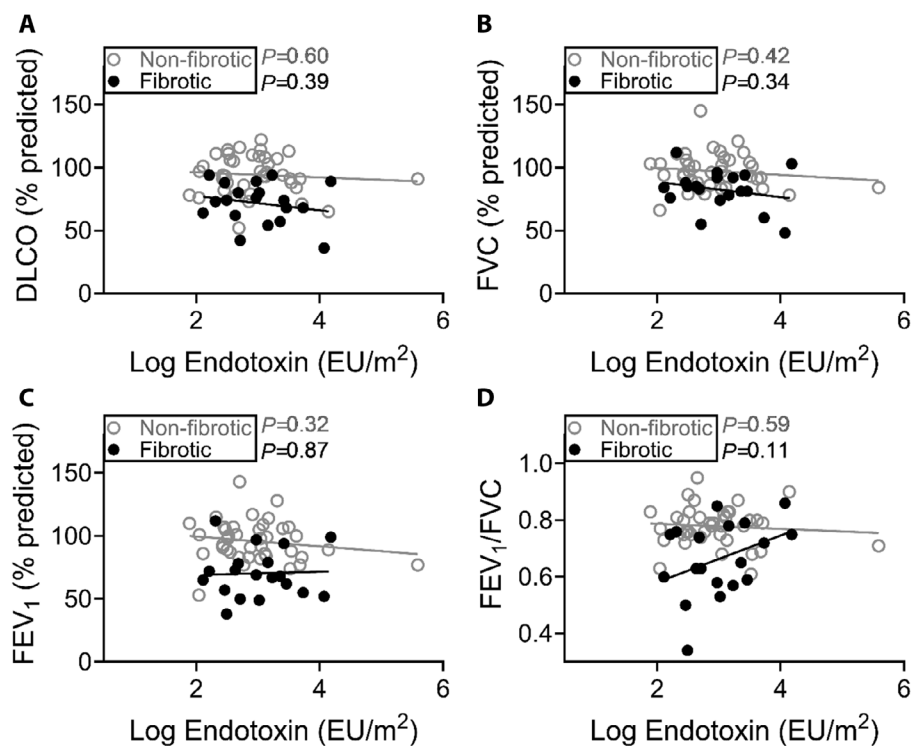
Figure S2. A) Percent predicted DLCO was not related to log-transformed endotoxin concentrations in participants with and without fibrosis (P=0.39; P=0.60, respectively); **B)** Percent predicted FVC was not related to log-transformed endotoxin concentrations in participants with and without fibrosis (P=0.34; P=0.42, respectively); **C)** Percent predicted FEV_1 was not related to log-transformed endotoxin concentrations in participants with and without fibrosis (P=0.87; P=0.32, respectively); **D)** FEV_1/FVC was not related to log-transformed endotoxin concentrations in participants with and without fibrosis (P=0.11; P=0.59, respectively); N=59 (non-fibrotic N=40, fibrotic: N=19).