ISSN 1124-0490 Volume 36 N. 3 - 2019 Pages 175-252
http://www.sarcoidosis.it

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VASCULITIS AND DIFFUSE LUNG DISEASES

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PUBLISHER
Mattioli 1885 - Strada di Lodesana 649/sx, Loc. Vaio - 43036 Fidenza (Parma)
Tel. +39 0524 530383 - Fax +39 0524 82537 - www.mattioli1885.com - edit@mattioli1885.com

Bibliographic Indices:
This journal is regularly listed in bibliographic services, including Current Contents/Clinical Medicine, the Science Citation Index, Sci Search, Research Alert and EMBASE/Excerpta Medica (Priority Journals)

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (3): 176-184

Pharmacological treatment of acute exacerbation of idiopathic pulmonary fibrosis: a retrospective study of 88 patients

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Abstract. *Background:* Acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF) is often fatal. Although pharmacological treatments have been studied, outcomes remain poor. This study evaluated the effectiveness of pharmacological treatments for AE-IPF. *Methods:* This retrospective study comprised 88 patients who received a diagnosis of AE-IPF and were admitted to our center during the period from January 2008 through April 2017. We reviewed the clinical features, treatments, and outcomes of the 88 patients. Cox proportional hazards regression analysis was used to identify variables that were significant predictors of 3-month death. *Results:* Data from 88 AE-IPF patients (age range, 56-81 years) were analyzed. In all patients, corticosteroid (CS) pulse therapy was performed an average of 1.7 times, and the initial CS maintenance dose was 1 mg/kg for 65 patients and 0.5 mg/kg for 23 patients. The combination treatments received were sivelestat in 83 patients (94%), recombinant human thrombomodulin (rhTM) in 45 patients (51%), pirfenidone in 41 patients (47%), and cyclosporine in 71 patients (81%). Univariate analysis showed that use of rhTM, and an initial CS maintenance dose of 0.5 mg/kg were associated with better 3-month survival. In multivariate analysis, both use of rhTM and an initial CS maintenance dose of 0.5 mg/kg were associated with better 3-month survival. Other treatments, including sivelestat, cyclosporine, pirfenidone, and polymyxin B-immobilized fiber column-direct hemoperfusion, were not associated with better 3-month survival. *Conclusion:* Addition of rhTM to CS, and a low initial CS maintenance dose (0.5 mg/kg), were associated with better 3-month survival in patients with AE-IPF. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (3): 176-184)*

Key words: pharmacological treatment, fibrosing interstitial pneumonia, acute exacerbation, corticosteroid maintenance therapy, recombinant human thrombomodulin

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, fibrosing interstitial lung disease characterized by the histological pattern of usual interstitial pneumonia (UIP). The clinical course of IFF may include periods of acute deterioration in respiratory function, which are termed acute exacerbations of IPF (AE-IPF) when a cause cannot be identified (1, 2). AE-IPF is associated with high morbidity. AE can also develop in patients with fibrotic non-specific interstitial pneumonia or interstitial pneumonia associated with collagen vascular diseases (CVD-IP). These AEs of interstitial pneumonias other than IPF can also be fatal (3).

Evidence regarding the effectiveness of pharmacological treatment for AE-IPF is limited. The international IPF guidelines include a weak rec-

Received: 11 October 2018
Accepted after revision: 6 Augusts 2018
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ommendation for corticosteroid (CS) treatment for most patients with AE-IPF (4), despite limited evidence of benefit. In clinical practice CS is given, usually in pulsed doses, to most patients who develop AE-IPF. Immunomodulators [cyclosporine (CsA), cyclophosphamide (CPA), or tacrolimus] are sometimes used in addition to CS. However, the evidence of benefit is not conclusive, as immunomodulators were found to be effective for AE-IPF in only a few small retrospective studies (5-9).

Sivelestat is a small-molecule (529 Da) neutrophil elastase inhibitor. Although a phase 3 study in Japan found that sivelestat improved investigator assessment of pulmonary function in patients with acute respiratory distress syndrome (ARDS) (10), several meta-analyses showed that the sivelestat did not improve mortality in ARDS (11). However, few studies have evaluated the effects of sivelestat for AE-IPF (12-13).

Recombinant human thrombomodulin (rhTM) is approved for treatment of disseminated vascular coagulopathy in Japan. rhTM convert plasma protein C into activated protein C, which deactivates coagulant factors and the pro-inflammatory effects of thrombin. Several small-scale studies showed that rhTM was beneficial for AE-IPF (14-16).

Pirfenidone treatment for chronic IPF slows disease progression (17). A small-scale retrospective study showed that pirfenidone was beneficial for AE-IPF (18).

Polymyxin B-immobilized fiber column-direct hemoperfusion (PMX-DHP) is a medical device that uses polystyrene fibers to immobilize polymyxin B and bind circulating endotoxins (19). Clinical reports suggest that PMX-DHP improves oxygenation in patients with ARDS, and AE-IPF (20).

We investigated the associations of various pharmacological treatments with 3-month outcome in patients with AE-IPF.

Patients and methods

Patients

This retrospective study investigated data from 253 consecutive patients with IPF admitted to Toho University Omori Medical Center during the period from January 2008 through April 2017. A total of 88 patients who had received a first clinical diagnosis of AE-IPF (65 with UIP and 23 with probable UIP, according to the 2018 international IPF guideline (21)) satisfied the inclusion criteria.

Data collection

Clinical data were collected to determine the characteristics of underlying IPF and IPF treatment before AE. We also collected data on respiratory function during the 6 months before AE. Covariates analyzed included PaO2/FiO2 ratio, white blood cell (WBC) count, and serological tests, namely, C-reactive protein (CRP), lactate dehydrogenase (LDH), Krebs von den Lungen-6 (KL-6), and surfactant protein D (SP-D), as well as D-dimer, fibrin/fibrinogen degradation products, brain natriuretic peptide (BNP), and estimated mean pulmonary artery pressure on cardiac ultrasonography, at AE onset.

Diagnosis of IPF and AE-IPF

We defined IPF as a probable UIP or definite UIP pattern on HRCT images, in accordance with the 2018 international IPF guideline. HRCT images of all the present patients were reviewed by 3 pulmonologists (H.S, T. I, S.S) and 1 chest radiologist (A. K). The Japanese Respiratory Society (JRS) classification of IPF disease severity (stage I-IV) (22) and the Gender-Age-Physiology (GAP) index (23) were used to determine IPF severity before AE.GAP index was calculated by gender, age, forced vital capacity (FVC) % predicted and diffusion capacity (DLco) % predicted and patients divided to severity of staging as stage I-III as previously described. Disease severity, pulmonary function, IPF treatment, and diagnostic findings on HRCT were assessed while IPF was chronic and stable, ie, before AE-IPF onset.

AE-IPF was defined on the basis of criteria proposed by Collard et al (1,2) and the JRS guidelines (2), with slight modifications, as follows: (1) previous or current diagnosis of IPF or probable UIP, (2) unexplained worsening or development of dyspnea typically of less than 1-month duration, (3) an HRCT scan showing new bilateral ground-glass opacities and/or consolidation superimposed on a background reticular or honeycomb pattern, (4) no evidence of pulmonary infection on bronchoalveolar lavage, endotracheal aspiration, or sputum culture and negative

results on blood tests for other potentially infectious pathogens (e.g. *Pneumocystis jiroveci*, cytomegalovirus), and (5) deterioration not fully explained by cardiac failure, fluid overload, pulmonary embolism, or other possible causes of acute lung injury. Infectious diseases were excluded by examination of several microbiological samples (e.g. cultures of sputum, blood, and urine were examined for mycobacteria, fungi, and bacteria). We also examined urinary antigens for *Streptococcus pneumonia* and *Legionella pneumophilia*, antigens for influenza A and B viruses (by using pharyngeal swabs), β-D-glucan, and serum antigen for *Aspergillus*. Left heart failure and pulmonary embolism were excluded by transthoracic echocardiography, tests of BNP and D-dimer, and contrast-enhanced CT. Using the classification of Akira et al (24), we classified the CT pattern of all patients at AE-IPF onset as diffuse, peripheral, or multifocal.

Treatment of AE-IPF

All patients were treated with high-dose CS pulse therapy (methylprednisolone 1,000 mg/day for 3 days). CS dose was tapered after pulse therapy (0.5-1.0 mg/kg/day). CsA (2.5 mg/kg/day) was combined with CS. Sivelestat was administered intravenously at a dose of 4.8 mg/kg/day for the first 14 days. rhTM was administered intravenously at a dose of 0.06 mg/kg/day for the first 6 days. Pirfenidone administration was continued in 22 patients who had received pirfenidone before AE onset. In patients who had not received pirfenidone before AE onset, pirfenidone was started at 600 mg/day within 4 days after onset in 6 patients and increased to a maintenance dose (1200-1800 mg/day).

PMX-DHP treatment administered sequentially with 2 Toraymyxin 20-R cartridge columns (Toray Industries, Tokyo, Japan) at a flow rate of 80-100 mL/min. Treatment was continued as long as possible beyond 2 hours. A double-lumen catheter was inserted into a central vein to provide blood access for direct hemoperfusion with PMX.

Endpoints

The primary endpoint was to elucidate the predictor of 3-month death after AE-IPF onset and evaluate the efficacy of pharmacological treatment. The secondary endpoint was treatment safety.

Statistical analysis

Continuous variables are expressed as median (range) unless otherwise stated and were compared using the Mann-Whitney U test. Categorical variables were compared with the χ^2 test. Survival was investigated by using the Kaplan-Meier method, and differences were assessed with the log-rank test. Cox proportional hazards regression analysis was used to identify variables that were significant predictors of death. The cut-off value was calculated by receiver-operating- characteristic curve analysis A p-value of less than 0.05 was considered to indicate statistical significance. All statistical analyses were performed by using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA).

Ethics

This study was approved by the Institutional Review Board of Toho University Omori Medical Center, in October 2017 (project approval number M17189).

Results

Patient characteristics

The clinical characteristics of patients before of AE onset (0-6 months) are shown in Table 1. We identified 88 consecutive patients (74 men and 14 women) who had been treated for AE-IPF. The median duration of observation from the first visit to our center was 13 months (range 1-137 months). Seventy-two patients (82%) had a smoking history. Twenty-two patients (25%) had a pathological diagnosis of UIP, as determined by analysis of a surgical lung biopsy specimen obtained 0-36 months before AE-IPF onset (n=18) or by autopsy (n=4).

Table 1 also shows the detailed clinical characteristics of patients at AE onset. WBC count, CRP, KL-6, SP-D were elevated at AE-IPF onset, and median PaO_2/FiO_2 ratio was low, at 247. HRCT imaging at AE-IPF onset showed diffuse ground-glass opacities and/or consolidation superimposed on pre-existing subpleural fibrosis. The diffuse CT pattern was the most frequent pattern at AE-IPF onset.

The detailed clinical characteristics of the patients before AE onset (0-6 months) are also shown

Table 1. Patient characteristics at AE-IPF onset and characteristics of underlying IPF before AE

Characteristic at onset of AE-IPF	(n=88)
Age, yr.	74.7 (56-89)
Male sex, no. (%)	74 (84.0)
Laboratory findings	
PaO2/FiO2 ratio	247 (45-485)
WBC count /mm3-	10650 (3300-16900)
CRP (mg/dl)	6.8 (0.2-24.3)
LDH (IU/L)	347 (193-647)
D-dimer (mg/ml)	5.1 (0.9-48.2)
FDP (μg/ml)	9.5 (2.5-107.9)
Serum makers	
KL-6 (U/ml)	1040 (366-10469)
SP-D (ng/ml)	317 (62-1070)
Estimated systolic PAP (mm Hg)	33 (10-65)
HRCT pattern of AE-IPF	
Diffuse/multifocal/peripheral, no. (%)	36 (41)/27 (31)/25 (28)
Characteristics of underlying IPF	
Smoking status (never/former/current)	
Smoking index	16/70/2
860 (0-3600)	
JRS severity stage ≥stage 3, no. (%)	48 (54.5%)
GAP stage ≥2, no. (%)	51 (58.0%)
Use of supplemental oxygen, no. (%)	40 (45.5%)
Diagnostic findings on HRCT	
Probable UIP/ definite UIP, no. (%)	23 (26.1)/65 (73.9)
Pathological UIP, no. (%)	22 (25.0)
Lung physiological features	
Forced vital capacity (L)	2.17 (1.50-3.51)
FVC, % of predicted value	73.7 (47.0- 109.1)
FEV1/FVC (%)	87.5 (67.1- 90.8)
Carbon monoxide diffusing capacity -% of predicted value	53.9 (28.8- 66.3)
IPF treatment before AE	
Pirfenidone	21/88 (24%)
N-acetylcysteine	31/88 (35%)
Corticosteroids	12/88 (14%)

IPF: fibrosing interstitial pneumonia, AE-IPF: acute exacerbation of fibrosing interstitial pneumonia, WBC: white blood cell, CRP: C-reactive protein, LDH: lactate dehydrogenase, FDP: fibrin/fibrinogen degradation products, KL-6: Krebs von der Lungen-6, SP-D: surfactant protein D, PAP: pulmonary arterial pressure, HRCT: high-resolution computed tomography, JRS: Japanese Respiratory Society, NAC: N-acetylcysteine, IS: immunosuppressant, CS: corticosteroids, UIP: usual interstitial pneumonia, rhTM: recombinant human soluble thrombomodulin

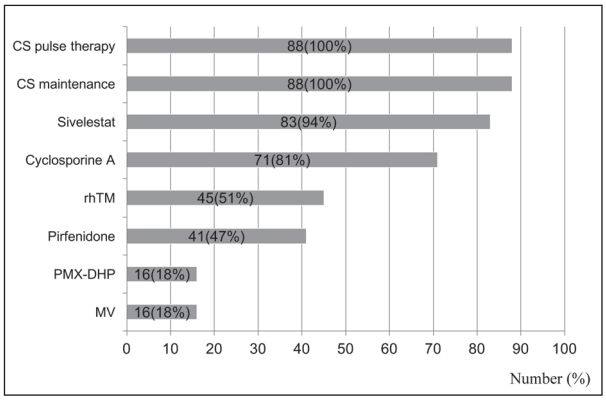
in Table 1. Over 50% of the patients had a GAP stage ≥2 and used supplemental oxygen. Median FVC and %FVC were 2.17 L and 73.7%. Twenty-one patients (24%) had received pirfenidone, 31 (35%) had received inhaled N-acetylcysteine, and 12 (14%) had received corticosteroids (CS) before AE-IPF onset.

Table 2 shows the details of pharmacological treatments for AE-IPF. CS pulse therapy was performed an average of 1.7 times, and the initial CS maintenance dose was 1 mg/kg for 65 patients and 0.5 mg/kg for 23 patients. Forty-five (51%) patients received rhTM, 41 (47%) received pirfenidone, 16 (18%) underwent PMX-DHP, 83 (94%) received sivelestat, and 71 (81%) received CsA at AE-IPF onset.

Predictors of 3-month death

During the observation period, 75 of 88 patients (85.2%) died. All deaths during the first 3 months were from respiratory failure caused by AE-IPF. Univariate analysis showed that the factors predicting 3-month survival were a PaO2/FiO2 ratio ≥250 (the cut-off value for PaO2/FiO2 ratio was calculated by receiver- operating- characteristic curve analysis) at AE onset (hazard ratio [HR], 0.36; 95% confidence

Table 2. Proportions of patients receiving various treatments at onset of acute exacerbation of fibrosing interstitial pneumonia. mber (%)



CS: corticosteroid, rhTM: Recombinant human thrombomodulin PMX-DHP: Polymyxin B-immobilized fiber column-direct hemoperfusion, MV: mechanical ventilation

interval [CI], 0.15-0.86; p=0.02), use of rhTM (HR, 0.44; 95% CI, 0.19-0.99; p=0.04), and an initial CS maintenance dose of 0.5 mg/kg after CS pulse therapy (HR, 0.15; 95% CI, 0.04-0.49; p=0.002) (Table 3). High value of LDH (HR, 1.003; 95% CI, 0.99-1.01; p=0.01) was associated with worse outcomes, Age, sex, other serological markers (including KL-6, SP-D), and pharmacological treatments such as sivelestat, CsA, pirfenidone and PMX-DHP were not associated with 3-month outcome. Analysis of IPF characteristics before AE showed that age, smoking status, IPF severity (as determined by JRS criteria and GAP stage), HRCT pattern (probable or definite UIP), lung function, and IPF treatment before AE that included pirfenidone were not prognostic factors (Table 3). Multivariate analysis showed that 3-month survival was associated with a rhTM treatment (HR 0.32; 95% CI, 0.12-0.86; p=0.02), and an initial CS maintenance dose of 0.5 mg/kg (HR, 0.13; 95% CI, 0.04-0.46; p=0.002).

Figure 1 shows Kaplan-Meier survival curves for the rhTM-treated and non-rhTM-treated groups. Survival at 3 months was significantly better in the rhTM group than in the non-rhTM group (62.3% vs 41.9%; p=0.04).

Table 4 shows the detailed characteristics of patients at AE-IPF onset and the features of underlying IPF before AE in the rhTM and non-rhTM groups. There were no significant differences between groups.

Figure 2 shows Kaplan-Meier survival curves for initial CS maintenance doses of 1 mg/kg and 0.5 mg/kg. Survival at 3 months was significantly better in the CS 0.5 mg/kg group than in the CS 1 mg/kg group (82.6% vs 41.5%; p=0.001).

Table 5 shows patient characteristics at AE-IPF onset and the features of underlying IPF before AE in patients receiving a low CS maintenance dose (0.5 mg/kg) and a high CS maintenance dose (1 mg/kg). A diffuse CT pattern was significantly more frequent and CRP concentration was significantly higher in those receiving a high CS maintenance dose. PaO2/FiO2 ratio was significantly lower in the high CS maintenance dose group.

Table 6 shows patient characteristics at AE-IPF onset and the features of underlying IPF before AE in patients with a definite or probable UIP pattern on HRCT. The proportion of males was significantly higher among those with a UIP pattern than among those with a probable UIP pattern. The proportion of patients with more advanced disease was higher for the UIP pattern than for the probable UIP pattern. Survival at 3 months did not significantly differ between the UIP and probable UIP groups (47.8% vs 47.7%; p=0.65) (Fig. 3).

Safety

Adverse events related to CS treatment were hyperglycemia in 6 patients. There were no severe adverse events in patients treated with cyclosporine A, sivelestat, or PMX-DHP during the observation period.Mild hemoptisis and hematuria developed on the day after rhTM administration in 1 patient. These symptoms improved within a few days and did not require suspension of rhTM treatment. Severe bleeding did not develop in any patient. There were no severe adverse events during the observation period in patients treated with pirfenidone, including gastrointestinal discomfort during administration of a prokinetic agent. One patient developed pneumonia and 1 patient developed pulmonary embolism during the observation period.

Discussion

Although a number of pharmacological treatments have been used for AE-IPF, the efficacy of such

Table 3. Results of univariate and multivariate Cox analysis for 3-month death

AE-IPF onset	HR	95%CI	p-value
Univariate analysis			
Age, yr.	1.01	0.95-1.08	0.18
Diffuse HRCT pattern	2.4	1.2-6.3	0.22
Laboratory findings			
PaO2/FiO2 ratio	0.99	0.986-0.998	0.02*
PaO2/FiO2 ratio ≥250	0.36	0.15-0.86	0.02*
WBC count/mm3-	1.00	1.00-1.00	0.37
CRP (mg/dl)	1.07	0.99-1.44	0.08
LDH (IU/l)	1.003	0.99-1.007	0.01*
KL-6 (U/ml)	1.00	1.00-1.00	0.57
SP-D (ng/ml)	1.001	1.00-1.00	0.18
FDP (μg/ml)	0.992	0.99-1.01	0.44
D-dimer (μg/ml)	0.979	0.94-1.02	0.36
Treatment			
CS maintenance 0.5 mg/kg	0.15	0.046-0.49	0.002*
Cyclosporine A	1.39	0.48-4.06	0.55
Sivelestat	0.34	0.06-1.84	0.46
rhTM	0.44	0.19-1.03	0.05
Pirfenidone	0.43	0.18-1.01	0.05
PMX-DHP	2.08	0.68-6.35	0.92
Mechanical ventilation	4.20	1.23-14.29	0.02*
Before AE-IPF			
Male sex	2.64	0.76-9.17	0.13
Smoking history	0.90	0.30-2.65	0.90
Advanced stage (JRS III, IV)	0.82	0.35-1.90	0.64
Advanced stage (GAP 2,3)	0.73	0.31-1.72	0.47
Definite UIP pattern on HRCT	0.99	0.38-2.58	0.99
Lung physiological features			
Forced vital capacity (L)	1.004	0.53-1.87	0.99
FVC % of predicted value	0.99	0.97-1.02	0.67
FEV1/FVC (%)	0.99	0.99-1.01	0.52
% DLco	1.01	0.98-1.04	0.55
Treatment of IPF before AE			
N-acetylcysteine	0.78	0.33-1.86	0.57
Pirfenidone	0.60	0.22-1.63	0.31
Corticosteroid	2.53	0.97-6.60	0.06
Multivariate analysis			
PaO2 / FiO2 ratio ≥250	0.42	0.12-0.85	0.08
CS maintenance 0.5 mg/kg	0.13	0.04-0.46	0.002*
rhTM	0.32	0.12-0.86	0.02*

CI: Confidence interval, HR: hazard ratio, AE-IPF: acute exacerbation of fibrosing interstitial pneumonia, HRCT: high-resolution computed tomography, WBC: white blood cell, CRP: C-reactive protein, LDH: lactate dehydrogenase, KL-6: Krebs von der Lungen-6, SP-D: surfactant protein D, FDP: fibrin/fibrinogen degradation products, rhTM: recombinant human soluble thrombomodulin, JRS: Japanese Respiratory Society, UIP: usual interstitial pneumonia, %DLco: carbon monoxide diffusing capacity % of predicted value, PMX: polymyxin B-immobilized fiber column hemoperfusion

treatments has not been confirmed in large prospective studies. This study examined the associations of 3-month death after AE-IPF with CS maintenance dose and use of an immunomodulatory agent, sivelestat, rhTM, pirfenidone, and PMX-DHP, which are used in combination with CS in Japan.

In clinical practice, most patients who develop AE-IPF receive CS, usually in pulsed doses. However, few high-quality studies have investigated optimal CS dosing, duration of administration, and tapering. In this study, 3-month survival was better for an initial CS maintenance dose of 0.5 mg/kg than

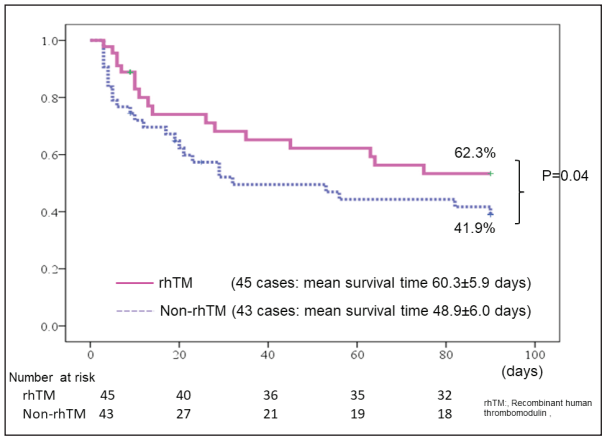


Fig. 1. Kaplan-Meier survival curves for 3-month survival after onset of acute exacerbation of idiopathic pulmonary fibrosis in patients who did and did not receive recombinant human soluble thrombomodulin (rhTM). Survival at 3 months was significantly better in the rhTM group than in the non-rhTM-treated group (62.3% vs 41.9%, p=0.04).

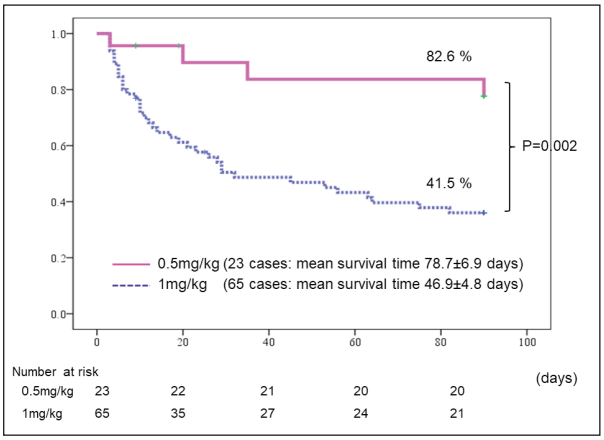


Fig. 2. Kaplan-Meier survival curves for 3-month survival after onset of acute exacerbation of idiopathic pulmonary fibrosis in patients treated with CS 0.5 mg/kg group and 1 mg/kg. Survival at 3 months was significantly better in the CS 0.5 mg/kg group than in the CS 1 mg/kg group (82.6% vs 41.5%, p=0.002).

Table 4. Patient characteristics at AE-IPF onset and features of underlying IPF before AE in the rhTM and non-rhTM groups

	rhTM (n=45)	Non-rhTM (n=43)	P-value
Age	73.3±6.9	74.9±6.0	0.38
Male gender	39/45 (81%)	35/43 (81%)	0.57
GAP stage ≥ 2	29/45 (87%)	22/43 (51%)	0.28
Diffuse HRCT pattern	20/45 (44%)	16/43 (37%)	0.52
CRP	7.9±5.9	8.2±6.7	0.88
LDH	356.5±86.2	363.8±148.7	0.16
KL-6	1729.2±1720.7	1287.3±833.5	0.11
SP-D	387.8±271.1	372.9±315.5	0.76
PiO_2/FiO_2 ratio	237.4±96.1	246.1±96.5	0.60

rhTM: recombinant human soluble thrombomodulin, GAP: Gender-Age-Physiology, HRCT: high-resolution computed tomography, CRP: C-reactive protein, LDH: lactate dehydrogenase, KL-6: Krebs von der Lungen-6, SP-D: surfactant protein D

Table 5. Patient characteristics at AE-IPF onset and features of underlying IPF before AE in patients receiving low-dose CS maintenance therapy (0.5 mg/kg) and high-dose CS maintenance therapy (1 mg/kg)

	CS maintenance dose 0.5 mg/kg) (n=23	CS maintenance dose 1 mg/kg (n=65)	P-value
Age	74.8±6.4	73.8±6.6	0.51
Male gender	15/23 (65%)	56/65 (86%)	0.004*
GAP stage ≥2	12/23 (52%)	39/65 (60%)	0.51
Diffuse CT pattern	5/23 (22%)	31/65 (48%)	0.03*
CRP	5.7±3.7	8.9±6.8	0.04*
LDH	352.8±81.3	362.6±131.7	0.74
KL-6	1364.5±928.3	1566.1±1500.5	0.55
SP-D	310.4±182.8	405.5±319.4	0.18
PaO2/FiO2 ratio	275.0±99.8	23.74±96.1	0.03*

CS: corticosteroid, GAP: Gender-Age-Physiology, HRCT: high-resolution computed tomography, CRP: C-reactive protein, LDH: lactate dehydrogenase, KL-6: Krebs von der Lungen-6, SP-D: surfactant protein D

for a dose of 1 mg/kg. The initial CS dose depended on AE-IPF severity and patient comorbidities. Thus, many physicians administered a low initial CS maintenance dose (0.5 mg/kg) after a satisfactory response to CS pulse therapy. In fact, the group treated with a low initial CS maintenance dose – which was associated with a significantly higher PaO2/FiO2, lower CRP concentration, and greater likelihood of a non-diffuse CT pattern – had better outcomes. In contrast, a previous study reported that high-dose prednisolone (>0.6 mg/kg) improved AE outcomes (24). Our results indicate that some AE-IPF patients do not require high-dose CS maintenance therapy. Among such patients low-dose CS maintenance therapy might reduce long-term adverse events from steroid use. Thus, a future prospective clinical trial should investigate the optimal initial CS maintenance dose in patients with similar AE-IPF severity.

Although immunomodulators are sometimes used in combination with CS, the evidence of benefit is not conclusive (5-9). CsA primarily inhibits calcineurin which suppresses expression of interleukin (IL)-2, the most essential cytokine in T cell activa-

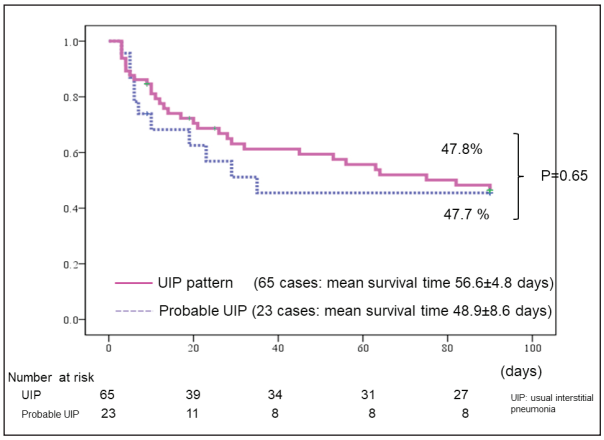


Fig. 3. Kaplan-Meier survival curves for 3-month survival after onset of acute exacerbation of idiopathic pulmonary fibrosis in patients with a definite UIP pattern and probable UIP. Survival at 3 months did not significantly differ between the groups (47.8% vs 47.7%, p=0.65)

Table 6. HRCT patterns of underlying IPF before AE, according to the international IPF guideline, 2018

	UIP pattern (n=65)	Probable UIP pattern (n=23)	P-value
Age	74.5±6.1	72.7±7.4	0.25
Male gender	59/65 (91%)	15/23 (65%)	0.004*
GAP stage ≥ 2	44/65 (68%)	7/23 (30%)	0.002*
Diffuse HRCT pattern	26/65 (40%)	10/23 (43%)	0.77
CRP	7.8±5.7	8.7±7.8	0.95
LDH	361.4±129.1	356.2±92.8	0.86
KL-6	1477.4±1065.5	1614.9±2026.5	0.68
SP-D	398.7±310.0	329.4±232.2	0.33
PiO_2/FiO_2 ratio	234.5±95.0	245.6±101.0	0.63

CS: corticosteroid, GAP: Gender-Age-Physiology, HRCT: high-resolution computed tomography, CRP: C-reactive protein, LDH: lactate dehydrogenase, KL-6: Krebs von der Lungen-6, SP-D: surfactant protein D

tion. T cells and alveolar macrophages are important in AE-IPF pathogenesis; thus, CsA may modulate the clinical course of IPF. Previous studies using a regimen of high-dose corticosteroid pulses followed by cyclophosphamide in patients with AE-IPF reported 3-month mortality rates of 45% and 73% (8-9). In this study, use of an immunomodulatory agent was not associated with 3-month survival.

Sivelestat is approved for ARDS in Japan. It is widely used for treatment of AE-IPF, which has a pathophysiology similar to that of diffuse alveolar damage (DAD) (12, 13). Neutrophil elastase may contribute to worsening of DAD pathophysiology and it is therefore reasonable to use this agent for AE-IPF. No large randomized controlled trials have examined the effectiveness of such agents for AE-IPF. In this study, sivelestat was well tolerated but was not associated with 3-month survival.

Several previous studies reported disordered coagulation, fibrinolysis, and endothelial damage in AE-IPF (15, 26). Collard et al reported significant elevations in plasma biomarkers of endothelial cell injury and coagulation in AE-IPF patients. (26). rhTM activates protein C, which deactivates coagulant factors and the pro-inflammatory effects of thrombin. Moreover, the N-terminal lectin-like domain of rhTM deactivates high-mobility group box 1 (HMGB1), which has anti-inflammatory effects. Elevation of serum HMGB1 was observed in patients with acute lung injury (27). rhTM treatment is likely to benefit AE-IPF patients.

A small-scale study showed that pirfenidone was beneficial for AE-IPF (18). In the present study, use of pirfenidone was not a predictor of 3-month survival; however, pirfenidone appeared to improve outcomes of patients with AE-IPF receiving rhTM. Pirfenidone also suppresses inflammatory cytokines such as transforming growth factor (TGF)-β and basic fibroblast growth factor (b-FGF), which are related to fibrosis progression and subsequent anti-inflammatory and antifibrotic effects. These results suggest that outcomes could be improved by suppressing both acute inflammation and subsequent fibrosis.

PMX-DHP was originally developed to remove endotoxins and improve hemodynamics and PaO2/FiO2 ratio in patients with sepsis. Recent studies suggest that PMX-DHP improves outcomes for patients with AE-IPF, and most of these studies report that PMX-DHP improves PaO2/FiO2 ratio. Abe et al reported a 3-month survival rate of 34.5% after AE-IPF with PMX-DHP treatment, which was better than the rate reported in a previous study (28). The mechanism by which PMX-DHP improves pulmonary oxygenation in AE is unclear. Several studies reported that PMX-DHP reduces inflammatory mediators, platelet-derived growth factor, vascular endothelial growth factor, and TNF-α (19) However, PMX-DHP therapy was not associated with survival in the present study.

Limitations

This study has several limitations. First, because it was a retrospective single-center study, prospective studies are needed in order to confirm our results. Second, initial prednisolone dose and use of an immunomodulatory agent, sivelestat, and PMX-DHP depended on AE-IPF severity and patient comorbidities; thus, patients with more severe disease received more-intensive treatment. This may explain why patients receiving these treatments had worse outcomes.

Conclusion

Addition of rhTM to CS, and a low initial CS maintenance dose (0.5 mg/kg), were associated with better 3-month survival among patients with AE-IPF. Decisions regarding whether to use high-dose CS, sivelestat, antifibrotic agents, rhTM, or PMX-DHP may depend on stratification of AE-IPF severity. Large, placebo-controlled, randomized trials will be necessary in order to confirm our results.

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Spirometry, cardiopulmonary exercise testing and the six-minute walk test results in sarcoidosis patients

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Abstract. *Background:* The 6-minute walking test, cardiopulmonary exercise testing, and spirometry are useful tools for evaluation of respiratory impairment and functional capacity in patients with lung disease. Sarcoidosis is a multisystem granulomatous disease of unknown etiology. *Objectives:* Since the pulmonary involvement can affect the quality of life in sarcoidosis patients, this study is aimed to evaluate the tests mentioned above in order to examine the functional capacity of sarcoidosis patients in different stages as well as the cause of exercise intolerance. *Methods:* This cross-sectional study was carried out on 50 Iranian patients with sarcoidosis. Patients were classified into three groups based on the findings of the chest radiography as well as the pulmonary CT scan, reported by an expert radiologist. Pulmonary, cardiac, and activity function have been evaluated in the patients, using cardiopulmonary exercise testing, the 6-minutes walking test, and spirometry. *Results:* In cardiopulmonary exercise testing, percent-predicted peak VO_2 (57.75±15.49, p=0.015) and percent-predicted O_2 pulse (70.54±17.37, p=0.013) were significantly lower in the third group, in comparison with the others. Also, VE/CO_2 (AT) (34.99±5.67, p=0.000) was significantly higher in the third group, in comparison with the other ones. Percent-predicted VO_2 showed a strong positive correlation with age (r=0.377, p=0.009), TSH (r= 0.404, p=0.007), and percent-predicted FVC (r=0.443, p=0.002). In addition, O_2 pulse had a positive correlation with BMI (r=0.324, p=0.026), percent-predicted FVC (r=0.557, p= 0.000), and percent-predicted FEV_1 (r=0.316, p=0.032). *Conclusions:* According to this study, ventilatory limitation, pulmonary involvement, and deconditioning are the main causes of activity limitations in sarcoidosis patients. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (3): 185-194)*

Key words: Respiratory function tests (D012129), Cardiopulmonary exercise testing (D005080), 6-minute walk test, Sarcoidosis (D012507), Spirometry (D013147)

Received: 3 September 2018
Accepted after revision: 5 March 2019
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Introduction

Sarcoidosis is a heterogeneous, inflammatory, multisystem, granulomatous disease of uncertain etiology (1-4). This disease can affect different organs, but the lungs are involved in more than 90% of the cases. Chest X-ray and spirometry are used to

evaluate the extent of pulmonary involvement. Their findings suggest that there is a lack of coordination between the level of breath shortness, spirometry, and chest radiography in sarcoidosis (5). Dyspnea and inability to exercise are affected by several factors including lung involvement and cardiovascular, musculoskeletal, and neurological reasons (6).

Several modalities are available in order to evaluate the functional exercise capacity (7). In recent years, the Six-Minute Walk Test (6MWT) has been used as a prognostic tool in patients with heart failure and pulmonary disease (8). According to The American Thoracic Society guidelines, 6MWT has been found to be a simple, low-cost, renewable, repeatable, and acceptable method which can be applied with minimal facilities. In addition, it has been increasingly used in analyzing the performance of athletic tolerance (9-11). Several studies have evaluated the efficacy of 6MWT in the prediction of mortality rate among patients with chronic pulmonary disease, as well as in those who are candidates of lung transplantation (12, 13).

Cardio-Pulmonary Exercise Testing (CPET) is used to assess the status of the heart, lung, and muscle function (14). Through this test, the functional lung capacity (FLC), amount of consumed oxygen, physical fitness, and respiratory, cardiovascular, or muscular status are estimated by measuring the respiratory gases (15, 16). Since the Pulmonary Function Tests (PFTs) are not reliable enough to predict the functional limitation during exercise in the sarcoidosis patients, CPET can be considered a helpful method for detecting exercise tolerance in such cases (17). Furthermore, some studies have proven the reliability of CPET in the detection of pulmonary gas exchange impairment (PGEI) in early radiographic stages (18, 19).

Since pulmonary involvement is a common presentation of sarcoidosis, this study is aimed to evaluate the pulmonary function of the patients as well as monitoring their pulmonary status in treatment centers. The 6MWT and CPET were used to investigate the functional lung capacity in sarcoidosis patients in different stages and find the correlation between 6MWT and CPET with other clinical markers.

Materials and Methods

Subjects and study design

This research is a cross-sectional study carried out on 50 Iranian patients who were referred to the referral respiratory hospitals of Iran due to dyspnea and exercise limitation. The diagnosis of sarcoidosis was based on biopsy-proven non-caseating epithelioid cell granulomas, according to the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) guidelines (20), clinical features, and radiological findings. The inclusion criteria comprised having a biopsy result proven of sarcoidosis and being more than 18 years old. Those who had any respiratory disorders, recognized muscular disease, tuberculosis, cardiac disease as well as the active/passive smokers were excluded from the study. None of these patients had another relevant medical history or comorbidity.

Demographic data, including age, sex, weight, height, and type of treatment were collected from patient's records. Clinical and paraclinical tests had also been conducted. Towards this end, levels of 25-hydroxyvitamin D, Hemoglobin concentration (Hb), Thyroid-Stimulating Hormone (TSH), Angiotensin Converting Enzyme (ACE), Calcium (Ca), and Erythrocyte Sedimentation Rate (ESR) had been measured in serum/blood/urine. Pulmonary Artery Pressure (PAP) and Ejection Fraction (EF) had been investigated by echocardiography, and stages of the disease and Pulmonary Artery Size had been measured through computed tomography scan (CT) and chest X-ray (CXR).

Based on the reports of expert radiologists, patients were classified into 3 groups in accordance with the findings of the chest radiography as well as the pulmonary CT scan. Group 1 consists of Stage 0 (radiologically normal) and Stage I (bilateral hilar lymphadenopathy without involvement of parenchymal); group 2, Stage II (bilateral hilar lymphadenopathy associated with parenchymal infiltrates) and Stage III (parenchymal infiltration without involvement of hilar lymphadenopathy); and finally group 3, Stage IV (evidence of pulmonary fibrosis) (20).

The study was approved by the ethics committee of Masih Daneshvari Hospital and Shahid Beheshti University of Medical Sciences, and informed consent was obtained from all participants. PFT,

6MWT, and CPET were performed on the same day for each case from July to December 2017.

PFT

Forced vital capacity (FVC) (% predicted), Forced Expiratory Volume in 1s (FEV_1) (% predicted), and FEV_1/FVC(%) were performed by pneumotachograph (Masterlab, Jaeger, Wurzburg, Germany) at the place of testing.

6MWT

Each patient had remained in a relaxed sitting position for at least 15 minutes before the beginning of the test. According to the mentioned guidelines, the participant walked along a 30-meter, flat, straight hall for 6 minutes (21). None of the patients received oxygen during the test. Heart rate was measured at the beginning as well as the end of the test and the distance walked in 6 minutes was measured as well (22). Peripheral capillary oxygen saturation (SpO_2) was monitored continuously and automatically every 30 seconds during the test.

CPET

Patients pedaled at a rate of 50 rpm/min for 3 minutes without resistance (unloaded phase). Work rate was then increased 10-20 watts (W) each minute. Patients were encouraged to take the test for approximately 10 minutes; otherwise, the test was ended due to the symptom limitation including leg pain (which was the most common symptom limitation), chest pain, dyspnea, fatigue, etc. (23) Peripheral capillary oxygen saturation (SpO_2), maximum oxygen consumption (VO_2 peak), carbon dioxide production (VCO_2), minute ventilation (VE), breathing reserve (BR), heart rate reserve (HRR), peak heart rate (HR), oxygen pulse (O_2 pulse), and VE/CO_2 of anaerobic threshold (AT) were collected by CPET, using Ergostick device of Geratherm company at Masih Daneshvari hospital, according to a standard protocol (23).

Statistical analysis

Statistical analyses were performed using SPSS 22.0 software. The qualitative data were reported as frequency and percentage, and the quantitative variables were reported as means, mean rank, and standard deviation. The Kolmogorov-Smirnov test was used to check the normality of the sample. PFT parameters were compared between groups using ANOVA followed by Tukey-HSD.

Clinical characteristics of the study population were compared among groups using ANOVA parametric testing followed by Tukey-HSD (for age, BMI, vitamin D level, ACE, Hb, ESR, TSH, Ca, and CaU) or Kruskal-Wallis followed by Mann–Whitney U test (for EF, PAP, and mPA). Diagnostic method and distribution of gender among groups were investigated using Fisher's Exact Test and chi-Square, respectively.

6MWT results were compared between groups using ANOVA parametric testing for HR-0, HR-6, and distance) and Kruskal-Wallis nonparametric testing (for SPO_2). CPET results were compared between groups using ANOVA parametric testing (for peak VO2, BR, O_2 pulse, and VE/CO_2) and Kruskal-Wallis nonparametric testing (for HRR and SPO_2). The correlations between parameters were evaluated using Pearson's correlation coefficient (r). $P<0.05$ was considered statistically significant.

The mean distribution of VO_2 peak (% predicted) and O_2 pulse (% predicted) between genders were compared using T-test.

Z-test was used to compare mean SPO_2 and HR before and 6 minutes after starting 6MWT.

Results

Subjects

The clinical characteristics of the patients are presented in Table 1. Pulmonary sarcoidosis was classified as stage 0 in six participants (11.8%), stage I in one participant (2%), stage II in 24 participants (47.1%), stage III in nine participants (17.6%), and stage IV in 10 participants (19.6%). Since lung function indices were not statistically different between Stages 0 and I as well as Stages II and III, patients were grouped according to their radiological stages as follow: Group one: Stages 0-I (n=7), Group 2: Stages II–III (n=33), and Group 3: Stage IV (n=10).

The most common diagnostic method for sarcoidosis was lung biopsy, followed by skin biopsy.

Table 1. Clinical and para clinical characteristics of the study population

Variable	All Subjects (N=50)	Stages 0-I (N=7)	Stages II-III (N=33)	Stages IV (N=10)	P-Value
Age, yrs	48.58±8.54	47.14±9.96	48.88±7.30	48.60±11.82	0.89
Gender, %					
Male	38%	14.29%	33.33%	70.00%	0.050*
Female	62%	85.71%	66.67%	30.00%	
BMI, kg/m^2	28.68±4.34	31.97±4.70	28.75±3.66	26.52±5.19	0.047*
Diagnostic method					
Lung biopsy	59.1%	0%	60.61%	100%	0.000*
Skin biopsy	30.6%	100%	24.24%	0%	
Neck Lymph nodes biopsy	10.2%	0%	15.15%	0%	
Mean time from diagnosis, yrs	5.47±5.48	5.14±4.14	4.48±5.40	9.33±5.48	0.042*
Vit D, ng/ml	20.75±12.36	17.19±10.20	22.23±13.61	17.98±8.55	0.470
Hb, g/dL	13.85±1.02	13.58±1.06	13.80±1.05	14.23±0.93	0.428
TSH, U/Ml	2.41±1.14	2.41±1.27	2.53±1.25	2.05±0.53	0.551
Ca, mg/dl	9.45±0.58	9.32±0.58	9.60±0.62	9.21±0.42	0.207
CaU, mg/day	150.61±87.92	198.50±111.02	129.88±27.75	159.38±122.52	0.600
ACE, U/L	71.56±35.01	86.20±44.35	71.44±35.74	63.78±28.48	0.529
ESR, mm/hr	23.87±17.99	23.86±12.98	25.22±22.02	20.44±7.75	0.805
Echocardiography findings					
PAP, mmHg	26.02±8.20	23.71±6.21	23.24±4.66	38.00±9.50	0.000*
EF, %	53.91±4.00	55.00±2.89	55.15±1.97	48.56±5.92	0.004*
CT findings					
Main Pulmonary Artery Diameter (mPA), mm	23.78±3.59	21.82±2.40	23.70±3.33	25.61±4.64	0.188

Data are presented as the mean ± SD or present for 50 patients; BMI=body mass index; Vit D=25-hydroxy vitamin D; Hb=Hemoglobin concentration; TSH=Thyroid-Stimulating Hormone; Ca=Calcium; CaU=Urine calcium level; ACE=Angiotensin Converting Enzyme; ESR=Erythrocyte Sedimentation Rate; PAP= Pulmonary Artery Pressure; EF=Ejection Fraction

Biopsy of neck lymph nodes was conducted in five patients.

About 54.4% of patients were receiving Prednisolone, 42.2% Methotrexate, and 3.3% Cyclosporineas the treatment. There was not any significant difference between levels of peak VO_2 and receiving Prednisolone, Methotrexate, or Cyclosporineas the treatment.

Females (mean ± SD: 74.30±14.59) reached a higher peak O_2 pulse (% predicted) compared with males (mean ± SD: 63.41±20.37) (female vs. male, P= 0.039). VO2 (% predicted) was not significantly different between females and males.

PFT, 6MWT, and CPET results

PFT, 6MWT, and CPET results are detailed in Table 2.

Since there were no significant differences between the SPO_2 of 6MWT and CPET tests, only the result of 6MWT is expressed (Table 2). SPO_2-0, SPO_2-6, and the distance were lower in group three in comparison with other groups (Table 3). On the other hand, except in group one, SPO_2 of all patients had decreased after six minutes. HR of patients in minute 0 and six in group three were higher, although this difference was not statistically significant (Table 2). HR had increased significantly after six minutes (P<0.000).

Correlations

The correlation of two variables of VO_2 (% predicted) and O_2 pulse (% predicted) with Age, BMI, TSH, ACE, ESR, Vit D, Hb, Ca, CaU, PAP, EF, main pulmonary artery diameter, PFT parameters, and distance had been evaluated (Table 4).

VO_2 (% predicted) showed a strong positive correlation with age, TSH, and FVC (% predicted) (Figure 1). There was not any correlation between VO_2 (% predicted) and BMI, ACE, ESR, Vitamin

Table 2. Results of PFT, 6MWT, and CPET

Variable	All Subjects (N=50)	Stages 0-I (N=7)	II-III (N=33)	IV (N=10)	P-Value
PFT					
FVC (% predicted)	79.01±21.53	90.00±27.03	83.25±16.93	58.20±18.47	0.001*
FEV1 (% predicted)	73.99±23.96	91.29±28.42	75.80±21.81	56.10±16.92	0.007*
FEV1/ FVC (%)	83.98±13.71	95.87±16.45	82.42±13.11	80.80±10.12	0.041*
6MWT					
SPO2-0	-	34.29	26.97	15.50	0.010*
SPO2-6	-	36.07	26.62	14.40	0.007*
HR_0 (beats/min)	86.38±12.07	85.29±10.37	85.18±9.81	91.10±18.68	0.393
HR_6 (beats/min)	124.22±20.37	118.00±19.53	121.64±18.10	137.10±24.55	0.072
Distance (meter)	436.60±92.59	465.86±49.73	467.73±74.13	313.40±66.43	0.000*
CPET					
Peak VO2	1.16±0.35	1.05±0.30	1.19±0.32	1.05±0.40	0.399
peakVO2 (% predicted)	57.75±15.49	50.50±8.17	61.81±13.95	47.40±17.51	0.015*
VO2/kg (ml/kg/min)	14.89±4.34	12.17±3.73	15.48±3.97	13.63±4.27	0.129
VE	52.63±15.30	43.33±10.84	53.06±13.92	54.60±19.84	0.304
VE(% predicted)	58.25±15.65	45.67±9.81	61.65±14.16	54.90±19.95	0.055
VE/VCO2 (AT)	34.99±5.67	33.52±4.81	33.36±4.08	41.28±6.63	0.000*
HR peak	143.21±20.60	131.17±21.37	145.47±17.34	140.50±26.67	0.286
HR%	84.81±11.64	75.50±9.01	86.80±10.15	83.00±14.79	0.080
HRR (beats/min)	-	35.00	21.77	26.95	0.086
O2 pulse	8.20±2.37	8.07±1.80	8.40±2.39	7.37±2.59	0.496
O2 pulse (% predicted)	70.54±17.37	76.17±20.06	73.77±14.66	56.30±18.57	0.013*
BR (breaths/min) (%)	36.16±23.18	34.73±15.57	37.37±23.06	32.35±29.45	0.838

Data are presented as the mean ± SD for all parameters except SPO2-0, SPO2-6, and HRR which are presented as mean rank in each group. Asterisk indicates Significant (P<0.05); PFT=Pulmonary Function Tests; FVC=forced vital capacity; FEV_1=forced expiratory volume in one second; 6MWT=6 min Walking Test; SPO2-0=oxygen saturation of 0 min; SPO2-6=oxygen saturation after 6MWT; HR=Heart Rate; Distance=traveled in 6MWT; CPET=Cardiopulmonary exercise testing; Peak VO2=peak oxygen uptake; VO2/kg=Volume of oxygen per kilogramme of body weight per minute; VE=Minute Ventilation; VE/VCO_2(AT)=ventilatory equivalent for carbon dioxide at anaerobic threshold; HRR=Heart Rate Reserve; O2 pulse=oxygen pulse; BR=Breathing reserve

Table 3. Comparing Variables' P-Values between groups

Parameters \ Stages	P-Value 0-I vs II–III	0-I vs IV	II–III vs IV
Peak VO2(%predicted)	0.188	0.907	0.021*
O_2 pulse (%predicted)	0.942	0.056	0.013*
VE/VCO_2(AT)	0.997	0.008*	0.000*
SpO_2-0	0.372	0.002*	0.000*
SpO_2-6	0.082	0.011*	0.014*
FVC(%predicted)	0.671	0.004*	0.002*
FEV_1(%predicted)	0.220	0.006*	0.044*
FEV1/FVC %	0.044*	0.060	0.937
Distance, meter	0.998	0.000*	0.000*

*Asterisk indicates Significant (P<0.05); Peak VO2=peak oxygen uptake; O_2 pulse=oxygen pulse; VE=Minute Ventilation; SPO_2-0=Peripheral capillary oxygen saturation of minute 0; SPO_2-6=Peripheral capillary oxygen saturation of minute 6, FVC=forced vital capacity; FEV1=forced expiratory volume in one second; Distance=traveled in 6MWT

D, Hb, Ca, CaU, PAP, EF, main pulmonary artery diameter, FEV(% predicted), FEV_1/FVC %, and distance.

There was a moderate bivariate correlation between O_2 Pulse (% predicted) and BMI; and FEV_1 (% predicted) and 6MWD. In addition, a strong positive correlation has been seen between O_2 Pulse (% predicted) and FVC (% predicted) (Figure 2). There was not any correlation between O_2 Pulse (% predicted) and Age, TSH, ACE, ESR, Vitamin D, Hb, Ca, CaU, PAP, EF, main pulmonary artery diameter, and FEV_1/FVC %.

Discussion

Due to the effects of sarcoidosis on multiple organs including lungs, heart, and rarely musculoskeletal system (5, 24) in addition to its effects on the patients' activity performance, there is a real need to evaluate the pulmonary function and activity performance in this disease. This cross-sectional study is aimed to assess the role of CPET, the 6MWT, and

Table 4. The correlation between VO2(%predicted) and O2 Pulse(%predicted) with Age, Gender, BMI, TSH, ACE, ESR, Vit D, Hb, PAP, EF, Main Pulmonary Artery Diameter, PFT results and distance

	PeakVO2(% predicted)		O_2pulse(% predicted)	
	p	r	p	r
AGE	0.009	0.377**	0.051	0.287
BMI	0.675	0.063	0.026	0.324*
TSH	0.007	0.404**	0.072	0.274
ACE	0.130	0.261	0.410	0.144
ESR	0.635	0.082	0.686	0.070
Vit D	0.145	0.218	0.909	-0.017
Hb	0.447	-0.116	0.917	0.016
CA	0.161	0.242	0.513	0.114
CAU	0.974	-0.009	0.670	-0.112
PAP	0.203	-0.191	0.186	-0.199
EF	0.653	0.068	0.110	0.239
Main Pulmonary Artery Diameter	0.314	-0.152	0.119	-0.233
PFT				
FVC(% predicted)	0.002	0.443**	0.000	0.557**
FEV_1(% predicted)	0.339	0.144	0.032	0.316*
FEV1/FVC %	0.423	-0.120	0.751	0.048
6MWD	0.058	0.278	0.011	0.369*

*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)

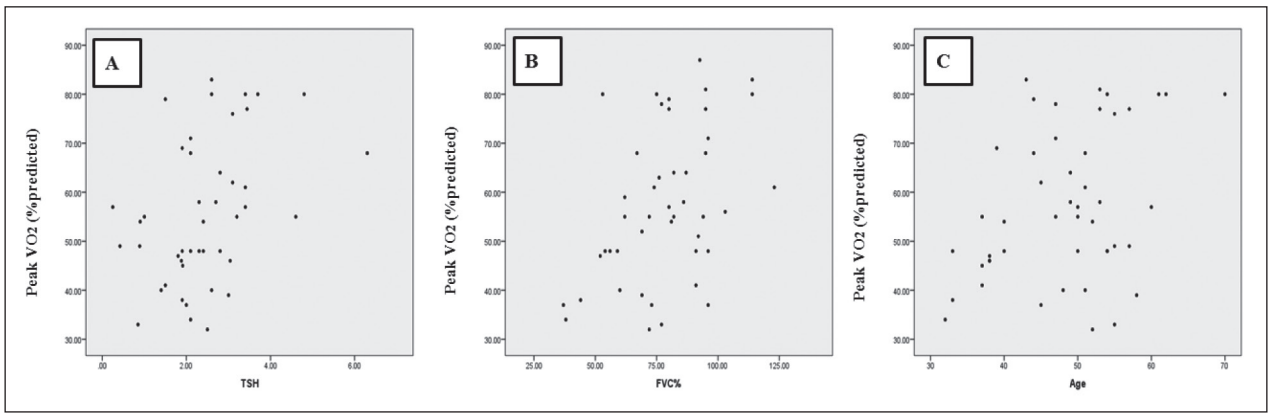

Fig. 1. Relationship of Peak VO2 (% predicted) with Thyroid-Stimulating Hormone (r=0.404; P=0.007) (panel A); FVC(% predicted) (r=0.443; P=0.002) (panel B) and age (r=0.377; P=0.009) (panel C). Correlation was determined using Pearson's correlation coefficient (r). P<0.05 was considered statistically significant (2-tailed)

spirometry test as the monitoring tools in sarcoidosis patients.

Findings of the current study, achieved through CPET, the 6MWT, and spirometry, revealed intolerance of sarcoidosis patients through CPET in advanced stages.

Percent-predicted VO_2 and O_2 pulse and their correlation were compared in different stages. The relationship between the above-mentioned parameters, clinical and paraclinical characteristics, and other parameters, such as the 6MWT, CPET, and PFT, have also been investigated in sarcoidosis patients.

The third group had reached a lower percent-predicted peak VO_2, percent-predicted O_2 pulse, and PFT in comparison with other groups. These findings are consistent with previous studies, in which the mentioned parameters decreased as well (Wallaert, 2011 #119) (25-28). Therefore, the assessments

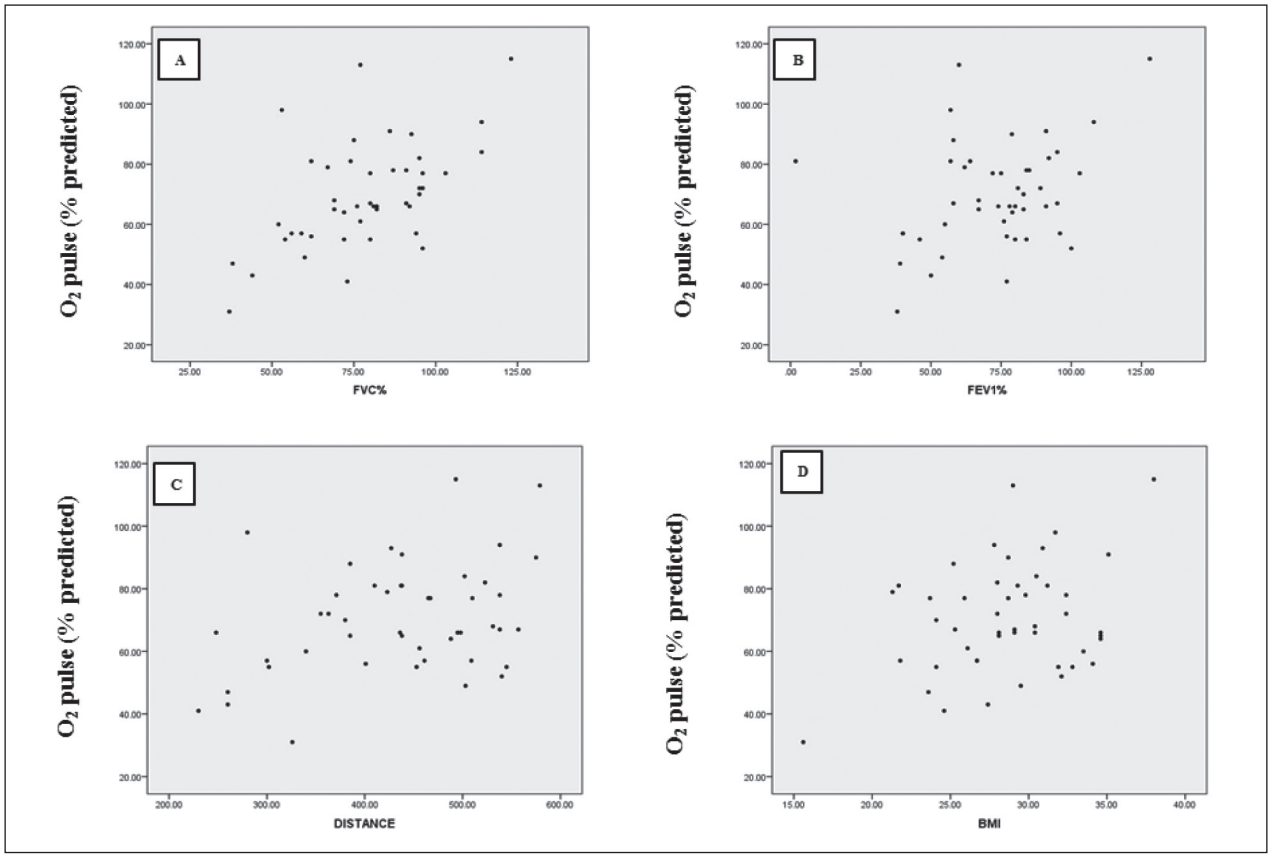

Fig. 2. Relationship of O2 pulse (% predicted) with FVC (% predicted) (r=0.557; P=0.000) (panel A); FEV1 (% predicted) (r=0.316; p=0.032) (panel B); distance (r=0.369; P=0.011) (panel C) and BMI (r=0.324; P=0.026) (panel D). Correlation was determined using Pearson's correlation coefficient (r). P<0.05 was considered statistically significant, with the exception of the correlation between O2 pulse (% predicted) and FVC (% predicted) which significance was considered as P<0.01 (2-tailed)

of percent-predicted VO_2 and O_2 are considered effective tools to predict the pulmonary and physical functioning in patients with sarcoidosis, especially in advanced stages. A lower percent-predicted peak VO_2 in the third group, in comparison with other groups, may be due to the severity of the disease in the constituents. Percent-predicted peak VO_2 was higher in group two, in comparison with group one. This contradictory result may either be related to the deconditioning in patients of group one or due to other reasons which are not related to cardio-pulmonary diseases. This finding is consistent with previous studies, in which the said parameter was also decreased in advanced stages (26-29).

Since VO_2/kg (ml/kg/min) depends on percent-predicted peak VO_2, we expected significant decreases in VO_2/kg (ml/kg/min) in the third group, in comparison with other groups. However, VO_2/kg (ml/kg/min) was not significantly lower in the third group. This is justified by the simultaneous reduction of the mean BMI at stage IV. Both mean BMI and percent-predicted peak VO_2 decreased in the third group, in comparison with other groups. Therefore, VO_2/kg in the third group was not different from the others. The decrease in BMI, in the third group, has been associated with increased breathing problems, due to which more energy is spent on breathing, or it may be because of the decreased appetite in patients with chronic diseases.

Percent VE was unexpectedly higher in group two, in comparison with group one, although this difference was not statistically significant. This finding poses the possibility of higher effort made by the patients of the second group, and also revealed that subjects in this group had performed the test for a longer period. It is obvious that subjects in the first group failed to continue the test, because of irrelevant reasons to ventilation and cardiovascular de-

fects. As expected, BR decreased in the third group. However, this difference was not statistically significant, which may be due to the small sample size in the third group.

In the current study, VE/VCO_2 (AT) increased significantly in stage IV, which was completely proportional to the severity of the disease in the third group. This finding was in agreement with the studies published by Kallianos (29) and Wallaert (25). Since the increased VE/VCO_2 (AT) is a gas exchange abnormally, this parameter could be used as a prognostic factor in chronic heart failure (29). Therefore, probably it can also be used as a predictor of mortality in sarcoidosis.

A decrease in percent-predicted peak VO_2 is correlated with a decrease of percent-predicted FVC in all cases and is in agreement with Lopes (27) and Wallaert (25) studies. This decrease correlates with the severity of the disease at advanced stages.

Two parameters of HR and SPO2 are measurable using both the 6MWT and CPET tests with no significant difference. So, the 6MWT, due to lower costs and easiness to perform, is more suitable to use instead of CPET for evaluating the parameters in advanced stages. This finding is in agreement with the American Thoracic Society guidelines for the 6MWT that find this test cheaper, easier to perform, more tolerable, and more reproducible, compared to the other tests (9-11).

To mention the 6MWT's weakness, in addition to that it is not possible to calculate the patients' VCO_2 and VE promptly, it also does not provide us with the cause of intolerance. On the other word, the reason behind stopping the test is not being defined by the 6MWT. For instance, in CPET after the interruption caused by exhaustion, it can be determined that it was due to deconditioning, but in the 6MWT we cannot be sure whether it is because of the exhaustion or respiratory reasons.

Although HR peak and percent-predicted HR were unexpectedly decreased in the third group, in comparison with the second one, this difference was not statistically significant. This incompatible result may either be related to the inability of cases in the third group to continue the test for a longer time or lower effort of these patients to continue the test.

If the halt in performing CPET will be related to cardiac insufficiency, in spite of the common belief that HRR will decrease, it may even increase in the said situation. Similarly, we witnessed that HRR had more escalation in the third group in comparison to the second group. The Lack of increase in HRR in higher stages can be explained by the inability of the patients in achieving tachycardia due to cardiac conductive disease.

The VO_2 /HR ratio, which is usually named as "oxygen pulse" (7, 30), significantly decreased in advanced stages. This finding was consistent with the results of Wallaert et al. (25) in which patients at stage IV had reached a lower O_2 pulse compared with those of the other stages. We also found a correlation between the 6MWD and O_2 pulse. Our study revealed that percent-predicted O_2 pulse was influenced by gender, BMI, percent-predicted FVC, and percent-predicted FEV_1. Therefore, percent-predicted O2 pulse could be a prognostic factor in sarcoidosis.

In the current study, as expected, SPO_2 decreased in the third group. The decrease of SPO2 is correlated with the severity of the disease. Therefore, evaluating the SPO_2 can be considered a convenient way to predict the mortality in sarcoidosis patients. In group one, the increase of SPO_2 after walking for six minutes was related to the increases in heart rate after physical activity.

The 6MWD decreases in many patients with sarcoidosis (31). Since the 6MWD is correlated with several factors (e.g., FVC and oxygen saturation), it seems that these parameters can be used to evaluate the functional status of patients with sarcoidosis. In the current study, the distance walked during six minutes was significantly less in patients at stage IV, which was consistent with the results of our last study (32) as well as the studies by Alhamad et al. (33) and Wallaert et al. (25).

We found that percent-predicted peak VO_2 was affected by age and TSH levels. This was in accordance with that of Artur et al. study, explaining that VO_2 is an age-related parameter (34). Regarding TSH levels, our result is inconsistent with that of Ittermann study (35). Sarcoidosis is known as one of the causes of subclinical hypothyroidism (36), and we also concluded that percent-predicted peak VO_2 which was affected by multiple factors, is affected by TSH levels as well.

Our study revealed a correlation between percent FVC and percent peak VO_2, which is consistent with Wallaert et al. (25) and Karetzky et al. (37) re-

sults; introducing percent FVC as a major significant predictor of percent peak VO_2. However, it is in contrast with Matthews study, also demonstrating the lack of correlation between PFT and percent peak VO_2 (38).

Since 25(OH) Vit D deficiency is known as a predicting factor, regarding the course of chronic sarcoidosis (39), we expected to witness the relationship between percent-predicted VO_2 and percent-predicted O_2 pulse with the mentioned biomarker; but it was not found as a contributing factor in this regard. This is likely to be due to the epidemy of moderate to severe vitamin D deficiency in Iran (40, 41).

Despite a reverse correlation that was seen between the chronicity of sarcoidosis and ACE levels (42) among the Iranian population, we did not find any significant correlation between ACE levels, percent-predicted VO_2, and O_2 pulse. ACE levels were lower in advanced stages, but the lack of a significant association may be due to the small sample size in our third group.

As far as we know, the correlation of PAP, EF, main pulmonary artery size, percent-predicted VO_2, and percent-predicted O_2 pulse were evaluated in sarcoidosis patients. For the first time, through the current study we encountered lack of the association between the mentioned parameters, percent-predicted VO_2, and percent-predicted O2 pulse and this may be probably due to the test sites which are situated at 1700 meters above sea level. Future parallel studies in lower areas may clarify this issue.

Since PAP increased significantly in the third group, it can be used as a predictor of disease severity in sarcoidosis. Considering that EF had a significant decrease in the stage IV patients, a decrement in EF can also be considered as a predictor of disease severity in sarcoidosis patients.

It is noteworthy that we did not classify our patients according to the type of pulmonary involvement. Although patients in the third group clearly had fibrosis involvement in their lungs, some cases may have had pulmonary vascular involvement, which undoubtedly has an effect on the results. This should be mentioned as the limitation of our study.

In conclusion, CPET revealed restriction in exercise capacity to a similar extent, disregarding the radiological stage in patients with sarcoidosis, while the 6MWT represents clinical weakness only in those with the most advanced disease.

Another finding of the current study is that pulmonary constraint is the main cause of activity limitation in sarcoidosis patients. However, it should be stated that deconditioning is as effective as the pulmonary constraint in activity limitation in sarcoidosis. The mentioned point is particularly crucial in the first stages of the disease because patients still do not have ventilation disorder. We recommend the use of non-pharmacological treatments and rehabilitation for them in order to tackle the deconditioning and furthermore improving the patients' dyspnea. It should be considered that dyspnea is not always caused by ventilatory problems urging us to prescribe immunosuppressors leading to worsening of the deconditioning.

In further studies, we are planning to start rehabilitation and physical exercise for patients with deconditioning and rerun the tests and investigate the impact on dyspnea.

Acknowledgments

The authors wish to thank all the sarcoidosis patients and all laboratory specialists who helped to get results of better quality.

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SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (3): 195-201

Radiologic and pathologic characteristics of myeloperoxidase-antineutrophil cytoplasmic antibody–associated interstitial lung disease: a retrospective analysis

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Abstract. *Background:* The association between interstitial lung disease (ILD) and myeloperoxidase (MPO)-antineutrophil cytoplasmic antibodies (ANCAs) has been described, but pathologic characteristics are not well characterized. *Objectives:* We assessed the radiologic and pathologic characteristics of ILD in MPO-ANCA–positive patients and the association between ILD and vasculitis, particularly microscopic polyangiitis (MPA). *Methods:* We retrospectively searched electronic health records to identify MPO-ANCA–positive patients with ILD who underwent surgical lung biopsy at our institution from January 1997 through August 2017. Demographic, clinical, imaging, and pathologic characteristics were analyzed. *Results:* We identified 18 MPO-ANCA–positive patients with ILD. The median (range) age was 58 (43-75) years, and the cohort included 10 men (56%), 10 former smokers (56%), and 11 patients (61%) had clinical evidence of systemic vasculitis (MPA) at the time of diagnosis of ILD. On high-resolution computed tomography, the most common radiologic pattern was "inconsistent with usual interstitial pneumonia" (UIP) (n=14 [78%]); the other 4 patients (22%) fulfilled the radiologic criteria for the UIP pattern. Honeycombing was seen in 15 patients (83%). Ten patients (56%) had the UIP pattern on biopsy: 4 of these patients had additional inflammatory changes that were not typical of UIP (as seen in patients with idiopathic pulmonary fibrosis), and the other 6 patients had other inflammatory patterns or findings. The presence or absence of MPA did not correlate with pathologic findings. *Conclusions:* MPO-ANCA–positive patients with ILD do not show the typical UIP pattern as seen in patients with idiopathic pulmonary fibrosis on surgical lung biopsy. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (3): 195-201)*

Key words: ILD, interstitial lung disease, microscopic polyangiitis, myeloperoxidase antibodies, UIP, usual interstitial pneumonia

Abbreviations
ANCA, antineutrophil cytoplasmic antibody
DLCO, diffusing capacity of lung for carbon monoxide
HR, hazard ratio
HRCT, high-resolution computed tomography
ILD, interstitial lung disease
IPF, idiopathic pulmonary fibrosis
MPA, microscopic polyangiitis
MPO, myeloperoxidase
NSIP, nonspecific interstitial pneumonia
OP, organizing pneumonia
UIP, usual interstitial pneumonia

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Accepted after revision: 19 March 2019
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Introduction

Microscopic polyangiitis (MPA) is a necrotizing, systemic small-vessel vasculitis that often involves the lungs, skin, kidneys, and peripheral nervous system and is associated with myeloperoxidase (MPO)-

antineutrophil cytoplasmic antibodies (ANCAs) (1). MPO-ANCAs are thought to be pathogenic because they promote the development of capillaritis (2). The association between interstitial lung disease (ILD) and MPO-ANCA was first reported in a case series in the 1990s (3). Other studies subsequently reported similar associations (4-11). ILD is observed in 2 settings: in patients with systemic vasculitis, usually MPA; and in patients with only positive MPO-ANCA serologic findings, some of whom later have MPA. It is unclear whether ILD observed in the presence of only MPO-ANCA differs from idiopathic ILD and ILD with overt MPA. Although the association between radiologic evidence of ILD and MPO-ANCA has been described, pathologic descriptions are limited.

The objective of this study was to describe the radiologic and pathologic characteristics of ILD associated with positive MPO-ANCA serologic findings. We also aimed to identify differences related to the presence of systemic vasculitis, particularly MPA.

Methods

Patient Selection

We used Advanced Cohort Explorer (Mayo Clinic) to retrospectively search for the electronic health records of all MPO-ANCA–positive adult patients with ILD who underwent surgical lung biopsy at Mayo Clinic, Rochester, Minnesota, from January 1, 1997, through August 31, 2017. Our search was conducted iteratively by using the following terms: first, "microscopic polyangiitis," "MPO," "MPO vasculitis," and "p-ANCA vasculitis"; second, "pulmonary fibrosis" and "interstitial lung disease"; and third, "surgical lung biopsy," "lung biopsy," and "video-assisted thoracoscopic surgery." For all identified patients, we performed a complete review of the health records to confirm the diagnoses and lung biopsy procedures. To avoid the limitations of transbronchial biopsy results, we focused on only surgical lung biopsies. We excluded patients with transbronchial lung biopsy and patients positive for perinuclear ANCA activity determined by only indirect immunofluorescence (ie, without MPO reactivity).

The Mayo Clinic Institutional Review Board approved this study (No. 17-002430). Patients who did not authorize the use of their electronic health records were excluded.

Clinical and Laboratory Data

Data extracted from the electronic health records included age, sex, race/ethnicity, smoking status, symptoms, laboratory results, MPO-ANCA status, urinalysis results, serum creatinine level, presence or absence of inflammatory markers, pulmonary function test results, treatment, outcome, and follow-up duration.

Diagnostic Criteria for MPA

MPA, when present, was diagnosed according to the definitions for vasculitides adopted by the 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides (1).

Radiologic Analysis

A thoracic radiologist (C.W.C.) reviewed all thoracic high-resolution computed tomography (HRCT) scans. The patterns were described according to the diagnostic criteria of the European Respiratory Society/American Thoracic Society for idiopathic interstitial pneumonias (12, 13). The extent and location of fibrosis were also determined. Follow-up HRCT scans, when available, were also reviewed.

Pathologic Analysis

A thoracic pathologist (E.E.Y. and/or T.V.C.) reviewed all available surgical lung biopsy slides and reports. Pathologic changes were described according to the appropriate classifications of the European Respiratory Society/American Thoracic Society for idiopathic interstitial pneumonias (12,13).

Statistical Analysis

Categorical variables were compared between patients with MPA and patients without MPA with the Fisher exact test. The Wilcoxon rank sum test was used to compare continuous variables between groups. $P<.05$ was considered statistically significant. Univariate Cox proportional hazards regression analysis was used to evaluate associations between variables and death. Kaplan-Meier analysis was used to estimate survival. JMP software (SAS Institute Inc) was used to perform the statistical analysis.

Results

We identified and evaluated 18 MPO-ANCA–positive patients. The median (range) age was 58 (43-75) years, and 10 men (56%) were included (Table 1). Most patients in our cohort were white (89%), and 10 patients (56%) had a history of smoking. The median (range) duration of respiratory symptoms before the diagnosis of ILD was 1 (0.16-30) year. Eleven (61%) patients had clinical evidence of active systemic vasculitis (MPA) at the time of diagnosis of ILD.

Fourteen patients (78%) underwent pulmonary function tests at the time of diagnosis of ILD. Six patients had a restrictive pattern evidenced by reduction in total lung capacity and reduction in diffusing capacity of lung for carbon monoxide (DLCO); 5, isolated reduction in DLCO; 1, isolated reduction in total lung capacity (DLCO not measured); and 2, normal pulmonary function test results. The median (range) percentage predicted forced vital capacity was 64% (32%-88%), and the median (range) percentage predicted DLCO was 54% (37%-88%). Eleven patients underwent echocardiography at the time of diagnosis of ILD, and the median (range) right ventricular systolic pressure was 36 (21-69) mm Hg. Three patients met the echocardiographic criteria for pulmonary hypertension (right ventricular systolic pressure ≥50 mm Hg) (14).

Table 1. Demographic and Clinical Characteristics

Characteristic	Value[a] (N=18)
Age, y	58 (43-75)
Men	10 (56)
White race/ethnicity	16 (89)
History of smoking	10 (56)
Microscopic polyangiitis	11 (61)
Respiratory symptoms[b]	17 (94)
Dyspnea	16 (94)
Cough	10 (59)
Chest pain	5 (29)
Results of pulmonary function tests	
Percentage predicted FVC[c]	64 (32-88)
Percentage predicted DLCO[d]	54 (37-88)
RVSP, mm Hg[d]	36 (21-69)

Abbreviations: DLCO, diffusing capacity of lung for carbon monoxide; FVC, forced vital capacity; RVSP, right ventricular systolic pressure.
[a] Values are shown as No. (%) or median (range); [b] Some patients had more than 1 symptom; [c] Determined for 11 patients at the time of diagnosis of pulmonary fibrosis; [d] Determined for 13 patients at the time of diagnosis of pulmonary fibrosis.

The HRCT findings are summarized in Table 2. The most common pattern was "inconsistent with usual interstitial pneumonia" (UIP), which was noted in 14 patients (78%) (unclassified or atypical, n=9; nonspecific interstitial pneumonia [NSIP], n=4; organizing pneumonia [OP], n=1). Only 4 patients (22%) met the radiologic criteria for the UIP pattern, but most patients had honeycombing (n=15 [83%]). The presence of ground-glass opacities (n=11 [61%]) and mosaic attenuation (n=3 [17%]) were the most common reasons for classifying findings on HRCT images as inconsistent with a UIP pattern (Figure 1). Presence of the UIP pattern on HRCT was not associated with the presence or absence of clinical vasculitis (MPA) at the time of diagnosis. Among the patients who had MPA at the time of diagnosis of ILD, 2 (18%) had the UIP pattern on HRCT and 9 (82%) had a pattern inconsistent with UIP. However, among patients who did not have active MPA at the time of diagnosis of ILD, 2 (29%) had the UIP pattern on HRCT and 5 (71%) had a pattern inconsistent with UIP (*P*=.61). Fibrosis was predominantly distributed in the lower lobe (n=10 [56%]), or a dif-

Table 2. Findings on High-Resolution Computed Tomography

Characteristic	No. of Patients (%) (N=18)
Pattern of interstitial abnormalities	
UIP	4 (22)
Inconsistent with UIP	14 (78)
Unclassified or atypical	9 (64)
NSIP	4 (29)
OP	1 (7)
Honeycombing	15 (83)
Cause of inconsistency with the UIP pattern[a]	
Ground-glass opacities	11 (61)
Mosaic attenuation	3 (17)
Diffuse distribution	2 (11)
Midlung distribution	2 (11)
Apical thickening	1 (6)
Consolidation	1 (6)
Subpleural sparing	1 (6)
Distribution of fibrosis	
Craniocaudal	
Lower	10 (56)
Diffuse	8 (44)
Axial	
Peripheral	13 (72)
Diffuse	3 (16)
Central	2 (11)

Abbreviations: NSIP, nonspecific interstitial pneumonia; OP, organizing pneumonia; UIP, usual interstitial pneumonia.
[a] Some patients had more than 1 finding.

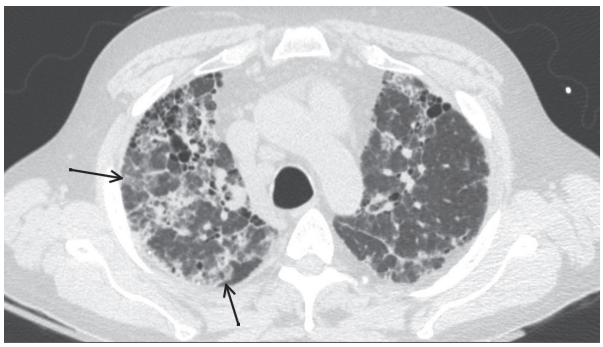

Fig. 1. Pattern Inconsistent With Usual Interstitial Pneumonia. Axial high-resolution computed tomographic image of the upper pulmonary lobes of a 55-year-old man with microscopic polyangiitis. This complex interstitial pattern is inconsistent with usual interstitial pneumonia according to the 2011 criteria of the European Respiratory Society/American Thoracic Society. Ground-glass opacities (black arrows), diffuse architectural distortion, scattered consolidation, and honeycombing are shown

fuse pattern was detected (n=8 [44%]); upper-lobe predominance was not detected in any patients. Regarding the axial distribution of fibrosis, peripheral changes were most commonly seen (n=13 [72%]).

The surgical lung biopsy results are summarized (Table 3). The UIP pattern was predominant in 10 patients (56%). In 4 of 10 patients with the UIP pattern, additional inflammatory changes were identified that are not typical of the UIP pattern in patients with idiopathic pulmonary fibrosis (IPF), including bronchiolitis, lymphoid hyperplasia, desquamative interstitial pneumonia, and OP. The NSIP pattern was predominant in 5 patients, and bronchiolitis was predominant in 1 patient. NSIP and the early UIP pattern could not be confidently distinguished in 1 patient. Only 1 of 18 biopsies showed active granulomatosis with polyangiitis (fibroinflammatory changes with granulomas) as the cause of the radiologic findings. The presence of a UIP pattern on biopsy was not associated with the presence or absence of clinical vasculitis (MPA) at the time of diagnosis. Among patients who had MPA at the time of diagnosis of ILD, 3 (27%) had the UIP pattern on biopsy, 1 (9%) had the UIP pattern with additional features, and 7 (64%) had a pattern inconsistent with UIP. However, among patients who did not have MPA at the time of diagnosis of ILD, 3 (43%) had the UIP pattern on biopsy, 3 (43%) had the UIP pattern with additional features, and 1 (14%) had a pattern inconsistent with UIP (*P*=.07).

Table 3. Pathologic pulmonary Findings of 18 patients

Patient No.	Histologic Findings
1	UIP pattern with areas showing features of DIP
2	Follicular bronchiolitis and cellular bronchiolitis with foci of OP and foci resembling NSIP
3	UIP pattern with a slight increase in lymphoid aggregates and focal OP
4	NSIP with follicular hyperplasia and DIP features
5	UIP pattern
6	NSIP with OP and follicular bronchiolitis
7	Fibroinflammatory changes with granulomas consistent with GPA
8	UIP pattern
9	UIP pattern with some associated chronic bronchiolitis, chronic pleuritis, and focal OP
10	UIP pattern
11	Unable to distinguish fibrosing NSIP and the early UIP pattern
12	NSIP with chronic bronchiolitis, OP, and early, focal, acute bronchopneumonia
13	Cellular NSIP with mild fibrosis and OP
14	Cellular and fibrosing NSIP
15	UIP pattern
16	UIP pattern
17	UIP pattern
18	UIP pattern with lymphocytic infiltrates and follicular lymphoid hyperplasia

Abbreviations: DIP, desquamative interstitial pneumonia; GPA, granulomatosis with polyangiitis; NSIP, nonspecific interstitial pneumonia; OP, organizing pneumonia; UIP, usual interstitial pneumonia

Follow-Up After Initial Evaluation

The median (range) follow-up period was 4.3 (0.6-13.4) years. During this period, active vasculitis developed in 3 of 7 patients without vasculitis (MPA) at the time of diagnosis of ILD at 7.2, 9.6, and 20.4 months after the initial diagnosis of ILD. Fifteen patients (83%) underwent follow-up HRCT, with a median (range) interval of 1.78 (0.24-11.39) years (HRCT was not performed at fixed intervals). Eleven patients had progression of interstitial findings (including 8 patients with moderate progression); 3, stable findings; and 1, improved findings.

Pulmonary function tests were repeated in 11 patients, with a median (range) interval of 2.37 (0.6-12.0) years (pulmonary function tests were not performed at fixed intervals). The median (range) change in percentage predicted forced vital capacity was –1% (–29% to 27%), and the median (range) change in percentage predicted DLCO was –10% (–26% to 25%).

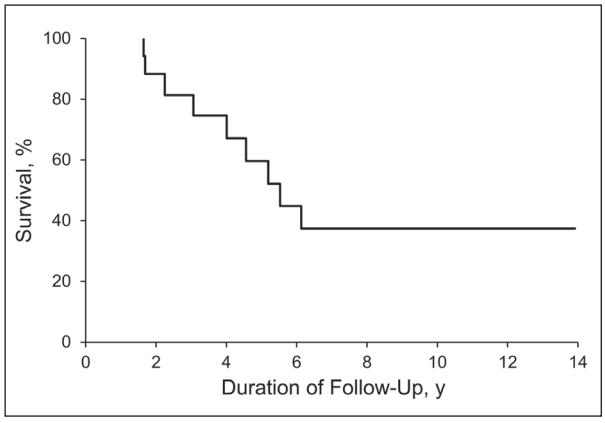

Fig. 2. Kaplan-Meier Estimate of Survival

Nine patients (50%) died during follow-up, and the most common cause of death was respiratory failure (n=6). One patient died of active vasculitis, and the causes of death could not be determined for 2 patients. The median survival of our cohort was 5.5 years (Figure 2). According to the univariate analysis, the predictors of death were DLCO at diagnosis (HR [hazard ratio], 0.89; 95% CI, 0.78-0.97; *P*=.007), DLCO at follow-up (HR, 0.82; 95% CI, 0.53-0.96; *P*=.008), and forced vital capacity at follow-up (HR, 0.95; 95% CI, 0.90-0.99; *P*=.02). All of these predictors are related to impaired lung function.

Discussion

In this retrospective study, we assessed the relationship between radiologic and pathologic characteristics of MPO-ANCA–positive patients with ILD who underwent surgical lung biopsy. The most common radiologic pattern in this cohort was inconsistent with the UIP pattern (as seen in patients with IPF), although more than half of patients had a predominant UIP pattern on biopsy. NSIP and bronchiolitis were also predominant pathologic findings in some patients. Of the 10 patients with a predominant UIP pattern, 4 had inflammatory changes that were not typical of the UIP pattern observed in patients with IPF. Radiologic and pathologic findings appeared to be independent of active clinical vasculitis at the time of diagnosis of ILD. Only 1 of 18 patients had pathologic characteristics of vasculitis (fibroinflammatory changes with granulomas) reported as the cause of the radiologic changes.

UIP has been described as a predominant pattern of fibrosis on HRCT in MPO-ANCA–positive patients (15, 16). However, only 4 of our patients (22%) had the typical UIP pattern on HRCT. Nevertheless, most patients in our cohort (15 patients [83%]) had honeycombing indicative of a clinically significant fibrosing process. In addition to honeycombing, some findings, particularly ground-glass opacities, made the radiographic diagnosis of UIP less likely. This discrepancy most likely reflects the bias associated with selecting patients for surgical lung biopsy who did not meet the radiographic criteria for UIP. Similar findings have been described by Foulon et al (10), who reported some degree of ground-glass opacities, honeycombing, reticular opacities, and traction bronchiectasis in all 17 patients in their study.

In our study, radiologic signs of fibrosis were predominantly distributed in the lower lobe. A similar finding is described in another study, in which 10 of 14 patients had lower-zone–predominant lung fibrosis (11). Similar lower-zone–predominant and peripheral-zone–predominant patterns of pulmonary fibrosis were described in another study of 19 patients (4). This is an important observation because the presence of lower-zone pulmonary fibrosis may distinguish ILD associated with MPO-ANCA and MPA from other types of ILD with upper-lobe predominance, such as chronic hypersensitivity pneumonitis.

Although the UIP pattern was the most common, predominant pathologic pattern in our patients, additional inflammatory changes that are not typical of the UIP pattern in patients with IPF were seen in 4 patients, including bronchiolitis, OP, lymphoid aggregates, and lymphoid hyperplasia. Five of our patients had a predominant NSIP pattern. In 1 patient, NSIP and early UIP could not be confidently distinguished, and bronchiolitis was the main finding in another patient. Thus, in 17 of 18 patients, the predominant histologic finding was the UIP pattern or NSIP, although patients with the UIP pattern showed additional inflammatory changes usually not seen in patients with IPF. Only 1 patient (Patient No. 7) showed vasculitis on surgical lung biopsy as the cause of the radiologic changes.

Prior studies of pathologic findings in MPO-ANCA–positive patients are rare. Homma et al (17) described biopsy and postmortem specimens obtained from 15 patients. Honeycombing was seen in 12 of these patients, but most of them also had

additional findings, including OP (n=9 [60%]), pleuritis (n=7 [47%]), lymphoid hyperplasia (n=6 [40%]), or vasculitis (n=5 [33%]). Foulon et al (10) reported 3 lung biopsies that showed only the UIP pattern. Ando et al (18) reported the UIP pattern in 6 patients who underwent surgical lung biopsy, but 2 patients had prominent lymphoid proliferation. Although relatively few patients were reported in these studies, the findings do not appear to be appreciably different from ours, with the provision that NSIP has not been described as a predominant pattern.

The median survival of our patients was 5.5 years. This is comparable to a median survival of 72 months for patients with MPA and pulmonary fibrosis reported by Tzelepis et al (8) and the mean survival time of 4.2 (range, 0.0-27.8) years reported by Arulkumaran et al (11). In the latter study, the authors reviewed 99 cases described in the literature and reported a median (range) survival of 5.3 (0.08-13.7) years. We identified 3 factors associated with death, all indicators of severity of pulmonary fibrosis. Kagiyama et al (5) identified older age, proteinase 3–ANCA positivity, and poor pulmonary function test results as negative prognostic factors. Over the years, pulmonary fibrosis tends to worsen in MPO-ANCA–positive patients; whether immunosuppressive therapy provides any beneficial effect on the progression of lung fibrosis is unclear. Treatment response could also differ according to the type of fibrosis. Most patients in our cohort died of respiratory failure, and other studies have reported similar findings (9, 10, 16). Thus, the overall prognosis of these patients could be directly related to the pulmonary fibrosis and not the underlying vasculitis. The prognosis of MPO-ANCA–positive patients with pulmonary fibrosis has been described as better than (19), similar to (17, 18) and worse than (5, 17) the prognosis of MPO-ANCA–negative patients with pulmonary fibrosis. This suggests that acute management of vasculitis may influence the reported outcomes of MPO-ANCA–positive patients with ILD and MPA.

Our study raises several important questions about the care of MPO-ANCA–positive patients with ILD. First, how should clinicians manage ILD? This question, which is particularly relevant in the era of antifibrotics, has 2 components: 1) Which patients (if any) should be treated with immunosuppressive therapy; and 2) Which patients (if any) should be considered for antifibrotic therapy? Because most of our patients had radiologic patterns of fibrosis that were not consistent with the UIP pattern, we cannot draw conclusions about patients with the typical UIP pattern. However, Chino et al (19) have shown that MPO-ANCA–positive patients with UIP but without overt MPA have more inflammation on biopsy than MPO-ANCA–negative patients with UIP. Their observation and our findings might suggest that MPO-ANCA–positive patients with ILD need an immunosuppressive treatment trial for ILD. Further standardized, multicenter investigations should evaluate the treatment responses of patients with ILD to immunosuppressive therapy and antifibrotics, particularly the responses of patients with radiographic or pathologic findings of UIP.

The prevalence of MPO-ANCA among patients with ILD is unclear. However, our study confirms the findings of other studies, which indicate that over the ensuing years overt MPA subsequently develops in more than 40% of MPO-ANCA–positive patients without overt MPA at the time of diagnosis of ILD (5, 10). Thus, the second important management question is how to address the risk of vasculitis among MPO-ANCA–positive patients. At the very least, patients should know about the signs and symptoms of active MPA and should be carefully monitored for the manifestations of MPA, particularly glomerulonephritis. Immunosuppressive treatment of ILD with inflammatory characteristics could also prevent the development of overt MPA.

The final management question raised by our findings is whether MPO-ANCA–positive patients with ILD who do not have a typical UIP pattern on HRCT, and therefore are considered for biopsy, should undergo this procedure, or whether they should first receive a treatment trial of immunosuppressive therapy.

Our study has several limitations. First, this is a retrospective study with a small sample size, and data are unavailable for some patients. Although we tried to identify excessive inflammation beyond what is generally seen in patients with the UIP pattern (eg, OP, NSIP, lymphoid aggregates), some patients may have had autoimmune features within the accepted criteria for UIP (eg, Patient No. 3 in Table 3). Second, our study is dependent on the biases of the clinicians who selected patients for surgical lung biopsy. Most patients at our institution with a radiologic pattern of definite UIP do not undergo surgical

lung biopsy, and the primary role of this procedure is to identify or exclude other causes of ILD that may be amenable to treatment. Consequently, our study may not be representative of the most common pattern of pulmonary fibrosis in MPO-ANCA–positive patients that can be detected with HRCT. Third, the chronologic evolution of lung fibrosis and active vasculitis was difficult to assess exactly because most patients were first seen elsewhere and the exact sequence of events was difficult to establish. However, because most patients had clinical features of active vasculitis (which develops acutely or subacutely) at the time of the biopsy, and because most patients had radiologic and pathologic evidence of the UIP pattern (a lesion that develops slowly), we speculate that pulmonary fibrosis usually precedes the onset of MPA. This is further supported by the observation (made here and by others) that systemic MPA will subsequently develop in many patients with only MPO-ANCA positivity (5).

In conclusion, in assessing the relationship between pathologic and radiologic findings in MPO-ANCA–positive patients with pulmonary fibrosis, we identified inflammation on surgical lung biopsies. This was mainly seen when the HRCT findings were not consistent with the UIP pattern. Our study shows the variability in the characteristics of ILD among MPO-ANCA–positive patients. Further multicenter investigations of larger cohorts of patients are needed to define outcomes and treatment responses according to the type of ILD.

Role of the Funding Source: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions: M.B.: collected data, analyzed and interpreted data, wrote the manuscript. E.E.Y.: collected pathologic data, analyzed and interpreted data, helped write the manuscript. T.V.C.: collected pathologic data, analyzed and interpreted data, helped write the manuscript. C.W.C.: collected radiologic data, analyzed and interpreted data, helped write the manuscript. J.H.R.: analyzed and interpreted data, helped write the manuscript. U.S.: senior author, research design, collected data, analyzed and interpreted data, statistical analysis, wrote the manuscript.

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SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (3): 202-208

High incidence of venous thromboembolism but not of coronary artery disease in granulomatosis with polyangiitis in first years after diagnosis

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Abstract. *Objectives:* Granulomatosis with polyangiitis (GPA), previously known as Wegener's granulomatosis, is one of antineutrophil cytoplasmic autoantibody (ANCA) – associated vasculitis. In patients with GPA an increased incidence of venous thromboembolism (VTE), mainly during active disease, has been described. The aim of the present study was to assess the incidence of VTE and its relation with classic risk factors for atherosclerosis, presence of coronary artery disease (CAD), echocardiographic parameters and laboratory findings in GPA patients. *Methods:* The group of consecutive patients with GPA were followed in the study. In all patients echocardiography and laboratory tests were performed. *Results:* Ninety six patients with GPA were followed for mean 3 years. In 16 patients (16.6%) VTEs occurred in association with GPA, of which 56% occurred 6 months before or one year after diagnosis of GPA. Classic risk factors for atherosclerosis were present in 77 patients (80.2%) at some moment during follow-up. In patients with VTE there were larger right ventricle diameter (p=0.041) and higher right ventricle systolic pressure (p=0.022) observed. VTEs occurred significantly less frequently in patients treated with cyclophosphamide (p=0.049). In this study group VTE occurred more frequently than CAD: 16 (16.7%) vs. 4 (4.2%); p=0,0049. Patients with VTE were younger than those with CAD (p=0.053) and had higher levels of ANCA-PR 3 (p=0.016). *Conclusions:* Patients with granulomatosis with polyangiitis in first years after diagnosis have higher risk of venous thromboembolism than coronary artery disease. This finding is probably related to hypercoagulability induced by the disease and its therapy. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (3): 202-208)*

Key words: vasculitis, granulomatosis with polyangiitis, venous thromboembolism, coronary artery disease

Introduction

Granulomatosis with polyangiitis (Wegener's; GPA) is an antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), which also includes microscopic polyangiitis and eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome). GPA is characterized by granulomatous inflammation and necrotizing vasculitis mainly affecting small- and medium-sized blood vessels and the presence of ANCA directed to specific antigens, particularly proteinase 3 (PR3-ANCA) and myeloperoxidase (MPO-ANCA). The inflammatory processes in GPA have a predilection for the kidneys and respiratory tract, but any organs, including cardiovascular system, can be affected (1).

An increased incidence of various cardiovascular events has been described among GPA patients (2-

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Accepted after revision: 29 April 2019
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4). In a retrospective analysis from the Danish National Hospital Register, patients with GPA showed an increased rate of cardiovascular events within the first 5 years after a diagnosis of GPA compared to the general population (3). Also in study by Aviña-Zubieta JA et al. patients with GPA have a significantly increased risk of myocardial infarction and a non-statistically significant trend toward an increased risk of ischemic stroke (4).

In recent years, the relationship between inflammation and thrombosis has been investigated and it turned out that immune and coagulation systems are functionally connected. Plasminogen has been described as an autoantigen in PR-ANCA patients. Its interaction with autoantibodies directed towards complementary PR3 is responsible for impairing fibrinolysis by blocking conversion from plasminogen to plasmin (5, 6). A procoagulant state caused by increase in endogenous thrombin and Factor VIII concentration was also reported in AVV patient in stable remission (7). Merkel PA et al. reported a high occurrence of pulmonary embolism (PE) and deep venous thrombosis (DVT) among GPA patients included in a randomized therapeutic trial (8). Of the observed venous thromboembolic events (VTEs), 81% occurred in patients with active or recently active vasculitis. A high incidence rate of VTEs was also calculated for patients with various ANCA-associated vasculitides in subsequent retrospective cohort studies (9-11).

The aim of the present study was to assess the incidence of coronary artery disease, PE, and DVT in a cohort of 96 patients diagnosed with GPA and followed for a median of 3 years.

Patients and methods

In this prospective cohort study, consecutive GPA patients who were hospitalized in the Department of Family Medicine, Internal and Metabolic Diseases at the Medical University of Warsaw in Poland between February 2010 and April 2017 were included. All patients were diagnosed with GPA according to current guidelines (12). Patients were entered into the study at the time point when a new diagnosis of ANCA-associated vasculitis was established and received initial treatment at our centre. Data collection included a full medical history, physical examination, laboratory studies and review of adverse events. Additionally, in all patients an echocardiography was performed. M-mode and two-dimensional standard echocardiography (Mindray M7, Shenzhen Mindray Bio-Medical Electronics Co.) followed by pulsed and continuous-wave Doppler recordings were performed by one experienced cardiologist. Five consecutive measurements were averaged for each parameter. All patients were tested for ANCA by indirect immunofluorescence and enzyme-linked immunosorbent assay (ELISA). Control visits were planned every three months. On each visit physical examination, laboratory studies, review of adverse events and ECG were performed. Additionally, every six months all patients had a scheduled echocardiogram. A patient was considered to have had a VTE if the event was clinically apparent and was confirmed by diagnostic studies (vascular ultrasonography or computed tomographic angiography). All patients with CAD diagnosis had coronarography performed.

All VTEs were counted in patients after diagnosis of GPA, adding a period of 6 months before the diagnosis of GPA was made. We added this period because, due to diagnostic delay, in most cases GPA had been active some time before the diagnosis was made. VTEs occurring in this period were considered GPA associated. VTEs occurring in association with a central venous catheter were excluded. In addition, we compared demographic and clinical characteristics and risk factors in patients who develop a VTE associated with GPA and those who did not develop a VTE. Finally, patients with VTE were compared to patients diagnosed with CAD.

The study protocol was approved by the ethics committee of the Medical University of Warsaw. Written informed consent was obtain from each participant.

Continuous variables are summarized as means and SD or as median and inter-quartile range. Qualitative variables are presented as counts and percentages . Comparisons between groups were made using Student's t-test for numerical, normally distributed data, the Mann-Whitney test for continuous variables not normally distributed and the Pearson's chi2 test or the Fisher's exact test (in case of minimum expected count less than 5) for categorical data. Pearson's or Spearman's correlation coefficients were calculated to investigate the associations between parameters. All hypotheses testing were two-tailed

with a p<0.05 type I error. All analysis were performed with SAS 9.2 software (SAS Institute Inc, Cary, NC, USA).

Results

Patients baseline characteristics is presented in Table 1. The median observation period was 3 years (range: 0.1-5.8 years) and none of the patients were lost to follow-up. Majority of patients (73%) were ANCA - positive at diagnosis. Classic risk factors for atherosclerosis were present in 77 patients (80.2%) at some moment during follow-up. Five or more internal involvement were observed in 22% of GPA patients. Over 84% of patients were treated with glucocorticoids, 16% with azathioprine and 47% with cyclophosphamide. In total VTE occurred in 16 (16.6%) patients; 4 VTEs (4%) occurred before and 12 (12,5%) after inclusion in the study. Among patients with VTE 13 had deep venous thromboses, 2 pulmonary emboli and one both. VTEs which occurred in association with GPA, in 56% occurred six months before or one year after inclusion to the study. Table 2 shows the differences between patients with and without VTEs. There were no difference between patients with and without VTEs in internal organs involvement. There were also no significant differences in age, classic risk factors for atherosclerosis, creatinine, ANCA antibodies and CRP levels. In echocardiography patients with VTE had larger right ventricle diameter (28.9±4.4 vs. 31.6±5.6; p=0.041) and higher right ventricle systolic pressure (32.8±5.4 vs. 36.3±5.9; p=0.022) observed. VTEs occurred significantly less frequently in patients treated with cyclophosphamide (51.9% vs. 25.0%; p=0.049). In this study group VTE occurred more frequently than coronary artery disease: 16 (16.7%) vs. 4 (4.2%); p=0,0049. The CAD events included 2 cases of myocardial infarction, one of unstable angina treated with CABG and one of unstable angina treaded with coronary angioplasty. Table 3 presents a comparison between patients with VTEs and CAD. There were no significant differences in classic risk factors, immunosuppresives use, internal involvement, creatinine and CRP levels. Patients diagnosed with CAD had higher interventicular septal diameter and all of them had left ventricle diastolic dysfunction. Patients with VTE were younger than those with CAD (50.7±16.2 vs. 66.5±10.8; p=0.053), had higher right ventricular systolic pressure (36.3±5.9 vs. 28.7±4.6; p=0.03) and had higher levels of ANCA-PR 3 (16.6 vs. 1.5; p=0.016).

Table 1. Patients characteristics at baseline

	N=96
Age, years	50,6±14,6
Sex, female, n (%)	62 (64,6%)
Hypertension, n (%)	64 (66,7%)
Hypercholesterolemia, n (%)	66 (68,7%)
Diabetes, n (%)	24 (25,0%)
Hypertension or Hypercholesterolemia or Diabetes, n (%)	77 (80,2%)
Coronary artery disease, n (%)	1 (1%)
Venous thromboembolism, n (%)	4 (4%)
Echocardiography	
Aorta diameter, mm	31,8±3,5
Left atrium diameter, mm	36,4±4,7
Interventricular septal diameter, mm	10,7±1,9
Posterior wall thickness, mm	10,4±1,3
Left ventricular diastolic diameter, mm	45,2±5,5
Left ventricular systolic diameter, mm	24,4±4,8
Right ventricular diameter, mm	29,4±4,7
Pulmonary artery diameter, mm	19,7±1,8
Vena cava inferior diameter, mm	17,1±2,4
Pulmonary acceleration time, ms	129±18
Right ventricular systolic pressure, mmHg	33,4±5,6
Left ventricular ejection fraction, %	62,9±6,7
Left ventricular diastolic dysfunction, n (%)	23 (30,7%)
TAPSE, mm	21,6±2,5
Internal involvement	
Eyes, n (%)	30 (31,2%)
Ears, n (%)	17 (17,7%)
Genitourinary, n (%)	1 (1,0%)
Musculoskeletal, (%)	40 (41,7%)
Upper respiratory tract, n (%)	81 (84,4%)
Lower respiratory tract, n (%)	69 (71,9%)
Kidney, n (%)	50 (52,1%)
Central nervous system, n (%)	11 (11,5%)
Peripheral nervous system, (%)	20 (20,8%)
Skin, n (%)	9 (9,4%)
Creatinine, mg/dl	1,6 (1,3-3,8)
Hs-CRP, mg/dl	1,5 (0,4-10)
Troponin I, ug/l	0,010
Five or more internal involvement, n (%)	21 (21,9%)
ANCA-positive, n (%)	70 (72,9%)
Anti-MPO-positive, n(%)	7 (7,3%)
Anti-PR3-positive, n (%)	63 (65,6%)

Discussion

In this prospective study, we investigated the incidence of venous thromboembolism and coronary

Table 2. Comparison between GPA patients with and without episodes of venous thromboembolism

	Patients without VTE, N=80	Patients with VTE, N=16	p
Age, years	52,6±14,4	52,7±16,2	0,978
Disease duration since diagnosis, years	2 [0,1-4,60]	2 [0,2-5,8]	0,996
Sex, female, n (%)	52 (65,0%)	10 (62,5%)	0,849
Hypertension, n (%)	55 (68,7%)	9 (56,2%)	0,339
Hypercholesterolemia, n (%)	56 (70%)	10 (62,5%)	0,555
Diabetes, n (%)	20 (25,0%)	4 (25,0%)	1,00
Hypertension or Hypercholesterolemia or Diabetes, n (%)	67 (83,7%)	10 (62,5%)	0,081
Coronary artery disease, n (%)	4 (5%)	0 (0%)	1,000
Echocardiography			
Aorta diameter, mm	31,7±3,3	32,2±4,3	0,603
Left atrium diameter, mm	36,2±4,7	37,1±4,8	0,499
Interventricular septal diameter, mm	10,7±1,3	10,6±1,4	0,852
Posterior wall thickness, mm	10,4±1,3	10,4±1,1	0,972
Left ventricular diastolic diameter, mm	45,0±5,5	46,0±5,2	0,513
Left ventricular systolic diameter, mm	24,2±4,9	25,2±4,4	0,416
Right ventricular diameter, mm	28,9±4,4	31,6±5,6	0,041
Pulmonary artery diameter, mm	19,6±1,9	19,9±1,6	0,655
Vena cava inferior diameter, mm	17,0±2,4	17,4±2,8	0,544
Pulmonary acceleration time, ms	129±16	131±24	0,748
Right ventricular systolic pressure, mmHg	32,8±5,4	36,3±5,9	0,022
Left ventricular ejection fraction, %	62,6±7,0	64,2±5,2	0,378
Left ventricular diastolic dysfunction, n (%)	21 (33,9%)	2 (15,4%)	0,321
TAPSE, mm	21,3±2,4	22,4±2,8	0,236
Glucocorticoids, n (%)	69 (86,2%)	12 (75%)	1,00
Azathioprine, n (%)	12 (15,2%)	4 (25%)	0,462
Cyclophosphamide, n (%)	41 (51,9%)	4 (25,0%)	0,049
Creatinine, mg/dl	0,9	1,0	0,219
Hs-CRP, mg/dl	0,40	0,50	0,794
Troponin I, ug/l	0,010	0,008	0,811
MPO-positive, n(%)	6 (7,5%)	1 (6,2%)	1,00
PR3-positive, n (%)	51 (63,7%)	12 (75,0%)	0,3871

artery disease (CAD) in a large homogenous cohort of GPA patients and the possible influence of disease duration and classic risk factors for CAD on the occurrence of VTEs and CAD episodes. We found an increased incidence of VTE in comparison to CAD in first years after the diagnosis of GPA. Interestingly, although most of GPA patients (80%) had CAD risk factors (hypertension, hypercholesterolemia or diabetes), they presented more frequently with VTE than with CAD episodes. However, some risk factors for atherosclerosis (cigarette smoking, obesity, hypercholesterolemia, hypertension and diabetes mellitus) are shared with venous thromboembolism. Our data are in line with the findings by Merkel et al. from Wegeners's granulomatosis Etanercept Trial (WGET). In the WGET trial 180 patients with GPA were followed for more than 2 years. In the end of the observation period, 29 of 180 GPA patients (16%) have had a VTE diagnosed. They found that an increased incidence of VTEs, mainly (83%) occurred 2 months prior to or following a diagnosis of active disease (8). The pathogenic background for the high VTE risk in GPA is poorly understood. The development of VTEs was not related to traditional clinical risk factors for venous thromboembolism in 2 studies of patients with ANCA-associated vasculitis (9, 10), while Allenbach et al. identified higher age, male sex, previous VTE and strokes as risk factors for VTEs in a retrospective analysis involving 1130 patients with systemic necrotizing vasculitides (11). Novikov et al. reported than majority of their patients with VTE were young, had no known risk factors for thrombo-

Table 3. Comparison between patients with venous thromboembolism and patients with coronary artery disease

	Patients with VTE, N=16	Patients with CAD, N=4	P
Age, years	50,7±16,2	66,5±10,8	0,053
Disease duration since diagnosis, years	2 [0,1 – 5,8]	2 [1 – 3,5]	0,8844
Sex, female, n (%)	10 (62,5%)	2 (50%)	1,00
Hypertension, n (%)	9 (56,2%)	4 (100%)	0,249
Hypercholesterolemia, n (%)	10 (62,5%)	4 (100%)	0,267
Diabetes, n (%)	4 (25,0%)	0 (0%)	0,538
Hypertension or Hypercholesterolemia or Diabetes, n (%)	10 (62,5%)	4 (100%)	0,267
Echocardiography			
Aorta diameter, mm	32,2±4,3	33,7±2,1	0,497
Left atrium diameter, mm	37,1±4,8	40,2±2,9	0,237
Interventricular septal diameter, mm	10,6±1,4	12,2±1,5	0,050
Posterior wall thickness, mm	10,4±1,1	11,7±1,0	0,051
Left ventricular diastolic diameter, mm	46,0±5,2	49,5±9,6	0,321
Left ventricular systolic diameter, mm	25,2±4,4	31,5±9,6	0,285
Right ventricular diameter, mm	31,6±5,6	32,0±3,3	0,884
Pulmonary artery diameter, mm	19,9±1,6	20,7±1,7	0,343
Vena cava inferior diameter, mm	17,4±2,8	20,2±2,9	0,095
Pulmonary acceleration time, ms	131±24	119±15,9	0,366
Right ventricular systolic pressure, mmHg	36,3±5,9	28,7±4,6	0,030
Tricuspid valve regurgitation velocity, m/s	2,8±0,4	2,4±0,1	0,017
Left ventricular ejection fraction, %	64,2±5,2	52,5±13,2	0,173
Left ventricular diastolic dysfunction, n (%)	2 (15,4)	4 (100)	0,0063
Glucocorticoids, n (%)	12 (75%)	3 (75%)	1,00
Azathioprine, n (%)	4 (25%)	2 (50%)	0,538
Cyclophosphamide, n (%)	4 (25%)	1 (25%)	1,00
Internal involvement			
Eyes, n (%)	6 (37,5%)	1 (25%)	1,00
Ears, n (%)	5 (31,2%)	1 (25%)	1,00
Genitourinary, n (%)	1 (6,2%)	0	1,00
Musculoskeletal, (%)	7(43,7%)	1 (25%)	0,619
Upper respiratory tract, n (%)	15 (93,7%)	4 (100%)	1,00
Lower respiratory tract, n (%)	14 (87,5%)	4 (100%)	1,00
Kidney, n (%)	10 (62,5%)	3 (75%)	1,00
Central nervous system, n (%)	3 (18,7%)	1 (25%)	1,00
Peripheral nervous system, (%)	5 (31,2%)	0 (0%)	0,530
Skin, n (%)	1 (6,2%)	0 (0%)	1,0
Five or more internal involvement, n (%)	6 (27,5%)	2 (50%)	1,00
Creatinine, mg/dl	1,0 [0,85-1,2]	1,1 [0,90-1,40]	0,924
Hs-CRP, mg/dl	0,50 [0,05-235]	2,25 [0,90-3,25]	0,298
Troponin I, ug/l	0,008 [0,006-0,060]	0,030 [0,006-0,020]	0,449
PR 3, U	16,6 [4,85-83,5]	1,5 [0,9-1,5]	0,016
MPO, U	1,7 [1,3-2,4]	2,3 [1,7-2,50]	0,448
MPO positive, n (%)	1 (6,2)	1 (25)	0,368
PR3 positive, n (%)	12 (75,0)	1 (25)	0,101

embolic events and developed VTE during first year after diagnosis (13). In our study higher incidence of VTE was not related to age, sex, CRP or ANCA antibodies levels. In a retrospective study by Stassen et al. in 198 patients with AAV increased risk of developing VTEs was observed. More than half of VTEs (52%) occurred during active disease, defined as 3 months before and after diagnosis or relapse of AVV. Also in this cohort there were no significant differences in classic risk factors between patients with and without AAV-associated VTE . In their study VTEs occurred less frequently in patients with PR3-ANCA (10). Weidner et al. retrospectively reviewed patients who were treated for AAV at a single centre during a 16-year period. This patient population with kidney involvement, included patients with microscopic polyangiitis and renal-limited vasculitis. Thirteen of 105 patients had VTEs during the observation and 12 of the 13 events occurred during periods of active vasculitis (9).

A retrospective study conducted in a Tertiary Reference Centre in Denmark also has confirmed that patients diagnosed with GPA have a significant risk of VTE both early and late during the course of their follow-up and are hospitalized several times for PE and DVT. They have also observed that within the first two years following the diagnosis of vasculitis, the incidence of PE and DVT were increased among the patients but the incidence of stroke was not increased during this time interval (14). Their results and our observations together suggest that manifestation of atherosclerosis (stroke, coronary artery disease) is less common complication than venous thromboembolism in first years after GPA diagnosis.

In Santana et al. study with confocal laser scanning microscopy, a significant association between pulmonary microvascular thrombosis and GPA was found. Their results suggests a possible role of microvascular thrombosis in the pathophysiology of pulmonary GPA and the potential benefits of anticoagulation therapy in pulmonary GPA (15). In our study increased risk for thromboembolism was unrelated to specific organ involvement. It was also independent of number of organs and systems involved.

The treatment with high doses of corticosteroids may also explain the increased incidence of VTE in GPA patients. In our study use of corticosteroids was equal in patients with VTE and without VTE, nevertheless, use of cyclophosphamide was related to lower incidence of VTEs. In 2011 a prognostic tool to define the 5-year cardiovascular risk was created for AAV patients based on data from four European Vasculitis Study Group (EUVAS) trials of GPA and MPA considering a total population of 535 patients. The results indicated that almost 12% of newly diagnosed GPA had presented at least one cardiovascular event, defined as cardiovascular death, myocardial infarction, coronary artery bypass graft/percutaneous coronary intervention or stroke (16). Also in a retrospective study conducted using Danish National Hospital Register on 293 patients with GPA, an increased risk of acute myocardial infarction was observed. Interestingly, this GPA population had an increased risk of cardiovascular events both in early (within 5 years) and in the late phase of the disease (3). In our study the incidence of CAD episodes was much lower and comparable to McGeoch et al. results. In their group of 517 patients with GPA only 3,3% had cardiac involvement and in two patients CAD was diagnosed (17). This differences could be related to short time of observation in our study. Furthermore, different diagnostic methods were used in other studies to evaluate coronary artery disease (ECG, echocardiography, magnetic resonance imaging or coronarography), whereas, in our observation all patients diagnosed with CAD had coronarography performed.

Conclusions

The present study demonstrated that granulomatosis with polyangiitis was associated with a much higher risk of venous thrombosis than coronary artery disease in first years after diagnosis, although classic risk factors for atherosclerosis were common in this study group. The underlying mechanism for this increased risk of VTE is unknown, but is likely to be associated with changes in endothelial function and with induction of hypercoagulability resulting from changes in pro and anticoagulant factors associated with inflammation and its therapy. Furthermore, patients with active GPA are not only at a higher risk for VTEs, but also at risk for bleeding, specifically those with severe lung and renal manifestations. For this reason, acute management of VTEs as well as initiation and duration of anticoagulation therapy is particularly challenging. Our study demonstrates that patients with GPA are rather at increased risk for venous thromboembolism than coronary artery disease, especially during first years after diagnosis. Therefore, we can recommend including active vasculitis among risk factors for venous thromboembolism. This study has also some limitations. It was carried out in one centre and no active screening for coronary artery disease or venous thromboembolism episodes was performed. More research is needed to clarify the cause of the high incidence of VTE in patients with GPA and to develop a strategy of screening to identify patients who require prophylactic anticoagulation.

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SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (0): 209-216

Role of cytomorphology in differentiating sarcoidosis and tuberculosis in subjects undergoing endobronchial ultrasound-guided transbronchial needle aspiration

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Abstract. *Background:* The role of cytomorphology in differentiating sarcoidosis from tuberculosis is not fully elucidated. Herein, we evaluate the utility of cytological features in differentiating between these two diseases in subjects undergoing endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA). *Methods:* Retrospective analysis of subjects who underwent EBUS-TBNA and had a final diagnosis of sarcoidosis or tuberculosis. The final diagnosis was based on the clinicoradiological features, microbiology and clinical course during follow-up (including response to treatment) at six months. A cytologist blinded to the clinical details and microbiology examined the aspirates. The primary outcome was the diagnostic accuracy of cytologist's impression to diagnose sarcoidosis as compared to the final diagnosis. *Results:* 179 (145 sarcoidosis, 34 tuberculosis) subjects were included. Granuloma was identified in 135 (75.4%) subjects; amongst these, the cytologist made a correct diagnosis in 62.2% cases, misdiagnosed 28.9% cases, and in 8.9% cases differentiating sarcoidosis from tuberculosis was not possible. The sensitivity, specificity, positive and negative predictive values (PPV and NPV) of the cytologist in diagnosing sarcoidosis was 62%, 64%, 90%, and 25%, respectively. The identification of a non-necrotic granuloma, along with a negative TST and the lack of endosonographic features favouring tuberculosis (heterogeneous echotexture and coagulation necrosis sign), provided the best specificity (97%) and PPV (99%) to diagnose sarcoidosis. *Conclusion:* Sarcoidosis cannot be reliably differentiated from tuberculosis based on cytomorphology alone. A combination of clinical features, endosonography, cytology and microbiology is required for accurate diagnosis. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (3): 209-216)*

Key words: bronchoscopy, endosonography, EUS, granuloma, tuberculin skin test

Introduction

Sarcoidosis and tuberculosis are the major causes of undiagnosed mediastinal lymphadenopathy in developing countries, including India (1). The differentiation between these two entities is challenging, particularly in high tuberculosis burden countries (2, 3). The exclusion of alternate causes of granuloma, especially tuberculosis, is essential for diagnosing sarcoidosis. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is the preferred initial modality for the evaluation of undiagnosed intrathoracic lymphadenopathy (4). Although tuberculin skin test (TST) is useful in differentiating sarcoidosis from tuberculosis, the dem-

Received: 1 February 2019
Accepted after revision: 19 April 2019
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onstration of *Mycobacterium tuberculosis* by smear, culture or molecular method provides the most definite evidence of tuberculosis (5). The conventional microbiological techniques have a poor sensitivity; however, the inclusion of Xpert MTB/RIF, has considerably improved the sensitivity of differentiating tuberculosis from sarcoidosis on EBUS-TBNA (6, 7).

The cytological features can be helpful in the diagnosis of granulomatous lymphadenopathy, even when the microbiology results are negative (8, 9). However, most of these studies have been performed on cervical lymph nodes and the differential did not include sarcoidosis (9, 10). The importance of differentiating these two common granulomatous diseases is even more relevant in subjects undergoing EBUS-TBNA for intrathoracic lymphadenopathy, where sarcoidosis is a major consideration. In our practice, cytopathologists diagnose sarcoidosis or tuberculosis based on cytomorphology alone. However, only a few studies have explored the cytomorphologic differences between sarcoidosis and tuberculosis (11-13). Herein, we evaluate the diagnostic performance of cytomorphology in diagnosing sarcoidosis, in patients undergoing EBUS-TBNA.

Methods

This was a retrospective analysis of prospectively collected data from January 2014 to March 2015. The study protocol was approved by the Institute Ethics Committee. A consent waiver was granted due to the retrospective nature of the study and the use of anonymized patient data. A part of the data has been published as an abstract (14) and some of the participants included in the current study were part of a previously published trial (15).

Study subjects: Consecutive subjects who underwent EBUS-TBNA were included if they fulfilled all the following: (i) age >18 years; (ii) enlarged intrathoracic lymph nodes ≥10 mm (short axis) on computed tomography (CT) of the chest; (iii) final diagnosis of sarcoidosis or tuberculosis. We excluded subjects with suspected or known malignancy.

Study protocol: After a thorough clinical history and physical examination, subjects underwent routine laboratory tests (TST, complete blood count, coagulation profile, liver and renal function tests), spirometry, chest radiography and CT of the chest. Intrathoracic lymph nodes and parenchymal abnormalities were assessed with CT scan.

EBUS-TBNA was performed as a day-care procedure in the bronchoscopy suite under moderate sedation (intravenous midazolam and pentazocine targeting a Ramsay sedation score of two). We used the EBUS bronchoscope (BF-UC180F; Olympus Medical, Japan) and a compatible ultrasound image processor (EU-ME1; Olympus Medical, Japan) for performing EBUS-TBNA (1). All subjects received premedication with intramuscular injection of atropine (0.6 mg) and promethazine (25 mg). Nebulized 4% lignocaine (2.5 mL), followed by two puffs of 10% lignocaine spray was administered for topical anaesthesia. 2 mL aliquots of 1% lignocaine was instilled over the vocal cords and the airways using the spray-as-you-go method (16). The EBUS scope was introduced transorally with the subject lying in the supine position. At least two lymph node stations were accessed (with two or more passes from each sampled node). The size, location (as per the IASLC lymph node map) (17) and endosonographic appearance (heterogeneous echotexture and coagulation necrosis sign [CNS]) (18) of the nodes were recorded. TBNA was performed using either a 21G or 22G EBUS-TBNA aspiration needle (Vizishot, NA-201 SX-4021A or NA-201 SX-4022A), under real-time ultrasound guidance (15). We employed continuous suction with a 20 mL VacLoc™ syringe and the catheter was moved back and forth for 15-20 times. Rapid onsite cytologic evaluation (ROSE) was not available. Endobronchial (EBB) and transbronchial lung biopsies (TBLB) were performed for subjects with suspected sarcoidosis.

Processing and reporting of the EBUS-TBNA samples: Smears were prepared from the aspirated material, both air-dried (for May-Grunwald Giemsa staining) and alcohol-fixed (95% alcohol for Ziehl-Neelsen staining to detect acid-fast bacilli [AFB], and haematoxylin-eosin staining). We also transferred the aspirated material in 0.9% sterile saline for mycobacterial culture and Xpert MTB/RIF. A single cytopathologist (NG) who was blinded to the clinical data, biopsies (EBB, TBB), mycobacterial culture, Xpert MTB/RIF, and the final diagnosis, reported the cytomorphological features of the slides. The following features were recorded: (i) adequacy (adequate, if the TBNA slide was diagnostic

or showed the presence of numerous lymphocytes); (ii) the presence of granuloma; (iii) granuloma density (<3, 3-6, 7-9, and >9 granulomas per smear); (iv) the presence or absence of necrosis; (v) grading of necrosis, if present (1: focal, 2: intermediate [neither focal nor extensive], 3: extensive); (vi) stain for AFB in cytology slides; and, (vi) the final impression of cytologist in TBNA smears where granulomas could be identified (favour sarcoidosis, favour tuberculosis, indeterminate).

Definitions: The final diagnosis of **sarcoidosis** was made in subjects with a consistent clinical and radiological presentation after six months of follow-up, when (a) granuloma was demonstrated on either TBNA, TBLB or EBB along with negative AFB, fungal stains, and no growth of mycobacteria on mycobacterial culture; and/or (b) clinical and radiological response after treatment with glucocorticoids or stable disease without treatment (in the absence of an alternate diagnosis) (2). **Tuberculosis** was diagnosed when two of the following criteria was fulfilled: (a) consistent clinical and radiological presentation; (b) smear positive for AFB and/or culture for *Mycobacterium tuberculosis* or Xpert MTB/RIF positivity; and, (c) clinicoradiological response to anti-tuberculosis treatment.

Study endpoints: The **primary objective** was to determine the diagnostic accuracy of the cytologist's impression to diagnose sarcoidosis on EBUS-TBNA smear, as compared to the final diagnosis and differentiate sarcoidosis from tuberculosis on the basis of cytomorphology. The **secondary objectives** were to assess the diagnostic performance of various cytomorphologic, endosonographic and clinical features of interest, either alone or in combination.

Statistical analysis: Data are presented as mean with standard deviation or number with percentage. We used the commercial statistical package SPSS (SPSS for Windows, version 22.0; IBM SPSS Inc; Armonk, NY) for the statistical analysis. Chi-square test and student t-test was used to analyse the differences between categorical and continuous variables, respectively. A p value <0.05 was considered significant. The sensitivity and specificity were calculated using a final diagnosis of tuberculosis and sarcoidosis, respectively. Diagnostic accuracy (by calculating sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV], expressed as percentages with 95% confidence intervals [CI]) of various parameters used to diagnose sarcoidosis was computed. We constructed Bayesian graphs demonstrating the variation in predictive values of a test (along y-axis) in relation to the disease prevalence (x-axis).

Results

We enrolled 179 subjects (mean age of 42.2 years, 59.8% males). A final diagnosis of sarcoidosis and tubercolosis was made in 145 (81%) and 34 (19%) subjects, respectively (Table 1). Granulomas were identified on cytology in 135 (75.4%) subjects (n=113 and n=22 in sarcoidosis and tuberculosis, respectively). Only these 135 subjects were included for the primary outcome analysis. Subjects with tuberculosis were younger (mean age of 34.2 years vs. 44.1 years, p=0.0002), and had a higher proportion of TST positivity (>10 mm; 67.6% vs. 6.2%, p=0.0001). The details of the mediastinal lymph node stations subjected to EBUS-TBNA are described in Table 1. The endosonographic appearance of heterogeneous echotexture (67.6% vs. 22.8%, p<0.0001), and CNS (23.5% vs. 2.8%, p<0.0001) were more frequently encountered in tuberculosis than sarcoidosis. A median of two lymph nodes and an average of two passes from each lymph node were obtained during EBUS-TBNA.

The adequacy of EBUS-TBNA slides, the identification of granuloma on cytology and the number of granulomas per smear were not different in subjects with sarcoidosis and tuberculosis (Table 2). Necrosis was rare in sarcoidosis compared to tuberculosis (6.2% vs. 55.9%); when present, it was always focal (Table 2). Xpert MTB/RIF was available in 130 (72.6%) subjects (16 and 114 subjects with the final diagnosis of tuberculosis and sarcoidosis, respectively). Of these, none in the sarcoidosis group and seven (43.7%) in the tuberculosis group were positive for Xpert MTB/RIF.

Primary outcome: On the basis of cytomorphology of granuloma (n=135), the cytologist was able to make a correct diagnosis in 84 (62.2%) cases, and misdiagnosis occurred in 39 (28.9%). The differentiation between tuberculosis and sarcoidosis was indeterminate on cytology in 12 (8.9%). The sensitivity, specificity, PPV, and NPV of the cytologist's impression to diagnose sarcoidosis was 62%, 64%, 90% and 25%, respectively (Table 3).

Table 1. Baseline clinical and endosonographic characteristics of study subjects undergoing endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) for suspected granulomatous intrathoracic lymphadenopathy

	Total (n=179)	Sarcoidosis (n=145)	Tuberculosis (n=34)	P value
Age in years, mean (SD)	42.2 (14.2)	44.1 (13.4)	34.2 (15.2)	0.0002
Male gender	107 (59.8)	86 (59.3)	21 (61.8)	0.48
TST negativity (<10 mm)	147 (82.1)	136 (93.8)	11 (32.4)	0.0001
Needle gauge				0.56
21G	95 (53.1)	76 (52.4)	19 (55.9)	
22G	84 (46.9)	69 (47.6)	15 (44.1)	
Lymph node stations* sampled during EBUS-TBNA				
Station 7	168 (93.9)	140 (96.6)	28 (82)	0.007
Station 4R	141 (78.8)	119 (82.1)	22 (64.7)	0.046
Station 4L	39 (21.8)	35 (24.1)	4 (11.8)	0.18
Station 10R	5 (2.8)	5 (3.4)	0	0.73
Station 11R	21 (11.7)	18 (12.4)	3 (8.8)	0.77
Station 10L	1 (0.6)	1 (0.7)	0	-
Station 11L	91 (50.8)	83 (57.2)	9 (26.5)	0.0001
Heterogeneous echotexture on EBUS	56 (31.3)	33 (22.8)	23 (67.6)	<0.0001
Coagulation necrosis sign	12 (6.7)	4 (2.8)	8 (23.5)	<0.0001
Central intranodal vessel	87 (48.6)	72 (49.7)	15 (44.1)	0.57
Number of lymph nodes sampled, median (IQR)	3 (2-3)	3 (2-3)	2 (2-3)	0.09
Number of passes per node, mean (SD)	2 (0.5)	2 (0.5)	2.4 (0.8)	0.02
Mean (SD) Duration of procedure, minutes	23.2 (5.9)	23.4 (5.8)	22.1 (6.4)	0.122

All values are presented as number (percentage) unless otherwise stated
CT- computed tomography; EBUS- endobronchial ultrasound; IQR- interquartile range; SD- Standard deviation; TBNA – transbronchial needle aspiration; TST- tuberculin skin test
*As per the IASLC lymph node map (17)

Table 2. EBUS-TBNA cytology features of subjects with granulomatous intrathoracic lymphadenopathy

Cytology parameter, n (%)	Total (n=179)	Sarcoidosis (n=145)	Tuberculosis (n=34)	P value
Adequacy	165 (92.2)	132 (91)	33 (97.1)	0.16
Granuloma identified	135 (75.4)	113 (77.9)	22 (64.7)	0.69
Absence of hemorrhage	56 (31.3)	36 (24.8)	20 (58.8)	0.002
Number of granulomas per smear*				0.11
<3	23 (17)	17 (15)	6 (27.3)	
3-6	19 (14.1)	19 (16.8)	0	
7-9	31 (23)	27 (23.9)	4 (18.2)	
>9	62 (45.9)	50 (44.2)	12 (54.5)	
Necrosis				0.0001
None	151 (84.4)	136 (93.8)	15 (44.1)	
Grade 1 (Focal)	16 (8.9)	9 (6.2)	7 (20.6)	
Grade 2 (Intermediate)	9 (5)	0	9 (26.5)	
Grade 3 (Extensive)	3 (1.7)	0	3 (8.8)	
AFB positivity**	15 (8.4)	0	15 (44.2)	0.0001
Cytologist's final impression based on the morphology (including AFB stain)*				0.003
Correct diagnosis	84 (62.2)	70 (61.9)	14 (63.6)	
Misdiagnosis	39 (28.9)	34 (30.1)	5 (22.7)	
Indeterminate	12 (8.9)	9 (8)	3 (13.1)	

AFB – acid-fast bacilli; EBUS-TBNA – endobronchial ultrasound guided transbronchial needle aspiration
*n=135 (subjects having granuloma) was used as the denominator to calculate these percentages
**AFB smear performed on the EBUS-TBNA aspirate slides by the cytologist

Secondary objectives: In subjects with sarcoidosis, mycobacteria were not demonstrated by either conventional microbiological investigations (smear or culture) or Xpert MTB/Rif, thereby yielding a sensitivity of 100%. The specificity was however low (44%) due to lack of demonstration of mycobacteria in a large number of cases of tuberculosis (Table 3). A negative TST, absence of necrosis on cytology, and

Table 3. Diagnostic accuracy of various parameters in diagnosing sarcoidosis among subjects undergoing EBUS-TBNA for granulomatous lymphadenopathy

Parameter	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Primary outcome*				
Diagnosis of sarcoidosis based on the cytomorphology of granuloma	62 (52-71)	64 (41-83)	90 (81-95)	25 (14-38)
Secondary outcomes**				
TST negativity (10 mm or less)	94 (89-97)	68 (49-83)	93 (87-96)	72 (53-86)
Absence of necrosis on cytology	94 (89-97)	56 (74-93)	90 (84-94)	68 (48-84)
Microbiology				
Negative Mycobacterial culture	100 (97-100)	44 (27-62)	88 (83-93)	100 (78-100)
Negative Xpert MTB/RIF#	100 (97-100)	44 (20-70)	93 (87-97)	100 (59-100)
EBUS-related characters				
Absence of heterogeneous echotexture or CNS in any node	68 (49-83)	77 (69-83)	91(84-95)	40 (27-54)
Combination of various parameters				
Identification of granuloma and negative TST	72 (64-80)	79 (62-91)	94 (88-97)	40 (28-53)
Non-necrotic granuloma and a negative TST	71 (63-78)	85 (69-95)	95 (90-98)	41 (29-53)
Identification of granuloma and a negative stain for AFB	77 (69-83)	68 (49-83)	91 (84-95)	40 (28-54)
Absence of endosonographic features suggestive of tuberculosis¶ and a negative TST	72 (64-80)	88 (73-97)	96 (91-99)	43 (31-55)
Presence of non-necrotic granuloma, negative TST and no endosonographic features suggestive of tuberculosis¶	55 (47-63)	97 (85-100)	99 (93-100)	34 (24-44)

AFB – acid fast bacilli; CI – confidence interval; CNS – coagulation necrosis sign; EBUS- endobronchial ultrasound; NPV – negative predictive value; PPV – positive predictive value; TBNA – transbronchial needle aspiration; TST – tuberculin skin test
Subjects with granuloma on cytology* (n=135) or the entire study cohort** (n=179) were used to calculate the primary and secondary outcomes, respectively (unless otherwise mentioned)
¶Heterogeneous echotexture and coagulation necrosis sign
#49 subjects in whom Xpert MTB/RIF was not available were excluded for this analysis

the absence of CNS during EBUS were other useful features suggesting the diagnosis of sarcoidosis (Table 3). The mere identification of a granuloma had a poor specificity (35%) to diagnose sarcoidosis. However, the identification of a granuloma and the presence of a negative TST improved the specificity to 79% (Table 3). The presence of a non-necrotic granuloma, negative TST and the lack of endosonographic findings favouring tuberculosis (heterogeneous node and CNS) provided the best specificity (97%) and PPV (99%) to diagnose sarcoidosis. The positive predictive value of the various tests decreased significantly with decreasing prevalence of sarcoidosis (Figure 1).

Discussion

The results of our study indicate that it is difficult to differentiate sarcoidosis from tuberculosis, solely based on cytomorphology. The absence of necrosis on cytology and a negative TST were useful features in diagnosing sarcoidosis. Necrosis was rare in sarcoidosis, and when present, it was never extensive. Although several findings shown in the current study are known, we reinforce the results using strict definitions for the final diagnosis, a large sample size, and the blinding of the cytologist to clinical and microbiological details (culture and Xpert MTB/RIF).

Granulomatous inflammation is a feature common to both tuberculosis and sarcoidosis. However, the identification of granuloma is neither sufficiently sensitive nor specific. A previous study investigating the rate of monocytopoiesis and monocyte recruitment in the granulomas, described the granuloma in tuberculosis to be "high turnover", and sarcoidosis to be a "low turnover" granulomas (19). The high turnover and the increased macrophage destruction could explain the frequent occurrence of necrosis in tuberculous granulomas. In our study, the absence of necrosis had a high sensitivity for diagnosing sarcoidosis (94%) and its presence was distinctly uncommon (6% of sarcoidosis had necrosis in a study of bronchial biopsies) (20). However, it lacked specificity (56% in our study) as several patients with tuberculosis present with non-necrotic granuloma. In a study of 212 tubercular lymphadenitis, three major morphologies were observed on fine-needle as-

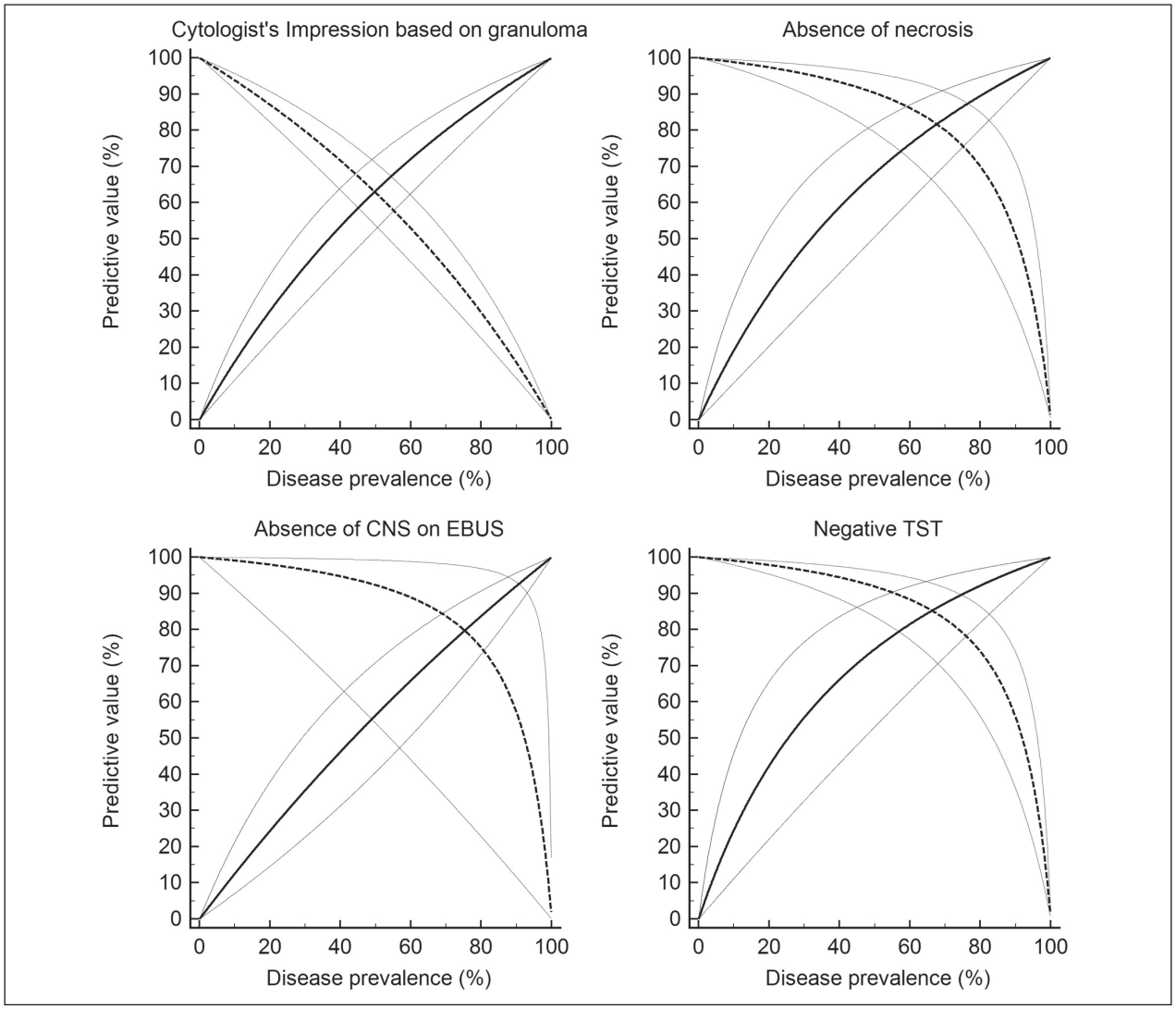

Fig. 1. Figure 1: Graph showing the relation between positive (solid line) and negative (dotted lines) predictive values of various study parameters to diagnose sarcoidosis (y-axis), with varying prevalence (along the x-axis). The graphs also show the 95% confidence interval for the PPV and NPV (lighter colour)

piration cytology (FNAC) that correlated with the bacillary load. These included epithelioid granuloma without necrosis (stain for AFB was positive in 5% of these cases), granuloma with necrosis (AFB-positive in 60.8%), and necrosis alone in the absence of granuloma (AFB-positive in 77.1%) (21). Thus, the most specific feature for tuberculosis on cytomorphology (necrosis) seem to correlate well with microbiology. The cytologic features are seldom helpful in the subset of cases (non-necrotic granuloma with negative microbiological investigations), where differentiation of sarcoidosis from tuberculosis is difficult. This explains the diagnostic challenge faced by the cytologist, as also shown in the current study.

While the ultrastructural (using an electron microscopy) size and shape of nuclei in the inflammatory cells of the granuloma have been shown to be different in sarcoidosis and tuberculosis (22), they are unlikely to be useful in routine practice. In a small study, the cellular composition (using monoclonal antibodies against surface markers of lymphocytes/macrophages) of both sarcoid and tubercular

granulomas have also been shown to be similar (11). Further, most of the evidence on cytomorphologic differentiation is from an era where molecular techniques to diagnose or exclude tuberculosis were not in vogue (20). In a recent study of 49 subjects undergoing EBUS-TBNA, cytomorphology alone was unable to differentiate sarcoidosis from tuberculosis (12), an observation similar to ours. Nevertheless, we found that certain features in cytology (necrosis), and a combination of cytologic features with TST are useful.

What does the current study add? We have systematically evaluated and provided the diagnostic accuracy of the various cytology parameters in diagnosing sarcoidosis among subjects undergoing EBUS-TBNA for suspected granulomatous lymphadenopathy. Misdiagnosing tuberculosis as sarcoidosis could be disastrous, particularly in high tuberculosis endemic region (in our study, 20% with suspected granulomatous lymphadenopathy had tuberculosis). The diagnostic accuracy of the cytology findings and EBUS signs would vary widely with varying prevalence of tuberculosis (and sarcoidosis).

Finally, our study has a few limitations. This was a single center study and all the slides were reported by a single experienced cytologist. Inter-observer correlation between cytologists was not evaluated. The EBUS findings of CNS and heterogeneous echotexture are frequently encountered in malignancies, another major indication for EBUS-TBNA. This would further alter the diagnostic accuracy of sonographic appearances in the real-world scenario. We primarily discuss the cytology findings, while in routine clinical practice the imaging findings (symmetric lymphadenopathy, presence of necrosis, and others), and involvement of extrapulmonary sites can provide additional clues to differentiate sarcoidosis and tuberculosis. We did find a significantly higher proportion of subjects with sarcoidosis having involvement of lymph node stations 11L, 4R and 7. Unfortunately, we have not systematically recorded and evaluated these differences in the current study. However, radiological appearance may be identical in both these diseases, and in fact, in a randomized trial of subjects with suspected sarcoidosis (based on imaging and clinical features), tuberculosis was diagnosed in 5.3% (15). Further, the results of our study cannot be extrapolated to immunocompromised hosts (such as HIV-AIDS) with suspected granulomatous adenopathy, where the morphologic features on cytology might be different.

In conclusion, cytomorphology alone is insufficient in differentiating sarcoidosis from tuberculosis in subjects undergoing EBUS-TBNA. A combination of microbiological, cytomorphological features (especially necrosis), TST, and endosonographic characteristics are helpful in the diagnosis of granulomatous mediastinal adenopathy.

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SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (3): 217-227

Effectiveness and tolerability of methotrexate in pulmonary sarcoidosis: A single center real-world study

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Abstract. *Background:* Pulmonary sarcoidosis patients who get disease progression despite corticosteroid treatment or can't tolerate corticosteroid required second-line drug. Methotrexate (MTX) is the most widely used in our clinical practice. Data on its safety and efficacy at different doses are still limited, especially for those without folic acid supplements. *Objective:* To report effectiveness of different MTX dosages and tolerability of MTX in pulmonary sarcoidosis without folic acid supplements. *Methods:* A retrospective study on pulmonary sarcoidosis patients receiving MTX therapy with various dose ≥3 months was conducted. The primary outcome was change in high-resolution computed tomography (HRCT) before and after MTX therapy. Other efficacy parameters included SGRQ score, prednisone dose change, discontinuation and relapse-free survival. Response-linked factors and safety outcomes were also analyzed. *Results:* Overall, 49 patients (81.7%) were assessed as MTX responders by HRCT and there was no significant difference in clinical response rate among three groups with different doses. The health-related quality of life (HRQL) of the responders improved obviously, which was evidenced by SGRQ score declining from 16.7(IQR: 7.9-26.4) to 10.7(IQR: 4.8-19.3) (P=0.029). The corticosteroids sparing effect was confirmed in "responders" group (P<0.001). When MTX was discontinued in 11 responders with complete improvement, 2 patients experienced relapses within 15.5 (range: 1-30) months (mean follow-up time of these 11 responders: 13.5±13.0 months). No clinical characteristics were found related to MTX effectiveness. Adverse events occurred in 31.7% of the patients, with gastrointestinal-related being the commonest. Drug discontinuation owing to adverse events occupied 6.7% of the subjects. *Conclusions:* Nearly 80% of the sarcoidosis subjects had well response to MTX. Its effectiveness was irrelevant to the treatment dosages and baseline characteristics. A quite low relapse rate was witnessed in those complete responders discontinuing MTX therapies. The steroid-sparing effect, well drug tolerability and low drug withdrawal rate were observed in these patients even without folic acid supplements in clinical practice. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (3): 217-227)*

Key words: sarcoidosis, methotrexate, effectiveness, tolerability

Introduction

Sarcoidosis is a multisystem disease of unknown cause, histologically characterized by the presence of noncaseating granulomas. The most frequently affected sites are the lungs, lymph nodes, skin, although it may occur virtually in any organ. The natural clinical course of this disease was unpredictable,

Received: 8 May 2019
Accepted after revision: 14 June 2019
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varying from spontaneous resolution to disease progression. It is reported that more than half of patients get remission within 3 years while approximately a third of patients have progressive disease giving rise to significant organ impairment (1). For patients who meet therapy indications, glucocorticoids are the first-line treatment to control their disease. Concerning the severe toxicity of corticosteroids by long-term use, tapering them to the lowest effective dose is needed in these patients. And second-line therapeutics (mainly including antimetabolites and cytotoxic drugs) are generally recommended for patients who experience relapse during tapering corticosteroids or are unresponsive to corticosteroids or intolerant to them.

Methotrexate (MTX) is the most commonly used as a second-line drug for sarcoidosis patients in our clinical practice (2, 3). It was reported that anti-inflammatory properties of MTX might be attributed to the release of adenosine from cells and the inhibition of polyamines (4). Only one randomized controlled trial (RCT) of 24 sarcoidosis patients by Baughman et al. (5) revealed the steroid-sparing effect of MTX in acute sarcoidosis. And the significant steroid-sparing effect, improvement in lung function and safety of MTX on sarcoidosis were found in two retrospective researches (6, 7) and one prospective study (8). The most frequently reported adverse events during MTX therapy were gastrointestinal problems, hepatic abnormalities and infection (6-8). Sarcoidosis patients in the former researches were all given folic acid supplements, but its evidence is lacking in sarcoidosis.

Additionally, until now, researches involving the effect and tolerability of MTX on pulmonary sarcoidosis are still insufficient, especially comparing different MTX dosages in sarcoidosis and analyzing factors related to its efficacy and occurrence of adverse event and prognosis of patients stopping MTX therapy. The present real-world study was conducted to report the effectiveness and safety of different MTX dosages on sarcoidosis patients without taking folic acid supplements in clinical practice and factors related to its effectiveness were investigated.

Methods

Patients

A retrospective single-center research was conducted on 60 subjects given MTX with different dose for pulmonary sarcoidosis from December 2011 to August 2018 in Peking Union Medical College Hospital (PUMCH), Dongcheng District, China. Inclusion criteria were as follows: (1) adult patients (≥18 years old) with the diagnosis of sarcoidosis proven by the biopsy, in accordance with WASOG/ATS/ERS criteria (9); (2) Patients met treatment indications according to WASOG/ATS/ERS criteria (10, 11). Furthermore, they had received MTX therapy for at least 3 months due to failure (refractory or recurrent) or intolerance (side effects or contraindication) of corticosteroid use. Sarcoidosis was considered refractory when the disease was not controlled or even progressive despite corticosteroid treatment at adequate dosage (12) for at least 3 months. Failure of former therapy was assessed and determined by two experienced pulmonologists and one radiologist during patients' regular clinical care (3). Patients treated by different pulmonologists in our hospital were collected to ensure that patients with various MTX doses were included in this study since different pulmonologists tend to choose different dose of MTX for pulmonary sarcoidosis patients according to their own experience and habits.

Study design

This real-world retrospective study was approved by the Regional Ethics Committee of our hospital (JS-1127/2016). Due to the retrospective nature of the study, informed consent was waived.

1. Data collection

Subjects who met our study inclusion criteria were included for further analysis. We collected the related data of these patients by paper and electronic medical records of our hospital healthcare system. These data included: (1) baseline data involving demographic information, smoking status, comorbidities, disease duration, prednisone dosage and Saint George's Respiratory Questionnaire (SGRQ) score at baseline, PFTs, HRCT, lung biopsy results, MTX

therapeutic dose; (2) data associated with effectiveness assessment including HRCT, SGRQ score and prednisone dose change; (3) information about adverse events, whether discontinued MTX therapy, whether relapse and date of occurrence.

2. Effectiveness and safety outcomes

The effectiveness of MTX on the included subjects who received therapy ≥3 months was assessed by HRCT. Two experienced respiratory specialists and two radiologists compared the chest images before and after MTX therapy in each patient. if the disagreement occurs, the chest images would be evaluated by another experienced radiologist. The chest imaging findings were scored, including bilateral hilar lymphadenopathy and the presence of lung infiltrates, on a scale of 1 to 4 (worsened, stable, partially improved and completely improved). A complete improvement was defined as the complete disappearance of lung lesions, without the occurrence of new lesions. A partial improvement was defined as ≥50% reduction of the pulmonary lesions. Patients who did not fulfill the complete or partial improvement criteria were classified into "non-responders" group. The health-related quality of life (HRQL) of patients was measured by SGRQ. SGRQ score (ranging from 0 to 100) was collected in these subjects, both pre-MTX and post-MTX therapy. Other effectiveness outcomes included corticosteroid sparing at the end of follow-up, discontinuation and relapse-free survival. A relapse was defined as the occurrence of new pulmonary sarcoidosis manifestations or a worsening of existing condition. Additionally, response related factors were also analyzed. Safety outcomes contained adverse events and drug discontinuation owing to adverse event.

Statistical analysis

We analyzed data with SPSS software version 19.0 for Windows (SPSS Inc., Chicago, IL)) and defined two-tailed $P<0.05$ as statistical significance. Categorical variables were expressed as numbers with percentages and normally distributed continuous variables as means ± SD. For non-normally distributed continuous variables, medians and interquartile range (IQR) were used (like SGRQ score and corticosteroids dosages in this study). Data were compared using Student's t-test between two groups of measurement data which fulfilled homogeneity of variance (calibration t-test for those not conformed to homogeneity of variance). For non-normally distributed continuous variables and rank variables, data were compared using Mann-Whitney U test (non-parametric tests) for independent samples. Chi-square test was used to compare data of categorical variables and Fisher's exact test was used as appropriate. Finally, a logistic regression model was performed to identify the factors associated with the efficacy of MTX therapy.

Results

1. Baseline characteristics of the included subjects

A total amount of 60 subjects consistent with our study criteria were included in our research. Baseline characteristics are shown in Table 3. A large number of patients (53/60, 88.3%) were in the pulmonary sarcoidosis stage II or III. More than half of the subjects (37/60, 61.7%) had extra-pulmonary organs involvement, including skin, lymph nodes, eyes, liver or spleen. Mostly, MTX was given to those refractory to corticosteroid (63.3%) or recurrent of the disease (25%). In total, 93.3% of patients (56/60) concomitantly received corticosteroid (mean dosage: 28.8±13.6 mg/day) and none of them had ever been given immunosuppressant for therapy (Table 3).

A majority of patients (37/60, 61.7%) were given MTX at the dose of 12.5mg weekly, while 12 patients (20.0%) at 10 mg weekly and 11 patients (18.3%) at 15 mg weekly. There was no significant difference in baseline characteristics, PFTs and chest radiographic staging proportions among three groups with various dosages (Table 1).

2. Effectiveness of MTX

2.1. The effect of various MTX dosages on HRCT

According to assessment of HRCT changes pre-MTX and post-MTX therapy, 90.9% (10/11) of patients with 15mg weekly were assessed as responders, while 75.0% (9/12) in "10 mg-weekly" group and 81.1% (30/37) in "12.5 mg-weekly" group, with no significant difference among them (P=0.579). Over-

Table 1. Baseline characteristics of three groups with different dosages

	All patients (n=60)	10 mg/week (n=12)	12.5 mg/week (n=37)	15 mg/week (n= 11)	*P* value
Age at start of therapy (years)	53.3±10.5	56.6±9.8	52.4±9.1	52.9±15.2	0.492
Male, n (%)	20 (33.3)	4 (33.3)	11 (29.7)	5 (45.5)	0.633
BMI (kg/m^2)	24.9±3.7	25.1±3.6	25.1±3.5	24.3±4.4	0.808
PFTs					
FEV1/FVC (%)	74.0±8.7	72.2±8.0	75.1±8.7	72.3±9.7	0.465
FEV1 (% predicted)	87.5±15.1	91.1±13.6	86.8±13.5	85.8±21.4	0.640
FVC (% predicted)	96.1±15.8	99.1±19.7	94.7±13.7	97.4±18.6	0.670
TLC (% predicted)	90.2±11.0	94.6±13.5	89.2±10.0	88.8±11.2	0.302
DLco (% predicted)	74.8±10.8	76.8±10.3	74.0±11.1	75.6±10.9	0.718
Chest radiographic staging, n (%)					0.628
I	4 (6.7)	1 (8.3)	3 (8.1)	0	
II	41 (68.3)	7 (58.3)	26 (70.3)	8 (81.8)	
III	12 (20.0)	4 (33.3)	5 (13.5)	3 (27.3)	
IV	3 (5.0)	0	3 (8.1)	0	

Table 2. The effect of various dosages (10 mg vs. 12.5 mg vs. 15 mg weekly) of MTX therapy on HRCT

HRCT score after MTX treatment	All patients (n=60)	10 mg/week (n=12)	12.5 mg/week (n=37)	15 mg/week (n=11)	*P* value
1, n (%)	1 (1.7)	0	1 (2.7)	0	
2, n (%)	10 (16.7)	3 (25.0)	6 (16.2)	1 (9.1)	
3, n (%)	32 (53.3)	8 (66.7)	18 (48.6)	9 (81.8)	
4, n (%)	17 (28.3)	1 (8.3)	12 (32.5)	1 (9.1)	
MTX responders, n (%)	49 (81.7)	9 (75.0)	30 (81.1)	10 (90.9)	0.579
MTX non-responders, n (%)	11 (17.4)	3 (25.0)	7 (18.9)	1 (9.1)	

all, 49 patients (81.7%) were confirmed as MTX responders (Table 2).

2.2. The effect of MTX on HRQL

The HRQL of patients in "responders" group improved significantly after treatment compared with the baseline value as evidenced by the remarkable decrease of SGRQ score from 16.7 (IQR: 7.9-26.4) to 10.7 (IQR: 4.8-19.3) (P=0.029). While HRQL of the non-responders improved at a lower level after treatment, not reaching the significant difference standard (P=0.554) (Figure 1).

2.3. Effect of MTX on daily corticosteroid dosages

Corticosteroid remission was obtained in patients of "MTX responders" group with a statistically significant decrease in the daily dose from 30 (IQR: 15-40) to 10 (IQR: 10-15) mg/day within an average duration of 55.4 weeks (P<0.001) (Figure 2, Table 3). Daily corticosteroid dose was also dropped after

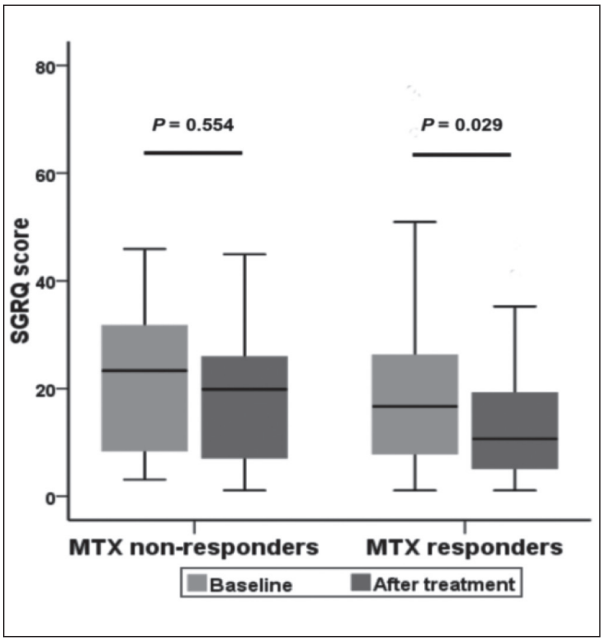

Fig. 1. SGRQ before and after MTX treatment in"responders" and "non-responders" group
SGRQ: Saint George's Respiratory Questionnaire.

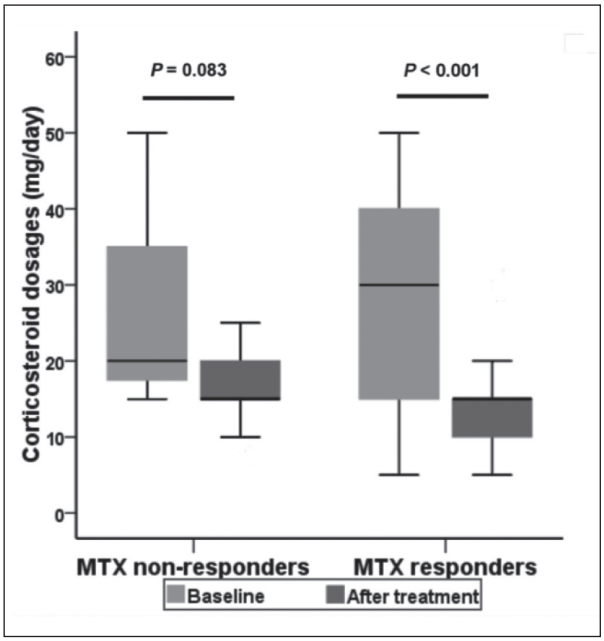

Fig. 2. Effect of MTX on daily corticosteroid dosages

MTX treatment in "non-responders" group from 20 (IQR: 17.5-35) to 15 (IQR: 15-20) mg/day within a mean duration of 42.9 weeks, though without statistical significance (P=0.083).

2.4. Therapy discontinuation and relapse

In total, MTX therapy was stopped in 17 patients (17/60, 28.3%) with a mean treatment duration of 54.6 weeks, containing 13 subjects in "responders" group (3 due to adverse events and 10 because of complete disappearance of lung lesions) and 4 in "non-responders" group (1 due to adverse events and 3 because of therapy failure). 4 patients in "non-responders" group were given azathioprine (AZA), cyclophosphamide, mycophenolate mofetil, tacrolimus respectively. Only one of these 4 patients remarkably improved after 1.5 years of AZA treatment and the other three remained unchanged. Disease relapses were observed in 2 patients (2/11, 18.2%) in "responders" group who achieved complete improvement (1 stopped due to adverse events and 10 because of complete disappearance of lung lesions), with a mean follow-up time of 13.5 months (range: 1-30). These two patients were given MTX therapy again. And their prognosis needed further follow-up.

3. Baseline characteristics of two groups ("responders" and "non-responders") and investigation of response-linked factors

Baseline characteristics of 60 subjects on the basis of the response to MTX were presented in Table 3. All the included subjects had biopsy-proven sarcoidosis and a larger proportion of them got the biopsy tissue from lung (78.3%) or skin (18.3%). The mean duration of therapy for the enrolled subjects was 53.1±33.5 weeks. The responders had longer MTX treatment duration than the non-responders (55.4±34.4 weeks vs. 42.9±28.8 weeks), though with no statistically significant difference (P=0.267). Meanwhile, the responders were older than the non-responders, with 54.2±10.8 years and 49.4±8.4 years respectively (P=0.168). The proportion of subjects at different chest radiographic stages of pulmonary sarcoidosis had no significant difference between "responders" and "non-responders" group (P=0.126). 18.2% (2/11) of the patients in the "non-responders" group had pleura involvement, higher than the "responders" group (6.1%, 3/49) (P=0.224). No other significant differences between these two groups ("MTX responders" vs. "MTX non-responders") were observed in any other baseline parameters, including sex, BMI, disease duration, smoking status and so on (Table 3).

HRCT performance of almost half proportion of "responders" group (59.2%) was only ground glass opacity (GGO) or nodular change/parenchymal masses, while combinations of several kinds of HRCT changes (54.6%) represented the most frequently in "non-responders" group, though without a statistically significant difference (*P*=0.627). Meanwhile, no significant difference in the other baseline indexes (PFTs and SGRQ score) was revealed between these groups (Table 4).

In the multivariate logistic regression analysis, no parameter (age (OR=1.001, 95% CI, 0.916-1.093, P=0.984), sex (OR=0.244, 95% CI, 0.034-1.777, P=0.164), BMI (OR=1.203, 95% CI, 0.921-1.571, P=0.175), chest radiographic stage (OR=4.828, 95% CI, 0.628-37.087, P=0.130), whether extra-pulmonary organ involvement (OR=0.414, 95% CI, 0.072-2.380, P=0.323), duration of the disease (OR=1.765, 95% CI, 0.737-4.226, P=0.202), MTX therapy period (OR=1.072, 95% CI, 0.944-1.218, P=0.281) was found significantly associated with the clinical response to MTX.

Table 3. Baseline characteristics related to treatment effectiveness ("responders" vs. "non-responders" group)

Baseline characteristics	All patients (n=60)	MTX responders (n = 49)	MTX non-responders (n = 11)	*P* value
Age at start of therapy (years)	53.3±10.5	54.2±10.8	49.4±8.4	0.168
Male, n (%)	20 (33.3)	16 (32.7)	4 (36.4)	0.813
BMI (kg/m^2)	24.9±3.7	25.3±3.8	23.4±3.0	0.131
Reason for MTX treatment, n (%)				0.133
Refractory to GCS	38 (63.3)	28 (57.1)	10 (90.9)	
Recurrence	15 (25.0)	14 (28.6)	1 (9.1)	
Others*	7 (11.7)	7 (14.3)	0	
Duration of treatment (weeks)	53.1±33.5	55.4±34.4	42.9±28.8	0.267
Smoking status, n (%)				1.000
Former smoker	2 (3.3)	2 (4.1)	0	
Never smoker	56 (93.3)	45 (91.8)	11 (100)	
Active smoker	2 (3.3)	2 (4.1)	0	
Disease duration, (years)	1.9±2.2	2.1±2.4	1.8±1.7	0.677
Chest radiographic staging, n (%)				0.126
I	4 (6.7)	3 (6.1)	1 (9.1)	
II	41 (68.3)	34 (69.4)	7 (63.6)	
III	12 (20.0)	11 (22.4)	1 (9.1)	
IV	3 (5.0)	1 (2.1)	2 (18.2)	
Pleura involvement, n (%)	5 (8.4)	3 (6.1)	2 (18.2)	0.224
Previous therapy, n (%)				0.478
Prednisone	51 (85.0)	42 (85.7)	9 (81.8)	
Hydroxychloroquine	3 (5.0)	3 (6.1)	0	
Prednisone+hydroxychloroquine	6 (10.0)	4 (8.2)	2 (18.2)	
Extra-pulmonary involvement, n (%)	37 (61.7)	30 (61.2)	7 (63.6)	1.000
Uveitis	10 (16.7)	7 (14.3)	3 (27.3)	0.371
Cutaneous	16 (26.7)	15 (30.6)	1 (9.1)	0.259
Lymph nodes	14 (23.3)	10 (20.4)	4 (36.4)	0.264
Liver	1 (1.7)	1 (2.0)	0	1.000
Spleen	1 (1.7)	1 (2.0)	0	1.000
Comorbidity, n (%)				
Diabetes mellitus	1 (1.7)	0	1 (9.1)	
Hypertension	4 (6.7)	2 (4.1)	2 (18.2)	
Coronary artery disease	1 (1.7)	1 (2.0)	0	
GERD	1 (1.7)	1 (2.0)	0	
Biopsy, n (%)				
Lung	47 (78.3)	38 (77.6)	9 (81.8)	1.000
Skin	11 (18.3)	10 (20.4)	1 (9.1)	0.670
Lymph nodes	2 (3.3)	1 (9.1)	1 (2.0)	0.336

GERD: gastroesophageal reflux disease; *: including those who had contraindications for corticosteroid use or refuse to corticosteroid therapy.

4. Adverse reactions and drug discontinuation

Totally, adverse events were reported in 19 subjects (19/60, 31.7%) with sarcoidosis. The most frequently reported side effects were gastrointestinal-related (11/60, 18.3%), headache/uneasy (6/60, 10.0%) and infection (5/60, 8.3%). Nausea and abdominal distension occurred the most often in gastrointestinal-related adverse events. Among 5 patients who got the infection during MTX therapy period, 3 patients experienced pulmonary infection. Laboratory abnormalities including aminotransferase elevations or WBC level declined were noted in 4 patients (4/60, 6.7%). No statistically significant differences in the occurrence of adverse events were observed between "responders" group and "non-responders" group (Table 5). MTX therapy was stopped due to adverse events in 4 patients (4/60, 6.7%), with 3 (3/49, 6.1%) in "responders" group and 1 (1/11, 9.1%) in "non-responders" group (P=0.566). The detailed information was presented in Table 5. There were no differences in the occurrence of these side effects among three subgroups with different dosage of MTX (10 mg/week, 12.5mg/week and 15mg/week) (P>0.05) (Figure 3).

Table 4. Baseline HRCT, PFTs and SGRQ

	All patients (n=60)	MTX responders (n = 49)	MTX non-responders (n = 11)	*P* value
HRCT performance, n (%)				0.627
Only hilar and ediastinal lymphadenopathy	4 (6.7)	3 (6.1)	1 (9.1)	
GGO	8 (13.3)	7 (14.3)	1 (9.1)	
Nodular change/parenchymal masses	25 (41.7)	22 (44.9)	3 (27.2)	
GGO and nodular	6 (10.0)	5 (10.2)	1 (9.1)	
Others*	17 (28.3)	12 (24.5)	5 (45.5)	
PFTs				
FEV1/FVC (%)	74.0±8.7	73.9±9.0	74.1±7.6	0.957
FEV1 (% predicted)	87.5±15.1	87.6±15.1	87.0±15.2	0.910
FVC (% predicted)	96.1±15.8	96.2±16.1	95.8±14.8	0.940
TLC (% predicted)	90.2±11.0	89.7±10.6	92.1±12.9	0.529
DLco (% predicted)	74.8±10.8	75.1±8.8	73.8±17.8	0.729
SGRQ score	17.1(IQR: 7.9-26.9)	16.7(IQR: 7.9-26.4)	23.3(IQR: 8.4-31.4)	0.731

GGO: Ground glass opacities; Others*: more than 2 changes including GGO, nodular change, thickening of the bronchovascular bundles, parenchymal bands or fibrosis; PFTs, pulmonary function tests; FEV1: forced expiratory volume in one second; FVC, forced vital capacity; TLC: total lung capacity; DLco, diffusion capacity of carbon monoxide

Table 5. Reported side effects during MTX treatment

Adverse event, n (%)	All patients (n = 60)	MTX responders (n = 49)	MTX non-responders (n = 11)	*P* value
Adverse event occurred	19 (31.7)	15 (30.6)	4 (36.4)	0.990
Gastrointestinal-related	11 (18.3)	9 (18.4)	2 (18.2)	1.000
Nausea, n (%)	6 (10.0)	5 (10.2)	1 (9.1)	1.000
Epigastric discomfort, n (%)	2 (3.3)	2 (4.1)	0	1.000
Anorexia, n (%)	3 (5.0)	2 (4.1)	1 (9.1)	0.462
Abdominal distension, n (%)	4 (6.7)	4 (8.2)	0	1.000
Gingivitis, n (%)	1 (1.7)	1 (2.0)	0	1.000
Infection, n (%)	5 (8.3)	4 (8.2)	1 (9.1)	0.395
Pulmonary	3 (5.0)	3 (6.1)	0	
The upper respiratory tract	1 (1.7)	0	1 (9.1)	
Oral cavity	1 (1.7)	1 (2.0)	0	
Headache/uneasy, n (%)	6 (10.0)	5 (10.2)	1 (9.1)	1.000
Fatigue, n (%)	3 (5.0)	1 (2.0)	2 (18.2)	0.084
Hepatic function or WBC declined, n (%)	4 (6.7)	4 (8.2)	0	1.000
Aminotransferase elevations, n (%)	3 (5.0)	3 (6.1)	0	1.000
WBC level declined, n (%)	1 (1.7)	1 (2.0)	0	1.000
Drug discontinuation due to AEs, n (%)	4 (6.7)	3 (6.1)	1 (9.1)	0.566

AEs: adverse events.

Discussion

In this single-center retrospective study, we mainly analyzed the effectiveness and safety of MTX on pulmonary sarcoidosis patient refractory to or intolerant of corticosteroid therapy. Our mean follow-up duration was 15.7 months (range: 3-63). More than 90% of the included patients had previously been treated with corticosteroid, without improvement or with relapse during corticosteroid tapering. Around 80% of sarcoidosis subjects were accessed as responders by HRCT. More than half of the patients received MTX at the dose of 12.5mg weekly and others at 10 mg or 15 mg weekly. No statistically significant difference was noted in the response to MTX among various dosages. One earlier prospective study of 50 sarcoidosis patients by Goljan-Geremek and colleagues (8) observed a dose-related effect of MTX in their cohort, showing that MTX total dose was significantly higher in the "MTX responders" group compared to the "non-responders" group. However, the treatment duration was signifi-

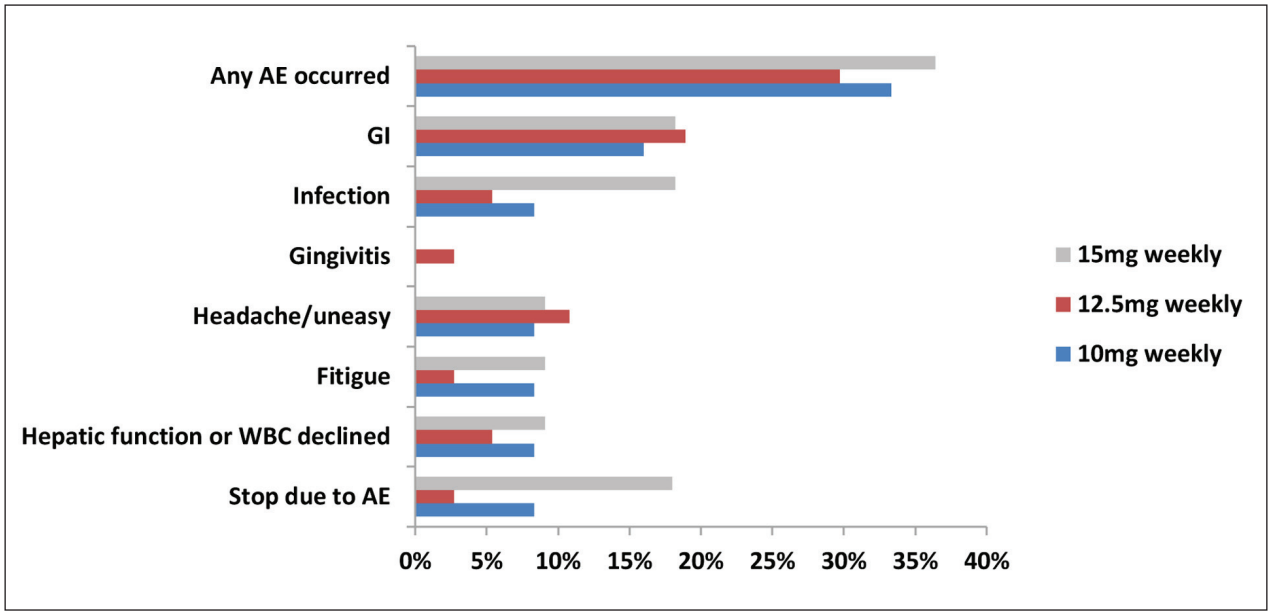

Fig. 3. Adverse events of various dose of MTX
No significant difference was found in every kind of event among three groups (P>0.05).
AE: adverse event; Skin: skin-related adverse event; GI: gastrointestinal related adverse event.

cantly longer in the "MTX responders" group of this study and the weekly dose-related effect of MTX on sarcoidosis was not for further analysis. Because the data related to the number of responders in different weekly doses of MTX were provided, we continued the statistical analysis of the relationship between MTX efficacy and various weekly dosages, revealing that no statistically significant difference (P=0.084) was observed in the proportion of responders on different MTX dosages (10 mg/week vs. 15 mg/week). This result was in coincidence with our study. The recommended initial dosage of oral MTX in sarcoidosis was 5–15 mg weekly by WASOG (13). Until now, no researches have been conducted to compare efficacy on different weekly dosages of MTX in sarcoidosis patients. Studies in rheumatic arthritis (RA) comparing different dosages of oral MTX showed dose-dependent efficacy in resistant RA patients (14). While the conclusion of dose-related effect of MTX was inconsistent in early RA patients (15, 16). More researches with large sample are needed for further analysis of the dosage related response to MTX on sarcoidosis patients.

In contrast to the present results, the literature has shown a lower response rate with improvement in only 55% of the subjects (8). The longer-lasting disease duration (12.34±20.49 years) and lower PFTs of the included subjects in that literature might explain this.

In addition to improvements in HRCT, MTX was also effective in lowering SGRQ score. Little studies had paid attention to the HRQL in sarcoidosis patients receiving MTX therapy. Only one research (8) in 2014 showed that 6-minute walk test (6MWT) improved from 531±106 m to 546±106 m and Borg score decreased from 1.22±1.9 to 0.75±1.3 in sarcoidosis patients given MTX, though without significant difference. And our study reported that HRQL of patients improved after MTX treatment as evidenced by the decline of SGRQ score both in "responders" group and "non-responders" group. This study showed that MTX had steroid-sparing potency both in "responders" group (from 30 (IQR: 15-40) to 10 (IQR: 10-15) mg/day within an average duration of 55.4 weeks) and "non-responders" group (from 20 (IQR: 17.5-35) to 15 (IQR: 15-20) mg/day within a mean duration of 42.9 weeks), though only with statistical significance in the former one. This was in coincidence with the earlier researches (7, 17).

Several studies have attempted to investigate the factors in association with response to drug therapy in sarcoidosis. The former studies had found that

pulmonary involvement was associated with poorer response to anti-tumor necrosis factor antagonists (18) or Acthar gel (19). However, studies concerning the factors associated with responsiveness to MTX in pulmonary sarcoidosis are rare. Only one (8) reported that pulmonary sarcoidosis patients who benefited from MTX therapy were those with initially impaired volume and capacity parameters. However, in our research, no relation between PFTs at baseline and the effectiveness of MTX in pulmonary sarcoidosis was found. The same results were observed in other parameters including age, sex, BMI, chest radiographic stage, whether extra-pulmonary organ involvement, duration of the disease, smoking status, MTX therapy period.

In addition to the above baseline characteristics, some laboratory results were also analyzed in the previous studies. One research (20) of 114 sarcoidosis patients reported that high baseline levels of serum angiotensin converting enzyme (sACE) correlated significantly with lung function improvement after MTX treatment, and the level of sACE decreased obviously after treatment. But sACE was still under debate because various researches reported conflicting conclusions. Until now, no definite evidence was provided for the use of sACE in the chest radiographic stage, monitoring of pulmonary sarcoidosis and predicting response to therapy (21-23). Furthermore, one study of 306 sarcoidosis patients by Doubkova et al. (24) showed that a higher CD4 to CD8 ratio (CD4/CD8) in the BALF was significantly related to spontaneous resolution. Another study (25) reported the same result that untreated patients with radiological improvement had higher numbers of CD4 cells and a higher CD4/CD8 in BALF than patients without improvement. A high CD4/CD8 was also related to better response to therapy (23). However, the lack of laboratory results in our study may not allow such kind of analysis and the relationship between these parameters and clinical responsiveness to MTX are needed to be analyzed in the future researches.

Responders to MTX were at risk of relapse after drug discontinuation. In our study, 2 relapses occurred in 11 responders with complete improvement (18.2%) who interrupted therapy with an average follow-up time of 13.5 months (range: 1-30). Owing to the limited sample, we were not able to do analysis to determine predictive factors for relapses. Therefore, larger sample size and longer follow-up time will be needed for further analysis. And 2 relapsed subjects were given MTX therapy again and the efficacy needed further assessment.

In the earlier researches, the sarcoidosis patients were given 5 mg folic acid weekly (7, 8). The recommendation on the prescription of folic acid during MTX treatment for sarcoidosis was based on some researches from rheumatoid arthritis (26, 27). However, until now, no studies on folic acid supplement during MTX treatment for sarcoidosis have been available. Therefore, considering shortness of evidence for folic acid supplements, our study focused on the safety of MTX on sarcoidosis without folic acid intake. It revealed that the occurrence rate of adverse events was 31.7% in our study with mean treatment duration of 53.1 weeks. The most frequently reported adverse events were gastrointestinal-related (11/60, 18.3%) and infection (5/60, 8.3%), obviously lower than the earlier research (7). And the occurrence rate of hepatic abnormalities in our study was dramatically lower than the previous data (8). However, long-term follow-up data on the safety of MTX were from rheumatoid arthritis (26), showing the lower morality (28) and discontinuation rate than other antirheumatic drugs owing to toxicity (29, 30). No studies with long-term follow-up data on safety profile of MTX for sarcoidosis was found.

There were several limitations to this study. First, due to the retrospective design of this study, nonstandardization of follow-up inevitably occurs, which might lead to missed side effects. But we have tried to minimize the loss of data through thorough investigation of patients' electronic and paper medical records. Second, no objective global assessment tools have been established to accurately assess sarcoidosis patients' response to a particular therapy (31). Furthermore, a large proportion of patients in our real-world clinical practice did not have regular lung function tests and most of them might take these tests in different hospitals, giving rise to the failure to analyze effectiveness by PFTs at different times according to one standardization. Third, laboratory results like sACE and CD4/CD8 in BALF were not available and analyzed in this study, so more researches are needed to investigate their relationship with response to MTX.

Conclusion

The present study has demonstrated efficiency of MTX with various dosages in pulmonary sarcoidosis as evidenced by the improvement of HRCT and HRQL. The steroid-sparing effect of MTX was observed both in "responders" group and "non-responders" group. No baseline characteristics were found in association with response to MTX. Additionally, this was the first study revealing the well tolerability of MTX therapy without folic acid supplements. Relapse rate was low after MTX withdrawal while relapse-linked factors are still needed to be analyzed and illuminated.

Authors' contributions: Chuling Fang, Qian Zhang and Zuojun Xu contributed to the construction of the database and the study design; Chuling Fang, Qian Zhang, Na Wang and Xiaoyan Jing contributed to the data collection; Chuling Fang, Qian Zhang and Zuojun Xu contributed to the data analysis; Chuling Fang contributed to the draft of the manuscript; and all authors contributed to the revision and final approval of the manuscript.

Funding: The research leading to these results has received funding from National Key Technologies R & D Program Precision Medicine Research (No.2016YFC0905700) and the National Natural Science Foundation of China (No.81670061).

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SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (3); 228-242

A prospective study of patients diagnosed with sarcoidosis: factors - environmental exposure, health assessment, and genetic outlooks

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Abstract. This original research is a directional study that determined the habits of individuals using four analyses to find statistical significance in the data collected from the surveys of 801 qualified of 1,340 individuals who agreed to participate. Results from the self-reported diagnosis of individuals affected by sarcoidosis produced seven statistically significant indicators of future research needed. The demographics revealed a significantly greater number of women and African-Americans participants than other minorities in the United States and suggested a sense of urgency to find a cure. Most important are the seven statistically significant findings that also gave credence to the researchers' four subdiagnostic classifications. They are acute sarcoidosis (AS) and chronic sarcoidosis with limited dissemination (CSLD), while more severe cases include those with chronic sarcoidosis with full dissemination including cutaneous involvement (CSFDIC) and chronic sarcoidosis with neurosarcoidosis (CSN). The most severe sarcoidosis cases (CSN) were on the "most likely" side of every statistically significant category except drinking alcohol, and the "least likely" to participate in physical activities. Conversely, the least severe case of sarcoidosis (AS) was the opposite. The complete list of statistically significant areas was related to alcohol use, tobacco use, ciprofloxacin use, environmental exposure to metals (copper, iron), infectious diseases (candidiasis), genetics, and physical exercise. Statistically, the most crucial study needed; emerged from the Rh blood grouping of the participants. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (3): 228-242)*

Key words: sarcoidosis, environmental exposure, genetics

Introduction

The modern history of sarcoidosis, an enigmatic multisystem disease, goes back to 1899 when the pioneering Norwegian dermatologist Caesar Boeck coined the term "sarcoid" to describe skin nodules characterized by compact, sharply defined foci of "epithelioid cells with large pale nuclei and a few giant cells (1). Sarcoidosis is a systemic granulomatous disease of unknown cause that primarily affects the lungs; however, the abnormal inflammatory disease process may affect any organ and tissue of the body. The lungs, the lymph nodes of the thorax and the neck, skin, and the liver are the most often involved. The hallmark histological feature of the disease is epithelioid cell granuloma derived from activated T cells and macrophages triggered by unknown immune stimuli such as bacterial protein or beryllium metal (2). There are over 200,000 living individuals with sarcoidosis in the United States. It is hard to say how many worldwide suffer from this disease (2). Globally; the disease is more common in people of Scandinavian, German, Irish, and Puerto Rico de-

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Accepted after revision: 1 June 2019
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scent (3). No one knows why this occurs. Granulomas of the lachrymal (concerned with the secretion of tears) and salivary glands are found in one-third of patients upon presentation. Since sarcoidosis mimics many other diseases, the number of people who have sarcoidosis is thought to be much higher than the number reported. The cause remains unclear; however, environmental, genetic, ethnic, and familial factors probably modify the expression of the disease. For example, African-Americans are at a greater risk of mortality and morbidity than are white Americans, and more often have a family history of sarcoidosis (4). More females are reported to have sarcoidosis than men. In fact, it is a two to one ratio over African American men. Current reported cases over the last several years show that the gender gap is narrowing. Often most patients that present with acute sarcoidosis (Löfgren syndrome) recover spontaneously, but some develop the chronic debilitating disease. Moreover, sarcoidosis is a multifactor disease of an abnormal immune response to unknown stimuli that produce granulomas in the organs and tissues, also referred to as Boeck's Sarcoid. The proposed study was conducted to identify possible significant factors related to patients diagnosed with sarcoidosis.

Methods

This study used a prospective survey that included nutrition, health history, environmental exposures, lifestyle, genetics, and demographic information. The survey further collected data on both external and internal risk factors that might predispose one to sarcoidosis. The final survey contained 121 questions and took most respondents between thirty and sixty minutes to complete. From the data, statistics were run exceeding the more salient ones included.

Before the start period of the grant, substantial work was done to construct the survey questions to learn more about the causes and effects of sarcoidosis. The first obstacle was to identify commonalities to complete this directional study. Identified were acute, and chronic, with two further distinctions of recurrent or progressive sarcoidosis. In general, published researchers suggested the differentiation of identified cases based on clinical phenotypes of sarcoidosis. Others, not only suggested identifiable subclassifications, they thought them necessary for the treatment of sarcoidosis patients (5). From these documents, four subclassifications seem to capture the manifestations of sarcoidosis that were explicable to participants, with an increase in severity as suggested by the debilitating conditions in the subdiagnostic names given. Subdiagnostic refers to the reliance on the participants to accurately self-report their diagnosis. The first subdiagnostic classification of *acute sarcoidosis* (AS), though called subdiagnostic is recognized, is also called Löfgren syndrome (6). Moreover, the last three are in the recognized chronic category, but not the new subdivision (5). Consequently, the order of increasing severity is the somewhat defined *chronic sarcoidosis with limited dissemination* (CSLD) (7), and *chronic sarcoidosis with full dissemination including cutaneous involvement* (CSFDIC) (8), and the more defined, *chronic sarcoidosis with neurosarcoidosis* (CSN) (9). Prior classification of sarcoidosis was based on chest radiographic features of the disease but didn't fully capture the chronic aspects of the disease (2). Subdiagnostic categories in this paper do not imply lack of lung involvement. The subdiagnostic categories were a way to organize the participants based on the severity of the condition. During this same period of classification, the investigators submitted an application to conduct research using human subjects to the Texas State University Institutional Review Board (IRB) for approval. The application was approved by the Texas State University IRB (IRB application number 2015Z8977) on June 18, 2015.

Participants for the study were recruited by posting a short web-based survey on 31 Sarcoidosis Support Groups on the social network Facebook. The criteria for inclusion in the study was a diagnosis of sarcoidosis as made by a licensed physician or healthcare provider. The short web-based survey explained the purpose of the study and requested that individuals with a diagnosis of sarcoidosis participate in the study. Those who agreed were asked to provide their names and email addresses. Using this technique, 1,340 individuals agreed to participate. Potential participants were later contacted using a custom email with an automatic login link to the research survey. Therefore, no identifying information was needed from this point forward. A sample size of 1000 was targeted. Numerous reminders were provided to participants, and ultimately, 801 individuals responded.

The Principal Investigator of the study developed a bank of possible research questions. From

the bank of questions, the research team refined the questions until everyone agreed on a pilot version. This version was field tested with six individuals diagnosed with sarcoidosis, three health professionals, and one faculty member in health professions. Based on their feedback, the survey was finalized (see Appendix A for a copy of the survey).

The recruitment and main surveys were administered electronically using Snap Surveys© software, which can be used to create and manage web-based surveys. The university subscribes to an Education Enterprise License for this robust software package that includes technical support, software updates, and other application maintenance as needed. Further, this software package allowed the researchers to build a customized data collection instrument while managing and controlling user access. Specifically, when constructing instruments, Snap Survey© software allows for custom design throughout the online instrument, the ability to utilize conditional logic and data entry validations (i.e., question routing), and the creation of distinct participant groupings (e.g., specific classes). Snap Survey© software is installed on a university server, rather than hosted by a third-party cloud storage company. For this specific project, this feature allowed the online version of this survey to be hosted on the Texas State University data center, which brought with it all the security and protection features associated with the university's IT infrastructure. Participants had the option to select a "choose not to answer" response to each question. Participants could also skip a question, resulting in a noted response of missing. Respondents were from all parts of the United States.

Results

Eight hundred and one participants responded to the survey. Demographic information, as well as self-reported subdiagnostic categories, are reported in Tables 1 and 2. Of the 801 respondents, 617 provided valid zip codes as seen in Table 1 along with the state in which respondents live. Representation came from all but two of the 50 states of the US, those being Montana and Nebraska. One respondent lives in the District of Columbia (1, 0.2%). The top five states with the most respondents in the study are Texas (49, 7.9%), New York (48, 7.8%), Michigan (25, 25%), Florida (37, 6.0%) and California (30, 5.0%). Hawaii, New Mexico, South Dakota, and Wyoming, each had one responded (Table 1). Of the 801 respondents, 30 live in the UK, 11 in Canada, seven (7) in Australia, five (5) in Scotland, four (4) in Ireland, one (1) each in Brazil, Netherlands, Slovenia, and New Zealand. Three respondents serve in the military living in the Middle East. Women constituted most of the 801 respondents. Notably, even the least common subdiagnostic classification chronic sarcoidosis with full dissemination, including cutaneous involvement (CSFDIC) was still well-represented among participants, comprising 15.9% of all responses. Chronic sarcoidosis with limited dissemination (CSID) was the largest group which represented 28.5% of all responses. Acute sarcoidosis represented the next largest at 20.7% (Table 2).

Table 3 summarizes responses related to eating habits. Participants questions about their eating habits were prospecting for its role in causing sarcoidosis in addition to its known ability to exacerbate conditions of sarcoidosis (10, 11). About half of the respondents ate breakfast (48.6%) with only 8.2% reporting never eating breakfast. Slightly more than half ate lunch (50.6%) with 2.4% reporting never eating lunch. The largest meal eaten by respondents daily was dinner (78.7%) (12).

Table 4 summarizes responses related to participants smoking habits and alcohol use. Of the 801 subjects in the study, 700 (87.4%) reported not to be a smoker. Of the 801 subjects in the study, 433 (54.1%) reported alcohol use, whereas 367 (45.8%) report no use. Medical marijuana was used by 47 (5.9%); the majority did not report using it (753, 94%).

Table 5 summarizes responses related to exposure to heavy metals. Exposure was defined to participants with the statement, "exposure means that one work or have worked in an industry where this metal was used, in an environmental contamination exposure situation, or that one had any contact inhalation or skin contact with the metal."

Table 6 begins the genetic look at 438 self-reported blood phenotypes matched to their subdiagnosis. Another 84 self-reported their phenotypes but not their subdiagnosis. Associations between subdiagnostic classification and responses to the other questions in the survey were explored for the 663 participants who reported a subdiagnostic classification. Only those associations that were statistically

Table 1. Frequencies and percentages of states in which respondents live

State	N	%	State	N	%
Alabama	8	1.3	Missouri	18	2.9
Alaska	3	0.5	Nevada	5	0.8
Arizona	7	1.1	New Hampshire	5	0.8
Arkansas	8	1.3	New Jersey	14	2.3
California	31	5.0	New Mexico	1	0.2
Colorado	9	1.5	New York	48	7.8
Connecticut	8	1.3	North Carolina	28	4.5
Delaware	3	0.5	North Dakota	3	0.5
District of Columbia	1	0.2	Ohio	27	4.4
Florida	37	6.0	Oklahoma	8	1.3
Georgia	23	3.7	Oregon	10	1.6
Hawaii	1	0.2	Pennsylvania	20	3.2
Idaho	3	0.5	Rhode Island	3	0.5
Illinois	18	2.9	South Carolina	20	3.2
Indiana	14	2.3	South Dakota	1	0.2
Iowa	5	0.8	Tennessee	12	1.9
Kansas	7	1.1	Texas	49	7.9
Kentucky	13	2.1	Utah	6	1.0
Louisiana	9	1.5	Vermont	2	0.3
Maine	4	0.6	Virginia	14	2.3
Maryland	15	2.4	Washington	12	1.9
Massachusetts	17	2.8	West Virginia	7	1.1
Michigan	25	4.1	Wisconsin	13	2.1
Minnesota	15	2.4	Wyoming	1	0.2
			Total	**617**	

significant are reported. Table 7 report crosstabulations of subdiagnosis with the response to those questions that were statistically significant at the .05 level. Neither gender nor race/ethnicity variables were significantly associated with the subdiagnostic category.

Cross Tabulations and Chi-Square Analyses (Table 7): The following are the results from the statistical analysis of the 663 participants who reported a subdiagnostic classification. Ciprofloxacin exposure was relatively common across all groups, with 47.8% of the total sample having reported some exposure. The past or present use of ciprofloxacin was statistically significantly associated with the subdiagnostic classification, $\chi^2(3) = 8.49$, $p = .037$. Table 7 shows this association. Comparison of observed cell counts with cell counts expected under the null hypothesis of statistical independence reveals that those with *acute sarcoidosis* (AS) reported the least exposure, followed by those with *limited dissemination* (CSLD).

Table 2. Sample of self-reported demographic characteristics and subdiagnostic categories

Variable	N	%
Subdiagnostic Category		
Acute sarcoidosis	166	20.7
Chronic sarcoidosis with limited dissemination	228	28.5
Chronic sarcoidosis with full dissemination, including cutaneous involvement	127	15.9
Chronic sarcoidosis with neurosarcoidosis	142	17.7
Choose not to answer	116	14.5
Missing	22	2.7
Gender		
Female	654	81.6
Male	143	17.9
Self-Identified	2	0.2
Missing	2	0.2
Hispanic		
No	744	92.9
Yes	40	5
Missing	17	2.1
Race		
American Indian/Eskimo/Aleut Alaskan Native	8	1
Asian/Pacific Islander	9	1.1
Black/African American	103	12.9
White	603	75.3
Two or More	28	3.5
Other	23	2.9
Missing	27	3.4

Those with a diagnosis of *chronic sarcoidosis with full dissemination, including cutaneous involvement* (CSFDIC) or *chronic sarcoidosis with neurosarcoidosis* (CSN) reported the greatest exposure.

Receiving a diagnosis of candidiasis was also associated with the subdiagnostic classification, $\chi^2(3) = 9.34$, $p = .025$. This association is shown in Table 7. As was the case with ciprofloxacin usage, the more severe the subdiagnostic classification, the more likely diagnosis of candidiasis was to be reported.

The association of subdiagnostic classification with tobacco usage (Table 7) was statistically significant, $\chi^2(3) = 7.89$, $p = .048$. Those with diagnoses of AS, CSLD, and CSFDIC reported the roughly equal incidence of tobacco usage, around 10%. Those with a diagnosis of CSN reported a higher incidence of usage at 19.0%.

The association of alcohol use with subdiagnostic classification was statistically significant, $\chi^2(3) = 10.87$, $p = .012$. Table 7 shows this association. Comparison of observed cell counts with cell counts expected under the null hypothesis of statistical independence reveals that those with either *acute sarcoidosis* (AS) or *chronic sarcoidosis with limited dissemination* (CSLD) are more likely to report the use of alcohol than would be expected by chance. Whereas those with *chronic sarcoidosis with full dissemination, including cutaneous involvement* (CSFDIC) or *chronic*

Table 3. Sample of self-reported eating habits

Variable	N	%
How often do you eat breakfast		
1-2 days a week	185	23.1
3-5 days a week	159	19.9
6-7 days a week	389	48.6
Never	66	8.2
Missing	2	0.2
How often do you eat lunch		
1-2 days a week	110	13.7
3-5 days a week	258	32.2
6-7 days a week	405	50.6
Never	19	2.4
Missing	9	1.1
How often do you eat dinner		
1-2 days a week	30	3.7
3-5 days a week	131	16.4
6-7 days a week	630	78.7
Never	2	0.2
Missing	8	1

Table 4. Key Variables related to substance use

Variable	N	%
Use of tobacco		
No	700	87.4
Yes	100	12.5
Missing	1	0.1
Use of medicinal marijuana		
No	753	94
Yes	47	5.9
Missing	1	0.1
Use of alcohol		
No	433	54.1
Yes	367	45.8
Missing	1	0.1

sarcoidosis with neurosarcoidosis (CSN) are less likely to use alcohol.

The association of copper exposure with subdiagnostic classification was statistically significant, $\chi^2(3) = 9.81$, $p = .020$. As shown in Table 7, those with AS and CSLD reported between four and five percent incidence of exposure, whereas those with

Table 5. Sample of self-reported exposure to metals

Variable	N	%
Have you ever been exposed to…		
Arsenic	20	2.50
Barium	19	2.40
Beryllium	21	2.60
Cadmium	16	2.00
Chromium	17	2.10
Cobalt	16	2.00
Copper	59	7.40
Iron	64	8.00
Lead	95	11.90
Mercury	57	7.10
Nickel	31	3.90
Platinum	14	1.70
Selenium	6	0.70
Thallium	5	0.60
Tungsten	12	1.50
Uranium	5	0.60
Zinc	34	4.20

CSFDIC and CSN reported the considerably higher incidence of exposure at 11.8% and 9.9%, respectively.

Iron exposure was also associated with subdiagnostic classification, $\chi^2(3) = 10.51$, $p = .015$. As shown in Table 7, the pattern of reported exposure by classification was highly similar to the pattern observed for copper exposure. Those with AS and CSLD reported the relatively low incidence of exposure at 4.8% and 6.1%, respectively. In contrast, those with CSFDIC and CSN reported the noticeably higher incidence of exposure at 12.6% and 12.7%, respectively.

Cross Tabulations and Odds Ratio Analyses (Table 8): The statistically significant Chi-Squares in the above six subdiagnostic classifications warranted additional analyses. Specifically, we were interested in analyzing if there were differences based on the severity of sarcoidosis and if the person had an acute versus the chronic case of sarcoidosis. This study was a case-control because we started with the outcome and looked back to see which individuals in the two groupings were exposed or used alcohol, tobacco, and so on. The first grouping divided the 663 participants into artificial categories of "Less" and "More" severe cases of sarcoidosis. Less severe cases included those with *acute sarcoidosis* (AS) and

Table 6. Sample of self-reported blood groups and subdiagnostic categories

Cases of sarcoidosis	Blood Type								Total
	A Positive	A Negative	B Positive	B Negative	AB Positive	AB Negative	O Positive	O Negative	
AS	21	5	16	2	7	2	47	14	114
CSLD	46	6	19	2	6	2	53	21	155
CSFDC	21	4	15	2	1	6	26	10	85
CSN	12	5	13	3	4	1	34	12	84
Total	100	20	63	9	18	11	160	57	438

Table 7. Cross tabulations and chi-square of subdiagnostic classification from exposure, usage, or infection

Exposures / Usages / Infection	Ciprofloxacin			Candida Albicans			Tobacco			Alcohol			Copper			Iron		
Statistical Differences (AS, CSLD, CSFDIC, CNS)	$\chi^2(3) = 8.49$, ***p* = .037**			$\chi^2(3) = 9.34$, ***p* = .025**			$\chi^2(3) = 7.89$, ***p* = .048**			$\chi^2(3) = 10.87$, ***p* = .012**			$\chi^2(3) = 9.81$, ***p* = .020**			$\chi^2(3) = 10.51$, ***p* = .015**		
Subdiagnostic Classifications	Yes	No	Total	Yes	No	Total	Yes	No	Total	Yes	No	Total	Yes	No	Total	Yes	No	Total
1. Acute sarcoidosis (AS)																		
Count	66	100	166	17	149	166	20	146	166	80	86	166	7	159	166	8	158	166
Expected Count	79.4	86.6	166	22	144	166	21	146	166	76.4	89.6	166	11.8	154	166	14	152	166
% within row	39.8	60.2	100	10.2	89.8	100	12	88	100	48.2	51.8	100	4.2	95.8	100	4.8	95.2	100
2. Chronic sarcoidosis with limited dissemination (CSLD)																		
Count	106	122	228	24	204	228	22	206	228	120	108	228	11	217	228	14	214	228
Expected Count	109	119	228	31	197	228	28	200	228	105	123	228	16.2	212	228	19	209	228
% within row	46.5	53.5	100	10.5	89.5	100	9.6	90.4	100	52.6	47.4	100	4.8	95.2	100	6.1	93.9	100
3. Chronic sarcoidosis with full dissemination, including cutaneous involvement of the disease (CSFDIC)																		
Count	68	59	127	19	108	127	13	114	127	45	82	127	15	112	127	16	111	127
Expected Count	60.7	66.3	127	17	110	127	16	111	127	58.4	68.6	127	9	118	127	11	116	127
% within row	53.5	46.5	100	15	85	100	10.2	89.8	100	35.4	64.6	100	11.8	88.2	100	12.6	87.4	100
4. Chronic sarcoidosis with neurosarcoidosis (CSN)																		
Count	77	65	142	29	113	142	27	115	142	60	82	142	14	128	142	18	124	142
Expected Count	67.9	74.1	142	19	123	142	18	124	142	65.3	76.7	142	10.1	132	142	12	130	142
% within row	54.2	45.8	100	20.4	79.6	100	19	81	100	42.3	57.7	100	9.9	90.1	100	12.7	87.3	100

chronic sarcoidosis with limited dissemination (CSLD), while more severe cases include those with *chronic sarcoidosis with full dissemination including cutaneous involvement* (CSFDIC) and *chronic sarcoidosis with neurosarcoidosis* (CSN). The second grouping divided the participants into two groups of acute sarcoidosis, which included only individuals in the AS classification versus chronic sarcoidosis, which included all other participants in the CSLD, CSFDIC, and CSN classifications. Table 8 report crosstabulations, the odds ratios, and Chi-Square based on severity and acute versus chronic. Alpha was set at the .05 level.

Table 8 presents the association of ciprofloxacin use with the severity of sarcoidosis. The result was statistically significant, with an Odds Ratio (OR) of 0.66 and confidence interval (CI) 0.49 to 0.91. This result indicates that the odds an individual with a less severe case of sarcoidosis is 0.66, or there is a 34% less chance of them reporting the use of ciprofloxacin than an individual with a more severe case of sarcoidosis.

Table 8 presents the association of ciprofloxacin use with the acute versus chronic sarcoidosis was also statistically significant, with an OR of 0.65 (CI

Table 8. Cross tabulations, odds ratio, and chi-square of severity and disease onset from subdiagnostic classification of exposure, usage, or infection

		Exposures / Usages / Infection	Ciprofloxacin			Candida Albicans			Tobacco			Alcohol			Copper			Iron		
			Yes	No	Total	Yes	No	Total	Yes	No	Total	Yes	No	Total	Yes	No	Total	Yes	No	Total
AS, CSLD	Less Severe	Count	172	222	394	41	353	394	42	352	394	200	194	394	18	376	394	22	372	394
		% within Exposure Case	43.7	56.3	100	10.4	89.6	100	10.7	89.3	100	50.8	49.2	100	4.6	95.4	100	5.6	94.4	100
CSFDIC, CNS	More Severe	Count	145	124	269	48	221	269	40	229	269	105	164	269	29	240	269	34	235	269
		% within Exposure Case	53.9	46.1	100	17.8	82.2	100	14.9	85.1	100	39	61	100	10.8	89.2	100	12.6	87.4	100
	Total	Count	317	346	663	89	574	663	82	581	663	305	358	663	47	616	663	56	607	663
		% within Exposure Case	47.8	52.2	100	13.4	86.6	100	12.4	87.6	100	46	54	100	7.1	92.9	100	8.4	91.6	100
				95% CI			95% CI			95% CI			95% CI			95% CI			95% CI	
			Value	Lower	Upper	Value	Lower	Upper	Value	Lower	Upper	Value	Lower	Upper	Value	Lower	Upper	Value	Lower	Upper
	Odds Ratio for Exposure Case (Less Severe / More Severe)		**0.66**	0.49	0.905	**0.535**	0.341	0.838	1.464	0.921	2.328	**1.61**	1.176	2.206	**0.396**	0.215	0.729	**0.409**	0.233	0.716
			$\chi^2(3) = 6.729, p = .009$			**$\chi^2(3) = 7.609, p = .006$**			$\chi^2(3) = 2.614, p = .106$			**$\chi^2(3) = 8.852, p = .003$**			**$\chi^2(3) = 9.366, p = .002$**			**$\chi^2(3) = 10.291, p = .001$**		
AS	Acute	Count	66	100	166	17	149	166	20	146	166	80	86	166	7	159	166	8	158	166
		% within Exposure Case	39.8	60.2	100	10.2	89.8	100	12	88	100	48.2	51.8	100	4.2	95.8	100	4.8	95.2	100
CSLD, CSFDIC, CNS	Chronic	Count	251	246	497	72	425	497	62	435	497	225	272	497	40	457	497	48	449	497
		% within Exposure Case	50.5	49.5	100	14.5	85.5	100	12.5	87.5	100	45.3	54.7	100	8	92	100	9.7	90.3	100
	Total	Count	317	346	663	89	574	663	82	581	663	305	358	663	47	616	663	56	607	663
		% within Exposure Case	47.8	52.2	100	13.4	86.6	100	12.4	87.6	100	46	54	100	7.1	92.9	100	8.4	91.6	100
				95% CI			95% CI			95% CI			95% CI			95% CI			95% CI	
			Value	Lower	Upper	Value	Lower	Upper	Value	Lower	Upper	Value	Lower	Upper	Value	Lower	Upper	Value	Lower	Upper
	Odds Ratio for Exposure Case (Acute / Chronic)		**0.65**	0.45	0.924	0.673	0.385	1.18	1.04	0.608	1.781	1.125	0.791	1.599	0.503	0.221	1.146	0.474	0.219	1.023
			$\chi^2(3) = 5.757, p = .016$			$\chi^2(3) = 1.930, p = .165$			$\chi^2(3) = .021, p = .885$			$\chi^2(3) = .427, p = .513$			$\chi^2(3) = 2.773, p = .096$			$\chi^2(3) = 3.768, p = .052$		

0.45 to 0.92). This result indicates that the odds an individual with an acute case of sarcoidosis is 0.65, or there is a 35% less chance of them reporting the use of ciprofloxacin than an individual with a chronic case of sarcoidosis.

Table 8 presents the association of candidiasis with the severity of sarcoidosis. The result was statistically significant, with an OR 0.54 (CI 0.34 to 0.84). This result indicates that the odds an individual with a less severe case of sarcoidosis is 0.54, or there is a 46% less chance of them reporting having candidiasis than an individual with a more severe case of sarcoidosis.

Table 8 presents the association of candidiasis with the acute versus chronic sarcoidosis with an OR 0.67 (CI 0.39 to 1.18). Since the confidence interval (CI) crosses the line of no effect (1), it is not statistically significant. In other words, no statistical difference exists between acute and chronic candidiasis at 95% confidence.

Table 8 presents the association of tobacco use with the severity of sarcoidosis with an OR of 1.46 (CI 0.92 to 2.33). Since the CI crosses the line of no effect (1), it is not statistically significant. In other words, no statistical difference exists between the severity of sarcoidosis for tobacco use at 95% confidence.

Likewise, Table 8 presents the association of tobacco use with acute versus chronic sarcoidosis with an OR 1.04 (CI 0.61 to 1.78). Since the CI crosses the line of no effect (1), it is not statistically significant. In other words, no statistical difference exists between the acute versus chronic sarcoidosis for tobacco use at 95% confidence.

Table 8 presents the association of alcohol use with the severity of sarcoidosis. The result was statistically significant, with an OR 1.6 (CI of 1.2 to 2.2). These results indicate that the odds an individual with a less severe case of sarcoidosis use of alcohol is 1.6 times that of the odds an individual with a more severe case of sarcoidosis. In other words, those with less severe cases are more likely to report the use of alcohol than those with more severe cases.

However, Table 8 presents the association of alcohol use with the acute versus chronic sarcoidosis with an OR 1.1 (CI 0.8 to1.6). Since the CI crosses the line of no effect (1), it is not statistically significant. In other words, no statistical difference exists between the acute versus chronic sarcoidosis for alcohol use at 95% confidence.

Table 8 presents the association of copper exposure with the severity of sarcoidosis. The result was statistically significant, with an OR 0.40 (CI 0.22 to 0.73). This result indicates that the odds an individual with a less severe case of sarcoidosis is 0.40, or there is a 60% less chance of them reporting exposure to copper than those with a more severe case of sarcoidosis.

Table 8 presents the association of copper exposure with the acute versus chronic sarcoidosis with an OR 0.50 (CI 0.22 to 1.15). Since the confidence interval (CI) crosses the line of no effect (1), it is not statistically significant. In other words, no statistical difference exists between acute and chronic association with copper exposure at 95% confidence.

Table 8 presents the association of iron exposure with the severity of sarcoidosis. The result was statistically significant, with an OR of 0.41 (CI 0.22 to 0.73). This result indicates that the odds an individual with a less severe case of sarcoidosis is 0.41, or there is a 59% less chance of them reporting exposure to iron than those with a more severe case of sarcoidosis.

Table 8 presents the association of iron exposure with the acute versus chronic sarcoidosis with an OR 0.47 (CI 0.22 to 1.02). Since the confidence interval (CI) crosses the line of no effect (1), it is not statistically significant. In other words, no statistical difference exists between acute and chronic association with iron exposure at 95% confidence.

Goodness of Fit Chi-Square Analyses (Table 10): We also ran the goodness of fit, Chi-Square analysis comparing the frequency of blood types in the sarcoidosis cases to that of the United States (US) general population. An initial check was statistically significant for the self-identified subdiagnostic classifications matched with their self-reported blood grouping; $\chi^2(3) = 30.511$, $p < 0.01$ at $\alpha = .05$ (table not shown) but did not reveal the root cause. Then a statistical analysis compared the ABO and Rh blood grouping of sarcoidosis participants to that in the United States (US) according to the American Red Cross (ARC) frequency distribution and Stanford School of Medicine (SSM) at $\alpha = .05$ (13, 14). The ARC gave the frequency distribution of eight phenotypes for four ethnic groups (Caucasian, African-American, American-Latino, and Asian), with slightly different percentages than SSM that had the

same eight phenotypes, but an unknown percentage compilation.

Matching the combined ABO and Rh blood types with self-identified subdiagnostic classifications were possible for 438 of the 663 participants (Table 6) and were statistically significant. ARC, $\chi^2(7) = 146.448$, $p < 0.01$, SSM, $\chi^2(7) = 95.852$, $p < 0.01$; at $\alpha = .05$. However, attempts to understand the phenomena resulted in the separation of the phenotypes and analyzing the assumed more important ABO phenotype, resulted in conflicting results. The ARC frequencies were NOT statistically significant for the 438 participants, $\chi^2(3) = 7.033$, $p = 0.071$, but was for the SSM, $\chi^2(3) = 50.975$, $p < 0.01$; at $\alpha = .05$.

Efforts to explain the apparent contradiction resulted in the comparison of the 438 participants Rh blood grouping alone and was statistically significant. ARC, $\chi^2(1) = 101.764$, $p < 0.001$ (Table 10), which was greater than SSM, $\chi^2(1) = 17.543$, $p < 0.01$; at $\alpha = .05$. The significant finding of the Rh blood grouping compelled the final comparison of all 522 self-reported Rh blood grouping (Table 9), without regards to the subclassifications, that included 84 unknown subdiagnosis, resulted in the greatest statistical significance. ARC, $\chi^2(1) = 126.128$, $p < 0.001$ (Table 10), again, greater than SSM, $\chi^2(1) = 22.503$, $p < 0.01$; at $\alpha = .05$.

Subclassification Association with Exercise: The Godin Leisure-Time Exercise Questionnaire (1985) was used to allow participants to self-report their level of physical activity (12). Of the 663 participants who reported a subdiagnostic classification, 659 provided sufficient information for the calculation of total physical activity (PA) score using Godin and Shephard's (1985) formula, $m = 19.03$, $sd = 21.59$. Inspection of a histogram of PA scores revealed a continuously distributed variable with many zeros (Figure 1). This pattern suggested that an important distinction among these participants was whether they reported any physical activity at all. In other words, the PA scores observed for these $n = 659$ participants could be parsimoniously conceptualized as a mixture of two distributions. One distribution with only the value of zero ($n = 218$), and separate, continuous distribution of PA scores for those participants who actually engaged in physical activity ($n = 441$, $m = 28.44$, $sd = 20.71$).

A dichotomous variable was thus created to indicate whether (1) or not (0) each participant with a non-missing PA score had a PA score that was greater than zero. The association of this dichotomous variable with subdiagnostic classification was statistically significant, $\chi^2(3) = 15.48$, $p = .001$. Inspection of means of this dichotomous variable (i.e., the proportion of individuals reporting greater than zero physical activity) for each subdiagnostic classification showed that AS ($n = 165$, $m = .72$), CSLD ($n = 227$, $m = .71$), and CSFDIC ($n = 126$, $m = .69$) were similar in the proportion of participants reporting some physical activity. In contrast, participants in the CSN subdiagnostic classification were notably less likely to report engaging in any activity ($n = 141$, $m = .53$).

Perhaps unsurprisingly then, while a one-way ANOVA did not show statistically significant variation of mean PA score across subdiagnostic classifications [$F(3, 655) = 1.93$, $p = .12$], means of these PA scores showed a similar descriptive pattern. Those participants with AS ($m = 20.75$, $sd = 23.53$), CSLD ($m = 19.60$, $sd = 19.83$), and CSFDIC ($m = 20.00$, $sd = 22.13$) had similar mean PA scores. Participants in the subdiagnostic classification of CSN had lower PA scores on average ($m = 15.26$, $sd = 21.24$).

Finally, this one-way ANOVA was performed again after excluding those participants with PA

Table 9. Subdiagnostic sarcoidosis vs. us population

	Sarcoid Observed Frequency	US Expected Frequency	Residual	ABO & Rh % <or > Expected	ABO % <or > Expected
A Positive	127	176.8	-49.8	-28%	-42%
A Negative	27	31.2	-4.2	-13%	
B Positive	70	42.1	27.9	66%	115%
B Negative	11	7.4	3.6	49%	
AB Positive	21	16.8	4.2	25%	-38%
AB Negative	11	29.7	-18.7	-63%	
O Positive	187	185.2	1.8	1%	109%
O Negative	68	32.7	35.3	108%	
Total	522				

Table 10. Rh blood grouping observed vs. expected significance

	Self-reported ABO, Rh Matched			Self-reported Rh		
	Observed	Expected	Residual	Observed	Expected	Residual
Positive	341	400.2	-59.2	405	477	-72
Negative	97	37.8	59.2	117	45	72
Total	438			522		
	$\chi^2(1) = 101.764$, $p < 0.001$			$\chi^2(1) = 126.128$, $p < 0.001$		

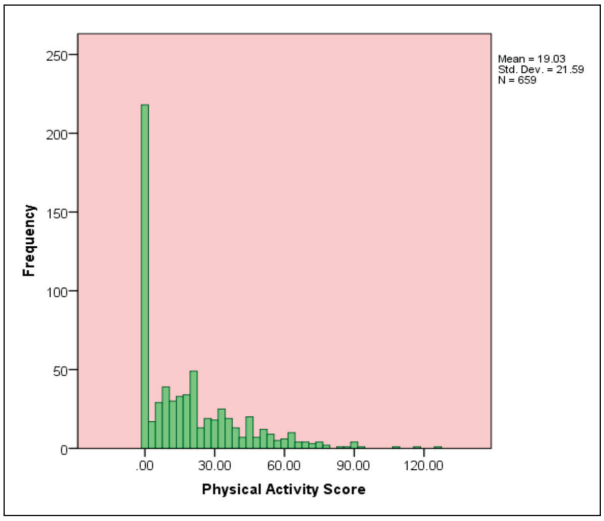

Fig. 1. Self-reported physical activity and activity frequency

scores of zero. There was no statistically significant variation of mean PA score across subdiagnostic classification [$F(3, 437) = .13$, $p = .94$], and mean PA scores across subdiagnostic classifications were descriptively homogeneous (AS, $n = 118$, $m = 29.01$, $sd = 23.11$; CSLD, $n = 161$, $m = 27.63$, $sd = 18.22$; CSFDIC, $n = 87$, $m = 28.97$, $sd = 21.20$; and CSN, $n = 75$, $m = 28.68$, $sd = 21.53$).

Discussion

More than three years are vested in this directional study of sarcoidosis using a prospective survey that covered demographic information, nutrition, environmental exposures, lifestyle, genetics, and health history. The investigators believe it is the single largest directional survey conducted of this population to date. Sarcoidosis is a systemic granulomatous disease of unknown cause that primarily affects the lungs and an abnormal inflammatory disease process that may affect any organ and tissue of the body. The lungs, the lymph nodes of the thorax and the neck, skin, and the liver are the most often involved. Although the hallmark histological feature of the disease is epithelioid cell granuloma derived from activated T cells and macrophages, the triggering stimuli are unknown but have indicators such as bacterial protein or beryllium metal (2).

Researchers of this study identified four subclassifications: *acute sarcoidosis* (AS) and *chronic sarcoidosis with limited dissemination* (CSLD), while more severe cases include those with *chronic sarcoidosis with full dissemination including cutaneous involvement* (CSFDIC) and *chronic sarcoidosis with neurosarcoidosis* (CSN). The subdiagnostic classification allowed further analysis of the data based on severity and, hopefully, can offer future insight into differential diagnoses, management, and additional research. The authors did not compare pulmonary function data to the four subcategories. That has not proven to be useful with past classifications; however, is an area of interest for future studies based on the results of this study (2). This directional study determined the habits of individuals and utilized four analyses to find statistical significance in the data collected from the surveys of 801 participants. Based on our analysis, seven statistically significant results emerged as indicators of future research needed. Namely, the "Cross Tabulations and the Chi-Square Analyses," the "Cross Tabulations and Odds Ratio Analyses," the "Goodness of Fit and Chi-Square Analyses," and the "Godin and Shephard's (1985)" formula were most valuable. These four statistics resulted in the seven statistically significant findings that gave credence to the four subdiagnostic classifications. These seven statistically significant findings are summarized in Figure 2 that reveals statistics that separates the four subcategories into a dichotomy of most likely and least likely occurrences. Six of the seven relates the frequency percentages, while the Rh D blood type reveals a dichotomy based on opposing (72 vs. -71) residual of observed versus expectancy that proved statistically significant. This study did not attempt to ascertain causality of the subcategories; rather, it focused on determining if these categories were statistically feasible. Moreover, the strength of this directional survey was not robust enough to speculate on causality but serves as a Pilot Study for more focused investigations.

Demographic diversity, including ethnicity, presented two points of interest. Considering this study was of those requesting it, the more than 80% of females in our results exceeds recent and robust studies of around 50% and may indicate a greater felt urgency for resolve in that populous (15, 16). While the demographics of those with sarcoidosis mimic that of the 2010 census for Whites (75.2, 72.4) and African-Americans (12.9, 12.6), it is more striking that African-American representation is two to three

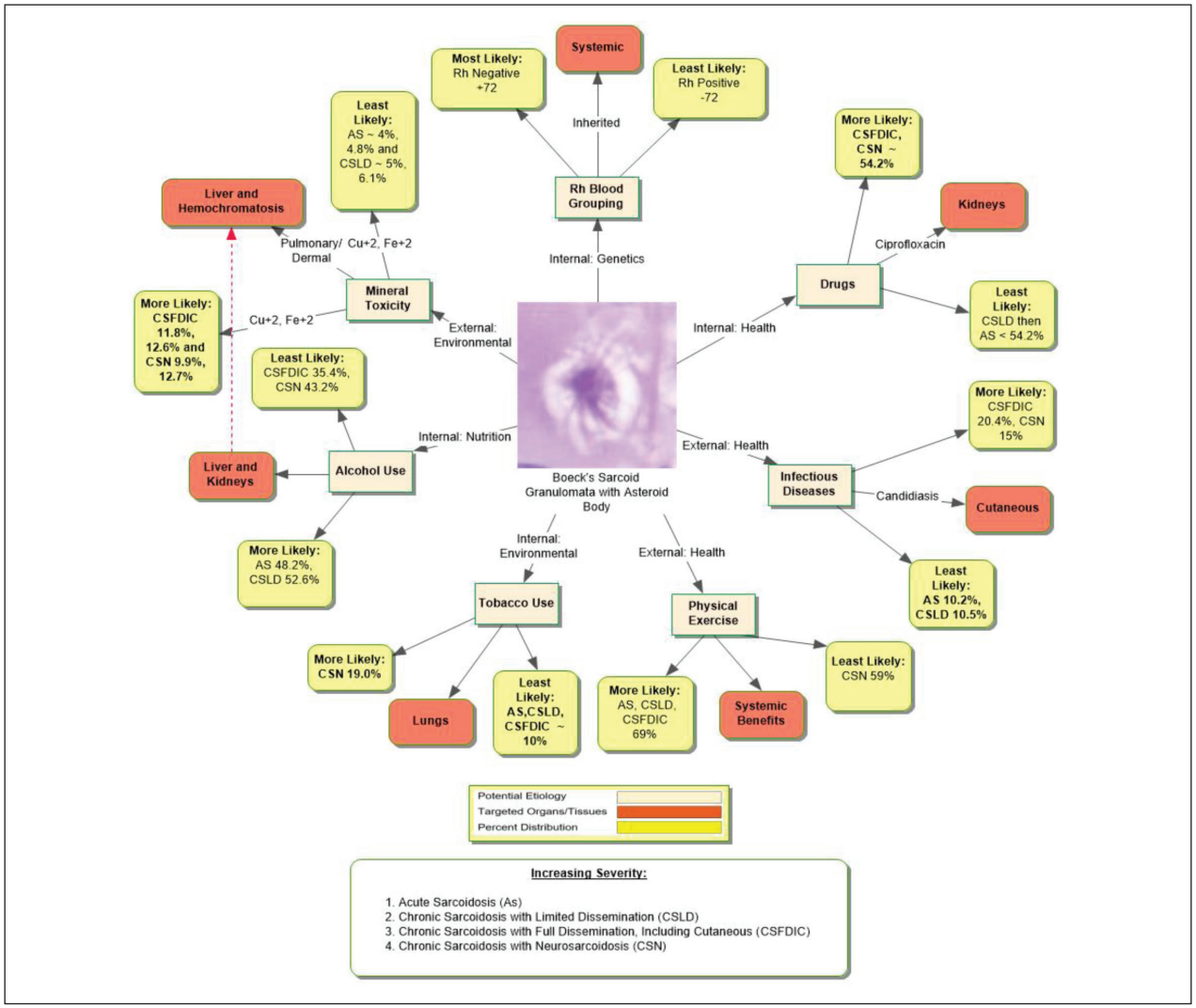

Fig. 2. Seven Statistically Significant Sarcoidosis Linked Dichotomies
*Boeck's Sarcoid – printed by permission of John Lee Sang, M.D., Central Texas Medical Center, San Marcos, Texas

times that of other closed groups such as Mexican Americans and Native Americans (17). It highlights studies demonstrating the disconcerting prevalence among African-American females, and may also reflect their desperation to find a cure (4).

The eating habits of the participants seem like those in the United States, with a greater percentage never missing the last meal of the day and did not reveal any statistical relationship to sarcoidosis. Likewise, 87.4% of the 700 reported nonsmokers is an expected response considering over 85% of people with sarcoidosis have the disease in the lungs. Nevertheless, the subdiagnostic classification with tobacco usage was statistically significant. However, tobacco is the only category that was statistically significant as a subcategory (Table 7) but not at levels of severity, and the acute or chronic onset of the disease (Table 7). Similarly, Gupta et al did not find an effect of tobacco smoke on disease severity in sarcoidosis (19). Perhaps a study with a larger number of participants can shed light on any association between tobacco use and sarcoidosis severity. The link between smoking and chronic obstructive lung disease has been well established. However, there is a dearth of research looking specifically at the impact tobacco use has on sarcoidosis and lung function across acute

and chronic categories. We cannot support the notion that tobacco use is protective against sarcoidosis either. It is known that tobacco affects the immune system among other pathophysiological effects of nicotine toxins and increased serum angiotensin-converting enzyme activity (ACE) tested for in clinical laboratories (20). Whether it is because of the symptoms which mimic COPD or the tendency not to smoke because of the risk of cancer, is not clear. Along with that, the low (5.9%) use of medical marijuana may reflect the same, although some evidence exists for the use of medical marijuana to alleviate the discomfort associated with chronic noncancer pain (21).

On the other hand, alcohol use did not seem to cause an adverse effect. In fact, a null hypothesis of statistical independence revealed that those with AS and CSLD are more likely to use alcohol than would be expected by chance and those with the more severe conditions (CSDIC, CSN) were less likely to use alcohol. Using the same groupings (AS, CSLD versus CSDIC, CSN) provided means of conducting an odds ratio that associated alcohol use with the severity of sarcoidosis. Results indicated that those with less severe cases are more likely to report the use of alcohol than those with more severe cases.

In contrast, environmental exposure presented copper and iron metals with similar statistical significance in the same areas of classification as alcohol, but unfavorable since past research has shown that environmental factors may contribute to sarcoidosis risk. Moreover, researchers associated the risk with individuals who work in occupations with potential metal exposures (22), and association exposure to inorganic particles (16). Workers exposed to beryllium dust or fumes have developed an immune response known as sensitization, a slowly progressive respiratory disease characterized by the formation of lung lesions called granulomas that resemble those found in sarcoidosis patients. These granulomas and accompanying fibrosis impair the lung's ability to expand fully and interfere with the normal gas exchange. Berylliosis has been suggested as a cause of pulmonary sarcoidosis and classified as a type of pneumoconiosis, a systemic granulomatous disease that mainly affects the lungs (17). Exposure to the minerals with statistical significance, copper, and iron, are known to work in tandem in the liver to produce hemochromatosis a condition that causes an iron overload that would affect other organs and tissue (20, 23). Also, high intakes of copper are known to cause gastrointestinal pain, erosion of epithelial cell lining, hemolytic anemia, kidney damage, and death, and therefore cannot be ruled out as contributing factors to sarcoidosis (24). Also, work environments that produce high levels of aerosolized inorganic particles, e.g., wood stoves, fireplaces, talc, human-made mineral fiber, silica dust, intense agricultural activities, continue to be places of higher risk for development of sarcoidosis (25).

Ciprofloxacin, like alcohol, copper, and iron, had similar statistical significance in the same areas of the null hypothesis of statistical independence and those with the more severe conditions (CSDIC, CSN). Unlike those mentioned, ciprofloxacin also has a significant statistical difference in subclassifications of acute and chronic sarcoidosis that revealed increased use of the drug with severity, regardless of the division. Notably, ciprofloxacin is the only category that resulted in a statistically significant difference in all categories. That is, it was statistically significant in subcategorization, levels of severity, and the acute or chronic onset of the disease. Admittedly, when considering the duration of the disease and speculations from the participants that their treatments resulted in other health issues, the relationship between the severity of the disease could be from other drug treatments, requiring additional research. However, ciprofloxacin is a widely used antimicrobial agent to treat bacterial infections of the respiratory and urinary tracts and implicated in cases of induced acute interstitial pneumonitis and hypersensitivity vasculitis (24, 26, 27). Therefore, we surveyed the cohort group to ascertain if ciprofloxacin might have an association with inducing sarcoidosis, and there is a statistically significant relationship between the drug and the severity of the disease.

Furthermore, since the cutaneous effect of candidiasis coincided with this study's greater number of women, where the infection is common, but also the greater number with sarcoidosis, and given its statistical significance (like alcohol, copper, and iron) warrants further investigation (24). In other words, is the drug a stimulus for sarcoidosis and candidiasis? Ciprofloxacin is known to cause an allergic response that results in acute interstitial nephritis (AIN) of the kidney (24). Paone et al. found that kidney granulomas cause interstitial nephritis with an elevated

serum angiotensin-converting enzyme (ACE) activity, and with a bronchoalveolar lavage fluid [BALF] lymphocytes, and a BALF CD4/CD8 ratio could potentially screen for sarcoidosis (28). Also, the fact that increased blood pressure and water retention indirectly results from the ACE activity suggests metabolic influence, and diabetes complication lends to other reasons for investigating the ciprofloxacin sarcoidosis axis.

Genetically, the greatest statistical significance is the Rh blood grouping of all 522 self-reports with an ARC, $\chi^2(1) = 126.128$, $p < 0.001$. This finding suggests the need to investigate the Rh blood group genotypes as the triggering stimuli for sarcoidosis. Specifically, the significantly increased Rh negative grouping suggests that the significantly decreased Rh-positive may have a commonality. To understand the commonality requires a look at the Wiener and Fisher-Race genotype (29). Since the Rh-negative with the greater frequency is "r" or "dce" haplotypes in the respective system, the closest match of Rh-positive with the greater frequency is "R^0" and "Dce" haplotypes respectively in the Wiener and Fisher-Race classification. Therefore, the genotypes of interest are R^0r or Dce/dce, for the Rh-positive participants and the Rh negative is rr or dce/dce respectively for the Wiener and Fisher-Race genotypes.

Concerning physical activity, overall, a statistically significant difference exists between inactivity and activity for those with sarcoidosis. This significance did not carry over to differences between the subcategories. Given that a participant reports engaging in some activity (i.e., the participant's PA score is greater than zero), the four subdiagnostic categories are approximately equal in the mean level of activity reported. Nevertheless, there was a decrease in activity with increased severity, with the first three categories exhibiting the least difference. Strikingly, the analyses together reveal that *chronic sarcoidosis with neurosarcoidosis* (CSN) participants are notably more likely than other participants to report engaging in no physical activity at all.

Because of the four subcategorical classifications (AS, CSLD, CSFDIC, CNS) by the researchers, through four statistical analysis, seven statically significant areas are of interest for future studies. Namely, the association with exercise using the Godin and Shephard's (1985) formula that indicated that those with the most severe cases, CSN, were less likely to engage in physical exercise. Although the other three were roughly the same, they exhibited a decrease in activity with the designated increase in severity. Second; drug use, specifically, Ciprofloxacin association with, third, Candidiasis, and their association with sarcoidosis. Fourth, the association of the seemingly positive effect of alcohol, and fifth, the statistical significance of tobacco use. Sixth, environmental exposure to copper and iron as causative stimuli for sarcoidosis. Seventh, and most significant, the association of sarcoidosis with increased Rh-negative grouping and the significantly decreased Rh-positive and what they have in common.

These seven findings reveal the benefit, if not the need, to standardize the classifications of sarcoidosis. It also indicates that the subclassification in this study is as valid and perhaps simpler than any identified and maintained throughout any single study. A simpler standard classification like the one presented, or the same seems more likely to advance researchers' ability to compare participants within and without the subcategories. Conceivably, such a standard subclassification could lead to other categories within them and assist in definitively proving or disproving causative, the exacerbating, or disabling agents, such as the seven (Figure 2) suggested (especially Rh genetics) having statistical significance in this study.

Acknowledgments

The greatest thanks go to the participants that made this study possible, and their desire for increased awareness and understanding of sarcoidosis. This study resulted from the collaboration of Clinical Laboratory Scientists, Respiratory Therapists, and Statisticians that produced a subdiagnostic classification survey that resulted in seven (Figure 2) statistically significant categories. Special thanks go to Sheridan Limmer, who conducted our initial literature review as a student in our Texas State University Clinical Laboratory Science Program, and upon completion received her Bachelor of Science in Clinical Laboratory Science (BSCLS). She also presented our findings in our State, Texas Association for Clinical Laboratory Science (TACLS), and National, American Association for Clinical Laboratory Science (ASCLS) meetings. Also, thanks go to Gail Ryser, our statistician who did our initial statistics and served as our consultant throughout the research.

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Cigarette smoking and risk of primary systemic vasculitis: a propensity score matching analysis

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Abstract. *Introduction:* Considering limited data about the association between smoking and primary systemic vasculitides (PSV), present study aims to investigate smoking habit in PSV patients compared to healthy subjects as well as to examine the effect of smoking on clinical characteristics, disease activity and disease outcome in PSV patients. *Methodology:* We included 126 patients diagnosed with PSV and 210 age- and sex-matched healthy controls. Demographic and clinical information and smoking history of patients and healthy controls were obtained by direct interview and questionnaire. Individuals who had smoked at least 100 cigarettes in their lifetime before the first symptom of vasculitis were classified as smokers; those who had never smoked or smoked less than 100 cigarettes in their lifetime were categorized as never smokers. Disease activity was evaluated by Birmingham Vasculitis Activity Score (BVAS). Disease outcome was assessed by vasculitis damage index (VDI) and the number of patients with disease in remission. Propensity score matching analyses (PSM) for reducing the heterogeneity between studied groups and calculating the actual effect of smoking in PSV was performed. *Results:* No significant differences were observed in clinical manifestations and disease outcome of patients including VDI and the patients with disease in remission between ever and never smokers. However, disease activity according to BVAS in ever smokers was significantly higher than never smokers (P=0.020). PSM resulted in 82 patients with PSV, and 164 matched healthy persons with similar baseline characteristics. By multivariate logistic regression and after adjustment for age, sex, marital status and educational status, ever smoking was not significantly associated with an increased risk of PSV compared with never smoking. *Discussion and conclusion:* Our study indicated a significant association between disease activity and smoking as well as a non-significant association between the clinical manifestations and disease outcome of PSV with smoking in Azeri population. Although further studies are needed to confirm these preliminary results, it seems that smoking may not be a significant risk factor for PSV. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (3): 243-250)*

Key words: smoking, primary systemic vasculitis, risk factors, propensity score matching

Introduction

Primary systemic vasculitides (PSV) are a heterogenous group of disorders in which inflammation in blood vessel walls develops and leads to mural structures damage and tissue ischemia without a known cause. PSV are relatively uncommon disorders, with annual incidence of 40 to 54 cases per 1 million persons (1). Although pathogenesis of PSV is not completely known, main mechanisms of vascular damage are immune complex deposition, anti-neutrophilic cytoplasmic antibodies (ANCA) (humoral response), and T-lymphocyte response with granuloma formation (cell-mediated) which result in endothelial cell activation, vessel obstruction and dependent tissue

Received: 14 February 2019
Accepted after revision: 29 April 2019
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ischemia (2, 3). Smoking is one of the environmental factors that have an important role in the genesis of aberrant immune response and development of numerous inflammatory diseases (4). Smoking is a well-established risk factor for rheumatoid arthritis (RA), systemic lupus erythematous (SLE), Graves' disease and multiple sclerosis and has also been associated with phenotypic variations in ankylosing spondylitis (5-8). Smoking is also associated with more intensive disease course in inflammatory diseases (8). Ghaussy et al. (9) found significantly higher SLE disease activity index (SLEDAI) in smokers compared to non-smokers. Smoking affects both innate and adaptive immune systems and plays dual roles in modulating immunity by either exacerbation of pathogenic immune responses or weakening of defensive immunity (4). Some proposed mechanisms for the role of smoking in autoimmune diseases pathogenesis are: a) promotion of vascular inflammation by increasing of neutrophil chemotaxis and recruitment of polymorphonuclears, monocytes and macrophages, release of H_2O_2, activation of nuclear factor-κB (NF-κB) and up-regulation of interlukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) (10-15); b) increasing IL-17 expression in the airway mucosa (16); c) increasing proteinase 3 expression by the endothelial cells (17); endothelial cells dysfunction, i.e. alteration in NO dependent vasodilation (18) and increase in endothelin-1 (ET-1) (19); d) inducing Survivin expression (20). Survivin is a multifunctional protein that is required for growth and differentiation of cells (20). Survivin triggers aberrant immune response by increasing of antigen presentation, prevention of autoreactive cells apoptosis and supporting synthesis of autoantibodies (21).

Data on the role of smoking in vasculitis are rare and limited to the subset of Behcet's disease (BD) (22-26), rheumatoid vasculitis (27, 28) and ANCA associated small vessel vasculitis (29-32). According to Turesson et al. (27) study, smoking is an independent risk factor for vasculitis and other types of severe extra articular RA. However, another study reports a lower proportion of active smokers in patients with ANCA associated small vessel vasculitis in comparison with the entire population of Germany (29). In addition, some studies report a positive relationship between smoking and the clinical features of BD (22, 23). However, other studies demonstrate that patients with BD have fewer oral aphthous ulcers (both in number and frequency) during periods of smoking compared to periods of abstinence (24-26). Since there are limited data about the assosciation between smoking and PVS, present study aims to investigate smoking habit in PSV patients compared with healthy subjects, as well as to examine the effect of smoking on clinical characteristics, disease activity and disease outcome in patients with PSV.

Methods and materials

Study design, patient sample, and data collection

This case-control study was conducted from October 2017 to April 2018 at the Connective Tissue Diseases Research Center (CTDRC). We included 126 patients diagnosed with PVS and 210 age- and sex-matched healthy controls. Patients older than 16 years of age who met either the American College of Rheumatology (ACR) 1990 classification criteria (33) or 2012 Chapel Hill Consensus Conference (CHCC) definition (34) for Takayasu arteritis (TAK), giant cell arteritis (GCA), polymyalgia rheumatica (PMR), polyarteritis nodosa (PAN), granulomatosis with polyangiitis (GPA, formerly Wegener's granulomatosis), eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg-Strauss syndrome), IgA vasculitis (IgAV, formerly Henoch-Schönlein purpura), hypocomplementemic urticarial vasculitis (HUV), cutaneous small-vessel vasculitis and central nervous system vasculitis (CNSV) consecutively, were recruited from the outpatient vasculitis clinic of CTDRC. Microscopic polyangiitis (MPA) was an exception because no ACR classification criteria were available. MPA cases were included if they met the 2012 CHCC definition. Because of diversity in the pathogenesis and clinical characteristics of PSV, we divided them to four groups of large vessel vasculitis including GCA, PMR and TAK; ANCA associated vasculitis including GPA, EGPA and MPA; immune complex small vessel vasculitis and cutaneous LCV including IgAV, HUS and CNSV; and medium vessel vasculitis (PAN and undifferentiated vasculitis with medium vessel involvement).

Detailed demographic and clinical information and smoking history of patients and healthy controls were obtained by direct interview and questionnaire. Smoking status was self-reported based on the fol-

lowing questions: 1. Have you ever smoked cigarettes? and 2. Do you smoke cigarettes now?. Those individuals who reported smoking were asked to report the total number of years smoked, and how many cigarettes and packs of cigarettes they smoked per day. They were then classified into the following groups based on their responses to the questions: individuals who had smoked at least 100 cigarettes in their lifetime before the first symptom of vasculitis, were classified as smokers; those who had never smoked or smoked less than 100 cigarettes in their lifetime, were categorized as never smokers; individuals who indicated they had smoked and were currently smoking, were classified as current smokers; and those who reported smoking but did not smoke at the time of data collection, were classified as past smokers. Current and past smokers were classified together as ever smokers for the purposes of this analysis. In case of smokers, smoking duration based on years and the number of smoked cigarettes per day were recorded. Pack-years were calculated as number of packs smoked per day multiplied by the number of years smoked. Patients' ages at the time of disease onset were included in this analysis. The controls were matched to the age that PSV cases had been when they first experienced a symptom referable to vasculitis. The vasculitis duration was calculated from the first symptom attributable to vasculitis. Disease activity at the time of disease diagnosis was measured by Birmingham Vasculitis Activity Score version of 3 (BVAS v.3) (35). Disease outcome at the time of disease diagnosis and in the last visit was assessed by vasculitis damage index (VDI) (36) and the number of patients with disease in remission.

The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (TUOMS) and performed according to the Helsinki humanity research declaration (2008). Furthermore, written informed consent was obtained from all the participants. During the study, all the personal information was kept confidential and other ethical and humanitarian considerations were performed accordingly.

Statistical analysis

Statistical analyses were performed using the SPSS statistical package (SPSS Inc., version 22). Variables were displayed as numbers (percentages), means ± SD or median (Min-Max), as appropriate. Between group comparisons were made by Chi-squared test, Independent-sample t test, or Mann–Whitney U test, as appropriate. Propensity score matching (PSM) analyses for reducing the heterogeneity between studied groups and calculating the actual effect of smoking in PSV was performed. Matching was performed based on demographic characteristics (age, gender, educational status and marital status). For each case with PSV, two healthy persons were selected as the control groups (ratio 2:1). Finally, 82 persons with PSV and 164 healthy unrelated persons analyzed. After propensity score matching, we carried out multivariate analyses with BD as the main outcome variable and smoking history as the main predictor variable to calculate odds ratios with 95% confidence intervals (OR, 95% CI). *P*-value less than 0.05 was considered significant.

Results

One hundred and twenty-six patients with PSV and 214 healthy individuals were enrolled for the purpose of the present study. No significant difference was observed in demographic characteristics of case and control groups (Table 1). PMR/GCA and GPA were the most common type of PSV (Table 2). Constitutional, cutaneous, renal and nervous system symptoms were the most common manifestations of PSV in the studied patients (Table 2). The smoking prevalence (including both past and current smokers) in the PSV group was 21.4% as compared to a 19.5% prevalence in the control group. Difference was not significant. There was no significant difference in the frequency of smoking in the four groups of PSV (Table 3). We compared demographic and clinical characteristics of ever and never smoker PSV patients (Table 4). Ever smokers were older than never smokers. Males were significantly more ever smoker. No significant differences were observed in clinical manifestations of PSV between ever and never smokers. Furthermore, no significant differences were observed in disease outcome of PSV including VDI and the patients with disease in remission between ever and never smokers. However, disease activity according to BVAS in ever smokers was significantly higher than never smokers. No correlation was observed between higher pack years and BVAS (r=-0.181, P=0.386).

Table 1. Demographic characteristics and smoking status of participants

Variables	Patient group (n=126)	Control group (n=210)	*P**
Age (years)	48.67±16.5	49.35±15.3	0.703
Gender			0.296
Female	70 (55.6)	112 (53.3)	
Male	56 (44.4)	98 (46.7)	
Education			0.725
Illiterate	34 (27.0)	51 (24.3)	
Primary school	43 (34.1)	77 (36.7)	
High school	25 (19.8)	45 (21.4)	
University	24 (19.0)	37 (17.6)	
Marital status			0.567
Single	47 (37.3)	71 (33.8)	
Married	79 (62.7)	139 (66.2)	
Smoking status			0.911
Never-smoker	99 (78.6)	169 (80.5)	
Current smokers	22 (17.5)	33 (15.7)	
Past smokers	5 (3.9)	8 (3.8)	
Ever smokers	27 (21.4)	41 (19.5)	
Pack-years of smoking	33.49±15.8	24.21±14.9	0.148

Categorical and quantitative variables were displayed as numbers (percentages) and means ± SD, respectively.
$P < 0.05$ was considered significant.
* *P* values indicate comparison between groups (Independent-sample t test or Chi squared, as appropriate).

Table 2. Clinical characteristics of vasculitis group

Variables	Number (n=126)	Percent
Types of vasculitis		
Polymyalgia rheumatica/giant cell arteritis	28	22.2
Granulomatosis with polyangiitis	26	20.6
Cutaneous small-vessel vasculitis	15	11.9
Undifferentiated vasculitis	15	11.9
Takayasu arteritis	12	9.5
Polyarteritis nodosa	10	7.9
Eosinophilic granulomatosis with polyangiitis	6	4.8
Hypocomplementemic urticarial vasculitis	5	4.0
IgA vasculitis	4	3.2
Microscopic Polyangiitis	4	3.2
Central nervous system vasculitis	1	0.8
Disease duration (months)	37.72±18.1	-
Clinical manifestations		
General symptoms	74	58.7
Cutaneous and mucous membranes involvement	57	45.2
Ophthalmic involvement	10	7.9
Ears, nose and throat involvement	26	20.6
Pulmonary involvement	25	19.8
Cardiovascular system involvement	19	15.1
Abdominal involvement	6	4.8
Renal involvement	40	31.7
Nervous System involvement	41	32.5
BVAS	10.6 (1-36)	-

BVAS: Birmingham Vasculitis Activity Score.
Data were displayed as numbers (percentages), means ± SD or median (Min-Max), as appropriate

Table 3. Smoking status in different groups of PSV in comparison with control group

Variables	Smokers (%)	*P*
PSV group		
ANCA associated vasculitis	11 (30.6)	0.617
Large vessel vasculitis	7 (17.5)	
Immune complex small vessel vasculitis and cutaneous LCV	4 (16.7)	
Medium vessel vasculitis (PAN and others)	5 (20)	
Control group	41 (19.5)	

PSV: primary systemic vasculitides;
Data were displayed as numbers (percentages).
* *P* values indicate comparison between groups (Chi squared).

Table 4. Comparison of demographic and clinical characteristics of PSV patients between ever and never smokers

Variables	Ever smokers (N=27)	Never smokers (N=99)	*P*
Age	54.85±13.9	46.99±16.8	0.028
Sex (Female/Male)	3/24	67/32	0.0001
General symptoms (%)	19 (70.4)	55 (55.6)	0.128
Cutaneous involvement/Mucous membranes (%)	12 (44.4)	45 (45.6)	0.517
Ophthalmic involvement (%)	3 (11.1)	7 (7.1)	0.356
Ears, nose, throat involvement (%)	9 (33.3)	17 (17.1)	0.068
Pulmonary involvement (%)	8 (29.6)	17 (17.2)	0.123
Cardiovascular system involvement (%)	4 (14.8)	15 (15.2)	0.602
Abdominal involvement (%)	2 (7.4)	4 (4.0)	0.389
Renal involvement (%)	11 (40.7)	29 (29.3)	0.201
Nervous system involvement (%)	9 (33.3)	32 (32.3)	0.147
BVAS at the baseline	13.96±8.9	9.69±5.8	0.020
VDI at the baseline	0 (0-3)	0 (0-3)	0.632
VDI at the last visit	0 (0-3)	1 (0-4)	0.595
Patients with disease in remission	23 (33.3)	89 (35.5)	0.540

PSV: primary systemic vasculitides; BVAS: Birmingham Vasculitis Activity Score; VDI: Vasculitis Damage Index.
Data were displayed as numbers (percentages), means ± SD, or median (Min-Max), as appropriate.
P< 0.05 was considered significant.
* *P* values indicate comparison between groups (Chi squared, Independent-sample t test or Mann–Whitney U test, as appropriate)

PSM resulted in 82 patients with PSV, and 164 matched healthy persons with similar baseline characteristics (Table 5). By multivariate logistic regression and after adjustment for age, sex, marital status, educational status and smoking status, ever smoking was not significantly associated with an increased risk of PSV compared with never smoking (Table 6).

Discussion

We performed a case-control study of smoking status among PSV patients and healthy controls. Based on our study, there was no significant difference in smoking status between PSV patients and healthy control group. Furthermore, there was no significant difference in clinical manifestations of PSV as well as disease outcome between ever smokers and never smokers.

There is little information about the risk of vasculitis and smoking. Similar to our study, Lane et al. (32) in a case control study on 74 patients with ANCA associated vasculitis could not find any significant difference in the prevalence of smoking in the vasculitis and control groups. Sessa et al. (31) in a study on 28 patients with ANCA-associated idiopathic systemic vasculitis all of whom had rapidly progressive renal failure, reported that 71% of their non-elderly patients (younger than 59) were heavy smokers which was not consistent with present study. Their study had no control group. They proposed that smoking had deleterious effect on the endothelium of the glomeruli and the renal micro vessels. Also, in a retrospective cohort study, Yamaguchi et al. (30) showed that

Table 5. Demographic characteristics of participants post propensity score matching

Variables	PSV group (n=82)	Control group (n=164)	*P*
Age (years)	46.5±14.2	47.2±11.1	0.711
Gender			0.916
Female	44 (53.4)	93 (56.7)	
Male	38 (46.3)	71 (43.3)	
Education			0.973
Illiterate (%)	25 (30.5)	48 (29.3)	
Primary school (%)	22 (26.8)	45 (27.4)	
High school (%)	18 (22.0)	35 (21.3)	
University (%)	17 (20.7)	37 (22.6)	
Marital status			0.973
Single (%)	29 (34.5)	55 (33.5)	
Married (%)	53 (63.1)	109 (66.5)	
Smoking status			0.904
Never-smoker	63 (75.8)	129 (77.5)	
Ever smokers	20 (23.2)	37 (22.5)	

PSV: primary systemic vasculitides.
Categorical and quantitative variables were displayed as numbers (percentages) and means ± SD, respectively.
P< 0.05 was considered significant.
* *P* values indicate comparison between groups (Independent-sample t test or Chi squared, as appropriate).

Table 6. Multivariate analysis of the association between smoking and PSV post propensity score matching

Variables	OR	95% CI for OR		Sig
		Lower	Upper	
Age (years)	1.005	0.978	1.034	0.713
Gender				
Female	-	-	-	-
Male	0.929	0.479	1.803	0.828
Education				
Illiterate (%)	-	-	-	-
Primary school (%)	0.856	0.328	2.236	0.750
High school (%)	0.985	0.417	2.324	0.973
University (%)	1.152	0.503	2.640	0.738
Marital status				
Married (%)	-	-	-	-
Single (%)	0.887	0.427	1.842	0.747
Smoking status				
Never-smoker	-	-	-	-
Ever smokers	1.063	0.481	2.346	0.880

PSV: primary systemic vasculitides; CI: confidence interval; OR: odds ratio.

smoking was a significant and dose-dependent risk factor for relapse of microscopic polyangiitis (MPA) in Japanese patients (hazard ratio, 7.48; 95% confidence interval, 2.73–21.0). Furthermore, Turesson et al. (27) who reported that smoking was an independent risk factor for vasculitis and other types of severe extra articular RA. In a case control study on 86 RA patients with vasculitis and 172 RA patients without vasculitis, Makol et al. (28) showed that smoking was a risk factor for rheumatoid vasculitis with the odd ratio of 1.98 (CI: 1.10-3.56). In Haubitz et al. (29) study on 197 patients with ANCA associated vas-

culitis in Germany, 14% of patients were smokers at the time of the first disease manifestation, which was significantly lower than the prevalence of smoking in the general German population (24.3% smokers). This difference was seen for both men and women, separately. They proposed that smoking was an environmental factor, which decreased the risk of ANCA associated vasculitis.

Our study did not show any association between smoking and organ involvement. Similarly, Hubitz et al. (29) study did not show any significant difference between smokers, non-smokers, or ex-smokers in disease manifestations, mortality, and development of end stage renal disease and relapse rate of ANCA associated small vessel vasculitis. Furthermore, no significant differences were observed in the clinical manifestations of BD patients in ever smokers and never smokers (37). However, disease activity in ever smokers at disease presentation was significantly more than never smokers (37) which was consistent with present study. Gür et al. (38) in a study on BD patients did not report any association between smoking and articular involvement. Moreover, Bilgin et al. (39) could not show any association between smoking and localization of inflammation in eye, duration and frequency of uveitis attacks in BD. In contrast, Lee et al. (22) showed that the frequency of vascular and gastrointestinal lesions in smokers was significantly more than non-smokers with BD. Another study on patients with BD showed a strong association between smoking with severity of disease and organ involvement (25). In another study, Krause et al. (40) reported that in 3 out of 12 smoker BD patients, smoking exacerbated the oral aphthous ulcers. Discrepancy between various studies might be due to differences in studied population, disease duration, and baseline disease activity as well as type, dosage and duration of medical therapies. Further studies would be required to clarify if and how smoking correlates with clinical parameters in PSV.

The limitations of the present study included the relatively small sample size and that information about the smoking status of the participants was obtained retrospectively and might have changed with the onset of vasculitis. The strengths of our study were the case-control design and the belonging of the patients to a single ethnicity; that was Azeri.

In conclusion, the results of present study indicated a significant association between disease activity and smoking as well as a non-significant association between the clinical manifestations and disease outcome of PSV with smoking in Azeri population. Although further studies are needed to confirm these preliminary results, it seems that smoking may not be a significant risk factor for PSV.

Acknowledgements

The results of this article are derived from the MD (specialty degree in Internal Medicine) thesis of Dr. Babak Alinejati registered in the Tabriz University of Medical Sciences, Tabriz, Iran. The authors wish to thank the Connective Tissue Diseases Research Center of Tabriz University of Medical Sciences for financial support. We would like to express our thanks to Dr. Leila Khabbazi who helped us in editing of the text. The authors wish to thank all the subjects for their participation in this research.

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The role of vitamin K in the etiology of diffuse alveolar hemorrhage

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With great interest, we have read the paper by Alexandre et al. (1).

They investigated Diffuse Alveolar Hemorrhage (DAH) in various disorders and concluded that "DAH appears to be a heterogeneous syndrome". The authors could not define a common pathway for DAH.

We would like to emphasize that in our opinion the underlying disorder is not the cause for DAH. DAH rather needs to be considered as an accompanying symptom. In other words, DAH is in all cases only a symptom associated with the diseases. We suggest that often, the pathophysiological basis for DAH is the same, *viz*. a genetically determined shortness of vitamin K.

We published that DAH in coumarin users in many cases develop idiopathic pulmonary fibrosis (IPF) or nonspecific interstitial pneumonia (2,3). Coumarins, like warfarin are used as anticoagulant. They are generally called vitamin K antagonists because they block the recycling and thus regeneration of vitamin K via inhibition of vitamin K epoxide reductase 1 (VKORC1). The crucial role of vitamin K deficiency as a risk factor or even trigger for fibrosing interstitial pneumonias (IP), was further strengthened by our finding, that patients who used coumarins and had at least one episode of DAH were almost without exception carriers of a *VKORC1* or *CYP2C9* variant allele or both. VKORC1 is important in the recycling of vitamin K. A lower activity of VKORC1 results in a lower regeneration of vitamin K. Coumarins are metabolized by the iso-enzyme CYP2C9 into inactive metabolites. Lower activity of this iso-enzyme will lead to higher levels of the coumarins and thus to more inhibition of VKORC1 by these compounds (4). Low vitamin K activity leads to low coagulation and increases the chance for DAH. The catalytic role of iron in the hemorrhage results in oxidative stress, which is a critical factor in fibrotic processes (5). Relative vitamin K deficiency can cause various health problems, whereas controlled vitamin K supplementation is a well-tolerated treatment (4).

Moreover, we recently described a family with IPF who had *VKORC1* variant alleles in all of the family members who had IPF and had *CYP2C9* variants in all but one (6). Coumarins are not only drugs but are also found in the human diet. Many other xenobiotics are substrates or inhibitors of CYP2C9 and inhibit the breakdown of coumarins. The decreased VKORC1 in combination with decreased CYP2C9 activity thus elicits DAH symptoms (4) and hence IPF.

Although we realize more options are possible to explain the occurrence of DAH in various pathologies, the role of vitamin K deficit should be considered, as the clinical relevance is substantial.

Received: 27 May 2019
Accepted after revision: 29 May 2019
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We recommend in case of unexplained DAH to genotype the patient and supplement with vitamin K when appropriate. Vitamin K supplementation is probably especially relevant for people who frequently have infections (and thus use antibiotics), use oral anticoagulants and/or come into contact with agents that affect coagulation, such as bacteria, fungi, cocaine and dyes.

Acknowledgement

The autors wish to thank dr. Petal Wijnen for carefully reading the manuscript.

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