ISSN 1124-0490 Volume 37 N. 4 - 2020
http://www.sarcoidosis.it

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VASCULITIS AND DIFFUSE LUNG DISEASES

OFFICIAL JOURNAL OF WASOG

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ITS – AIPO - Associazione Italiana Pneumologi Ospedalieri
Via Antonio da Recanate, 2
20124 Milano
Tel. +39 02 36590367
Fax +39 02 67382337
www.aiponet.it
aiposegreteria@aiporicerche.it

Società Italiana Di Pneumologia / Italian Respiratory Society (SIP/IRS)
Via San Gregorio, 12
20124 – Milano – Italy
Tel. +39 0249453331
Fax + 39 0287036090
mail segreteria@sipirs.it
web www.sipirs.it

PUBLISHER
Mattioli 1885 - Strada di Lodesana 649/sx, Loc. Vaio - 43036 Fidenza (Parma)
Tel. +39 0524 530383 - Fax +39 0524 82537 - www.mattiolihealth.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, PubMed/Medline, PubMed Central (Priority Journals)

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (4); e2020009 DOI: 10.36141/svdld.v37i4.10726

Risk and outcome of COVID-19 infection in sarcoidosis patients: results of a self-reporting questionnaire

Robert P. Baughman[1], Elyse E. Lower[1], Mindy Buchanan[2], Paola Rottoli[3], Marjolein Drent[4, 5, 6], Jacobo Sellares[7, 8, 9], Michelle Terwiel[4], Marjon Elfferich[6, 10], Joel Francesqui[7], María Rita Barriuso Cabrerizo[11], Nadera Sweiss[12], Filippo Martone[13], Tamara Al-Hakim[2], Marc A. Judson[14]

[1] University of Cincinnati Medical Center, Department of Medicine, Cincinnati, OH, USA; [2] Foundation for Sarcoidosis Research, Chicago IL, USA; [3] Specialization School of Respiratory Diseases, Dept of Medical, Surgical and Neurological Sciences, Siena University, Italy; [4] ILD Center of Excellence, Department of Pulmonology, St Antonius Hospital, Nieuwegein, the Netherlands; [5] Department of Pharmacology and Toxicology, Faculty of Health and Life Sciences, Maastricht University, Maastricht, the Netherlands; [6] ild care foundation research team, Ede, the Netherlands; [7] Servei de Pneumologia, Respiratory Institute, Hospital Clínic, IDIBAPS, Universitat de Barcelona, Barcelona, Spain; [8] Centro de Investigación Biomedica en Red-Enfermedades Respiratorias (CibeRes, CB06/06/0028), Spain; [9] Interstitial Lung Diseases Core member, European Reference Network for Rare Diseases of the Respiratory System (ERN-LUNG), Spain; [10] Hospital Gelderse Vallei, Ede, the Netherlands; [11] ANES (Asociación de Enfermos de Sarcoidosis). Madrid, Spain; [12] Division of Rheumatology and Medical Director of the Arthritis Clinic and Bernie Mac Sarcoidosis Translational Advanced Research Center (STAR), University of Illinois Chicago, Chicago IL, USA; [13] Amici Contro la Sarcoidosi Italia ONLUS, Italy; [14] Department of Medicine, Albany Medical College, Albany NY, USA

Abstract. *Background:* It has been suggested that sarcoidosis patients, especially those on immunosuppressive medications, are at increased risk for COVID-19 infection and more severe disease. *Methods:* A questionnaire was developed in four languages (English, Dutch, Italian, and Spanish). The questionnaire queried whether patients had been infected with COVID-19 and outcome of the infection. Risk factors for COVID-19 infection were collected. *Results*: A total of 5200 sarcoidosis patients completed the questionnaire with 116 (2.23%) reporting infection and 18 (15.8%) required hospitalization. Increased hazard ratio (HR) for COVID-19 infection were seen for those with a COVID-19 infected roommate (HR=27.44, p<0.0001), health care provider (HR=2.4, p=0.0001), pulmonary sarcoidosis (HR=2.48, p=0.001), neurosarcoidosis (HR=2.02, p<0.01), or rituximab treatment (HR=5.40, p<0.0001). A higher rate of hospitalization was found for those with underlying heart disease (HR=3.19 (1.297-7.855), p<0.02). No other feature including race, other immunosuppressive agent, age, or underlying condition was associated with a significant increased risk for infection or more severe disease. *Conclusion*: The overall rate of COVID-19 was 2.23%, suggesting an increased rate of COVID-19 infection. However, when an analysis of the questionnaires of sarcoidosis and non-sarcoidosis patients was performed in one localized area over this time period, the rate of COVID-19 infection was similar in both groups. Sarcoidosis patients who cohabitated with COVID-19 infected individuals, worked in health care, had pulmonary or neurologic sarcoidosis, or were treated with rituximab had an increased risk for COVID-19 infection. No significant increased risk for hospitalization could be identified based on age, race, gender or any specific immunosuppressive treatment. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (4): e2020009)*

Key words: COVID-19, sarcoidosis, immunosuppression

Received: 29 September May 2020
Accepted after revision: 29 October 2020
Correspondence: Robert P. Baughman MD,
200 Albert Sabin Way, Room 1001,
Cincinnati, OH USA 45267
E-mail: bob.baughman@uc.edu

Supported in part by NIH grant 2UL1TR001425-05A
Supported in part by the University of Cincinnati and Albany Medical Center

Introduction

The COVID-19 pandemic has dramatically changed and challenged the practice of medicine. Both sarcoidosis patients and their health care providers are concerned that sarcoidosis may increase the risk of contracting COVID-19 and may be associated with poor outcomes from COVID-19 infection (1). Sarcoidosis patients may have several risk factors associated with an increased rate and a poor outcome from COVID-19 infection including underlying lung disease and the use of glucocorticoids and other immunosuppressive agents (2;3). However, it is not clear that use of immunosuppressive therapy alone is a risk factor for increased rate of COVID-19 infection (4;5). A worse outcome from COVID-19 infection may be result of the common presence of other co-morbidities including heart disease, diabetes, and hypertension (2;6-8). Because of the potential risk of COVID-19 infection in those receiving immunosuppressive medications, sarcoidosis experts have proposed modifying the treatment of sarcoidosis (9). However, these recommendations were based almost exclusively on expert opinion and extrapolation from other medical conditions because of the absence of sarcoidosis-specific outcome data concerning COVID-19 infection.

Because of the lack of specific information concerning risk of acquiring COVID-19 infection and its outcomes in sarcoidosis patients, we initiated an IRB approved questionnaire survey of sarcoidosis patients between April and July 2020 to investigate the prevalence of COVID-19 infection, clinical outcomes and possible risk factors for contracting COVID-19 in several sarcoidosis cohorts across several countries. The questionnaire was distributed through several platforms and was available in four languages: English, Dutch, Italian, and Spanish. Five versions of the questionnaire that were minimally different (vide infra) were distributed to various cohorts. One version of the questionnaire was distributed to sarcoidosis patients who participated in a previously described registry (10). In order to compare these findings with another high-risk group, we also surveyed a cohort of breast cancer patients during this time period. The results of these five questionnaires were pooled and analyzed to provide data concerning the frequency, severity, potential risk factors and outcomes for COVID-19 infection in sarcoidosis patients.

Methods

A questionnaire regarding COVID-19 infection was developed by three of the authors (RPB, EEL, and MAJ). The questionnaire was approved by the University of Cincinnati Institutional Review Board and is shown in Supplement S-1. IRB approval for the FSR questionnaire was obtained from Advarra (Columbus, MD), where the registry number for the FSR Registry is Pro00008556 and modification number for this sub-study is: MOD00087736.

The questionnaire queried whether patients had been infected with COVID-19. If infected, they were asked to provide details regarding level of care (treated at home, hospital, or intensive care unit). They were also queried concerning risk factors for COVID-19 infection including household contacts and whether they were health care providers. The patients were asked if they had underlying medical conditions associated with increased risk for COVID-19 infection. They were also asked to provide information regarding their age, sex, and self-declared race. They provided their current residence, including their state for those living in the United States. Patients with sarcoidosis were queried about the duration of disease, specific organ involvement, and current and past immunosuppressive therapy. The questionnaire responses were collected in an anonymous manner with no patient identifiers captured. At time of completing the questionnaire, patients were asked to provide consent for use of their questionnaire responses. The distribution was meant to reach as wide an audience as possible. Patients were enrolled without incentives, since the survey was anonymous. Respondents were asked to complete the questionnaire even if they did not have any symptoms related to COVID-19 infection. There was no restriction for patients to complete more than one questionnaire.

A total of five questionnaires were distributed (Supplement S-1 to S-4). Table 1 summarizes the features of the five questionnaires. All were based on the University of Cincinnati/Albany Medical Center (UC/AMC) questionnaire. The same questionnaire was distributed to those patients who were Foundation for Sarcoidosis Research (FSR) registry. The questionnaire was translated into Dutch and distributes by the Dutch Sarcoidosis Society (Sarcoidose.nl) and the ild care foundation. The Italian Asso-

Table 1. Summary of Questionnaires used in Study

Title of questionnaire	Questionnaire version	Invitation distribution	Sarcoidosis patient group	Control group	Dates of Survey	Method of capture	Comments
UC/AMC	UC/AMC English	Posted on FSR website and all patients see at Cincinnati clinic	World wide including Cincinnati OH USA	Cincinnati OH USA	4/1/2020 to 7/1/2020	REDCap	For those in USA, state also indicated
FSR	UC/AMC English	Invitation to those in FSR registry	World wide	N/A	4/6/2020 to 7/1/2020	SurveyMonkey	
Dutch	Dutch version UC/AMC	Members of Sarcoidose.nl, and advertisement at the ILD Center of Excellence, Nieuwegein, the Netherlands	Netherlands	N/A	6/29/2020 to 7/29/2020	SurveyMonkey	Collected also if on no medication for sarcoidosis and patient's BMI
Italian	Italian version of UC/AMC	e-mail contacts of the Italian Association for Sarcoidosis patients (ACSI)	Italy	N/A	4/1/2020 to 7/1/2020	Google Forms	
Spanish	Spanish version of UC/AMC	recruited through membership of the Spanish association of patients with sarcoidosis (ANES-Asociación Española de) using e-mail contact	Spain	N/A	May 2020	Google Forms	

UC/AMC: University of Cincinnati/Albany Medical Center; FSR: Foundation for Sarcoidosis Research; N/A: not available; ild care: interstial lung disease care; BMI: body mass index
REDcap: Research electronic data capture https://redcap.research.cchmc.org/
SurveyMonkey: www.surveymonkey.com
Google Forms: https://www.google.com/intl/en-US/forms/about/

ciation for Sarcoidosis patients (ACSI) prepared the Italian version of the COVID-19 questionnaire. The Spanish association of patients with sarcoidosis (ANES-Asociación Española de) prepared a Spanish version of the COVID-19 questionnaire.

Statistics

The hazard ratio (HR) with 95% confidence interval (CI) was calculated for various factors for each questionnaire using a statistical software package (MedCalc Software limited, Ostend, Belgium). For those questions which were identical (except for language), the results were summed. HR were calculated for the individual questionnaires and for summary data when available. A p value of less than 0.05 was considered significant.

Results

Supplement Figures S1-S5 show the numbers of patients who completed the questionnaire for each site, including those who did not give consent for final analysis or were excluded for other reasons. Table 2 summarizes the values for 5200 sarcoidosis patients who were analyzed. A total of 116 (2.23%) reported COVID-19 infection. The overall rate of COVID-19 infection is shown for each site and ranged from 0.8 to 4.76%. As shown in the table, there was no significant difference in the rate of infection based on sex, race, or age. In the Dutch questionnaire an additional question revealed that 96 out of 973 COVID-19 negative patients (10%) reported COVID-19 symptoms but were not tested.

The results of the individual questionnaires are provided in Supplement S5-S9. Table 3 summarizes

Table 2. Rate of COVID-19 infection for five questionnaires and total

	UC/AMC	FSR	Dutch	Italy	Spain	Total
Total number Sarcoidosis patients	1972	1616	996	511	105	5200
Number COVID positive	66	13	23	9	5	116
Rate of COVID	3.35%	0.80%	2.31%	1.76%	4.76%	2.23%
Percent COVID-19 based on gender						
Male	3.70%	0.72%	1.42%	1.44%	3.57%	2.18%
Female	3.24%	1.59%	2.98%	1.68%	5.19%	2.81%
Percent COVID-19 based on race						
Black	2.88%	3.23%	NA	NA	NA	2.95%
White	3.62%	1.15%	2.31%	1.83%	4.90%	2.66%
Mean age of patients with or without COVID-19 infection						
Age COVID-19 positive, years	54.5 ± 11.39*	54.4 ± 11.29	55.3 ± 6.0	55.8± 8.63	49.80 ± 9.20	53.5 + 9.47
Age, COVID-19 Negative, years	53.0 ± 9.60	56.6 ± 10.39	55.0±10.8	51.8 ± 9.74	44.88 ± 8.82	52.4 + 9.90
Percent COVID-19 infected versus current prednisone therapy						
Yes	3.08%	1.55%	2.02%	0.38%	4.76%	2.68%
No	3.52%	0.62%	2.38%	3.00%	4.76%	2.08%
Percent COVID-19 based on living with COVID-19 infected roommate						
Roommate COVID positive	55.3%	40.0%	26.8%	62.5%	0.0%	52.7%
No roommate with COVID	2.33%	0.68%	1.26%	0.80%	4.95%	1.53%
Percent COVID-19 based on occupation as health care provider						
Health care provider	5.74%	1.20%	3.88%	4.26%	18.75%	5.46%
Not health care provider	3.09%	0.78%	2.13%	1.52%	2.33%	2.82%

NA: not analyzed because less than 10 patients who were this race

*Mean ± standard deviation

Table 3. Hazard ratio for developing COVID-19 infection: Summary of all five questionnaires

	Percent with feature	Total pos	Total neg	Total number	Percent Pos	Hazards Ratio	95% CI	P value
Social factors								
Roommate COVID positive	**1.81%**	**39**	**55**	**94**	**41.49%**	**27.44**	**19.798-38.048**	**<0.0001**
No roommate with COVID		**77**	**5016**	**5093**	**1.51%**			
Health care provider	**8.85%**	**22**	**436**	**458**	**4.80%**	**2.41**	**1.532-3.799**	**0.0001**
Not health care provider		**94**	**4626**	**4720**	**1.99%**			
Current treatment for sarcoidosis								
Current Prednisone								
Yes	**30.93%**	**36**	**1567**	**1603**	**2.25%**	**1.02**	**0.689-1.503**	**>0.10**
No		**79**	**3501**	**3580**	**2.21%**			
If taking prednisone:								
Prednisone >10 mg or more	**22.29%**	**16**	**520**	**536**	**2.99%**	**0.98**	**0.567-1.690**	**>0.10**
Prednisone < 10 mg		**57**	**1812**	**1869**	**3.05%**			
Hydroxychloroquine								
Yes	**9.20%**	**8**	**417**	**425**	**1.88%**	**0.80**	**0.391-1.628**	**>0.10**
No		**99**	**4095**	**4184**	**2.36%**			
anti-TNF monoclonal antibodies (infliximab, adalmumab)								
Yes	**7.89%**	**8**	**389**	**397**	**2.02%**	**0.89**	**0.437-1.812**	**>0.10**
No		**105**	**4531**	**4636**	**2.26%**			
Cytotoxic (methotrexate, azathioprine, mycophenolate, leflunomide)								
Yes	**22.54%**	**27**	**1141**	**1168**	**2.31%**	**1.05**	**0.688-1.615**	**>0.10**
No		**88**	**3926**	**4014**	**2.19%**			
Rituximab								
Yes	**1.28%**	**7**	**53**	**60**	**11.67%**	**5.3993**	**2.621-11.123**	**<0.0001**
No		**100**	**4528**	**4628**	**2.16%**			
Comorbidities								
COPD								
Yes	**11.43%**	**16**	**578**	**594**	**2.69%**	**1.25**	**0.744-2.108**	**>0.10**
No		**99**	**4503**	**4602**	**2.15%**			

(continued)

Table 3 ***(continued).*** Hazard ratio for developing COVID-19 infection: Summary of all five questionnaires

	Percent with feature	Total pos	Total neg	Total number	Percent Pos	Hazards Ratio	95% CI	P value
Diabetes mellitus								
Yes	**10.25%**	**9**	**524**	**533**	**1.69%**	**0.74**	**0.375-1.445**	**>0.10**
No		**107**	**4558**	**4665**	**2.29%**			
Heart disease								
Yes	**10.40%**	**9**	**375**	**384**	**2.34%**	**1.034**	**0.522-2.048**	**>0.10**
No		**75**	**3234**	**3309**	**2.27%**			
Hypertension								
Yes	**19.62%**	**24**	**1003**	**1027**	**2.34%**	**1.07**	**0.686-1.666**	**>0.10**
No		**92**	**4115**	**4207**	**2.19%**			
Organ involvement from sarcoidosis								
Lung								
Yes	**73.09%**	**101**	**3696**	**3797**	**2.66%**	**2.48**	**1.446-4.249**	**0.001**
No		**15**	**1383**	**1398**	**1.07%**			
Cardiac								
Yes	**9.02%**	**15**	**442**	**457**	**3.28%**	**1.5**	**0.878-2.555**	**>0.10**
No		**101**	**4509**	**4610**	**2.19%**			
Neurologic								
Yes	**8.33%**	**18**	**415**	**433**	**4.16%**	**2.02**	**1.234-3.307**	**0.0052**
No		**98**	**4664**	**4762**	**2.06%**			
Demographic features								
Sex								
Male	**32.42%**	**31**	**1420**	**1451**	**2.14%**	**0.7784**	**0.518-1.117**	**>0.10**
Female		**83**	**2941**	**3024**	**2.74%**			
Race								
Black	**32.78%**	**36**	**1399**	**1435**	**2.51%**	**0.9346**	**0.633-1.379**	**>0.10**
White		**79**	**2864**	**2943**	**2.68%**			
Duration of disease								
Sarcoidosis > 5 years	**71.61%**	**75**	**2735**	**2810**	**2.67%**	**1.1012**	**0.713-1.700**	**>0.10**
Sarcoidosis < 5 years		**27**	**1087**	**1114**	**2.42%**			

†Data not available from Dutch registry
CI: confidence interval; anti-TNF: anti-tumor necrosis factor antibody; COPD: chronic obstructive pulmonary disease

the hazard ratio (HR) for developing COVID-19 for all five sites. Forty-one percent of those with a COVID-19 infected roommate had COVID-19 infection (HR=27.44 (19.798-38.048, 95% confidence intervals, p<0.0001)). We did not collect information about which person was diagnosed first with COVID-19. All but the Spanish questionnaire identified a significant increased risk for COVID-19 for those with a roommate with COVID-19. In the Spanish questionnaire, only two patients reported a COVID-19 infected roommate. Neither of these sarcoidosis patients had COVID-19 infection at the time of completing the survey.

The overall risk for health care workers to have COVID-19 infection was 2.41 (1.532-3.799, p=0.0001), with nearly five percent of heath care workers who had sarcoidosis reporting COVID-19 infection. For the Spanish questionnaire, 18.8% of heath care workers reported COVID-19 infection, while less than six percent for all other questionnaires. The HR was only significant for the UC/AMC and Spanish questionnaires.

For sarcoidosis immunosuppressive therapy, rituximab treatment was associated with an increased risk of COVID-19 infection (HR=5.40 (2.621-11.123), p<0.0001). Only the UC/AMC and FSR included more than one patient treated with rituximab. However, both of these identified an increased risk with rituximab use. There was no significant increase in risk for COVID-19 infection for those on any other immunosuppressive therapy. For those receiving prednisone, there was no increased risk for patients prescribed 10 mg or more a day versus a lower dose. A total of 425 patients were prescribed hydroxychloroquine. There was no difference in the risk for COVID-19 among the other questionnaires or for the summary data of all sarcoidosis patients compared to all others. In the Dutch questionnaire, patients who reported to receive any medication for sarcoidosis had a decreased COVID-19 risk (HR=0.40, 0.165 to 0.958, P<0.05). There was no significant difference for the larger UC/AMC questionnaire (HR=1.49, 0.916 to 2.437, p>0.10) or for the combined data.

There was no increased risk with any of the associated with age, race, sex, duration of disease, or the comorbidities investigated. In the Dutch questionnaire, there was no difference in HR for those with a BMI above 25.

There was an increased risk for sarcoidosis patients with lung involvement (HR=2.48 (1.446-4.249), p=0.001). However, the FSR was the only individual questionnaire which identified this as a significant risk. The summary identified neurosarcoidosis as an increased risk factor for COVID-19 infection (HR=2.02 (1.234-3.307), p<0.01). For neurosarcoidosis, there was a significant HR seen for the Spanish and FSR questionnaire and borderline for UC/AMC (p=0.0619).

Most patients responding to the UC/AMC questionnaire provided their country of residence or state of residence if they lived in the United States, and the percentage of patients with COVID-19 infection by residence is shown in Table S-10. There was no significant difference in rates of infections between the United States and non-United States residents. For the United States, there was a wide range but not a significantly different rate of infection between states.

For the UC/AMC questionnaire, we analyzed the risk of COVID-19 infection for two groups of patients seen by either EEL or RPB at the University of Cincinnati from April1 to June 30, 2020. During that time, 547 cancer patients were seen at University of Cincinnati (UC cancer). Two (0.37%) reported COVID-19 infection. Only 3 of 541 (0.55%) sarcoidosis patients seen at the UC sarcoidosis clinic during this time reported COVID-19 infection. The hazard ratio for COVID-19 infection in sarcoidosis patients at the University of Cincinnati was not significantly higher than those with cancer (HR=1.52, 0.254 to 9.041, P>0.10).

Outcome of COVID-19 infection

Table 4 shows the reported rate of hospitalization for those with COVID-19 infection for each questionnaire and for the total group. A mean of 15.8% (range 13 to 27%) of COVID-19 infected patients were hospitalized with about one-third cared for in the intensive care unit at some time during hospitalization.

For 105 of the COVID-19 infected patients we had information regarding current immunosuppressive therapy. Nineteen (18.1%) of these were hospitalized. Table 5 summarizes these outcomes and calculates the HR for hospitalization for various immunosuppressive therapies. There were no

Table 4. Outcome of COVID-19 infected patients

	UC/AMC	FSR	Dutch	Italy	Spain	Total
Home	57	8	20	7	4	96
Hospitalized (%)	9 (17.6%)	3 (27.3%)	3 (13.0%)	2 (22.2%)	1 (20%)	18 (15.8%)
Unknown		2				

Table 5. Outcome of sarcoidosis patients infected with COVID-19 versus immunosuppressive therapy*

DRUG	Home	Hospital	Percent in hospital	HR	95% CI	p
All patients	86	19	18.1%			
Prednisone						
Yes	29	5	14.7%	0.75	0.293-1.901	>0.10
No	57	14	19.7%			
If prednisone						
Prednisone ≥ 10 mg	13	2	13.3%	0.76	0.186-3.106	>0.10
Prednisone < 10mg	47	10	17.5%			
anti-TNF monoclonal antibodies (infliximab, adalmumab)						
Yes	5	2	28.6%	1.65	0.473-5.740	>0.10
No	81	17	17.3%			
Hydroxychloroquine						
Yes	7	1	12.5%	0.67	0.103-4.416	>0.10
No	79	18	18.6%			
Cytotoxic (methotrexate, azathioprine, mycophenolate, leflunomide)						
Yes	17	7	29.2%	1.97	0.873-4.440	>0.10
No	69	12	14.8%			
Rituximab						
Yes	6	1	14.3%	0.78	0.121-5.006	>0.10
No	80	18	18.4%			

*Data not available on all patients.
HR: hazard ratio; anti-TNF: anti-tumor necrosis factor antibody;

Table 6. Outcome of COVID-19 infection versus underlying comorbidities*

	Home	Hospital	Number pos	Rate	HR	95% CI	P
Total							
DM	9	2	11	11.46%	1.19	0.308-4.585	>0.10
no DM	72	13	85				
HTN	14	5	19	19.79%	2.03	0.784-5.234	>0.10
no HTN	67	10	77				
COPD	12	3	15	15.63%	1.35	0.432-4.217	>0.10
no COPD	69	12	81				
Heart disease	8	5	13	13.54%	3.19	1.297-7.855	0.0115
no heart disease	73	10	83				
Any feature	**35**	**7**	**42**	**43.75%**	**1.12**	**0.444-2.854**	**>0.10**
No feature	**46**	**8**	**54**				

DM: diabetes mellitus; HTN: hypertension; COPD: chronic obstructive pulmonary disease; HR hazard ratio.
*Summary data from UC/AMC, FSR, and Dutch

significant HR with any specific therapy. For the 96 COVID-19 infected patients from three questionnaires (UC/AMC, FSR, and Dutch), we were able to analyze the clinical outcome versus presence of diabetes, hypertension, COPD, and heart disease. Table 6 shows the results of this analysis. Only those with underlying heart disease had an increased rate of hospitalization (HR=3.19, 1.297-7.855, p<0.02).

Discussion

In this analysis of five surveys of sarcoidosis patients from the USA and Europe concerning COVID-19 infection, we found evidence that the rate of COVID-19 infection in sarcoidosis patients was higher than in the general population. We found that sarcoidosis patients who were healthcare workers or were living with a person infected with COVID were at higher risk of COVID infection; these data have been reported in the general population (12;13) and support the well-established fact that COVID-19 is highly infectious and is easily transmitted to individuals who are in close proximity to an actively infected person (14). In regard to immunosuppressive therapy, only rituximab was associated with increased risk for COVID-19 infection in sarcoidosis patients. The lack of association of prednisone use with the development of COVID-19 infection held even when comparing ≥ 10 versus < 10 mg/day. In sarcoidosis patients with diabetes, hypertension, heart disease, and co-existing chronic obstructive pulmonary disease no increased risk of acquiring COVID-19 infection was identified, even though these comorbidities have also been identified as risk factors for COVID-19 infection (6;8;15).

The current data surveyed sarcoidosis patients mostly in United States and Europe from April through July 2020. This time frame co-indices with the onset of the pandemic in these two parts of the world. In our analysis of a questionnaire administered to 5200 sarcoidosis patients, 2.23% or 22,308 cases per million had become infected with COVID-19 during this time. During the time period in which this questionnaire was administered, the number of confirmed cases of COVID-19 in the United States was estimated as 1,060 per million (https://en.wikipedia.org/wiki/Template:COVID-19_pandemic_data/United_States_medical_cases). The same site reported that the cumulative rate of COVID-19 infection in Spain was 5197 per 1 million, Italy 3853 per 1 million, and Netherlands 3141 per 1 million. How-

ever, the rate of infection for COVID-19 infection in the general population may be higher. For example, a recent survey using serologic testing found that 2.5% of the Italian population had acquired COVID-19 infection by July 15, 2020 (http://www.salute.gov.it/imgs/C_17_notizie_4998_0_file.pdf). While there was some variation in the rates of COVID-19 infection from the various questionnaires, overall there was no significant difference in the rate of COVID-19 infection. This was also true when examining the UC/AMC questionnaire, which included 1601 (3.1% infected) responders from US versus 361 (4.2% infected) from outside the United States.

These data suggest that the rate of COVID infection is higher in sarcoidosis patients than the general population. However, the rate of COVID-19 infection varies by the time period as well as from country to country and even within the United States. It is therefore possible, that the rate of COVID-19 in sarcoidosis patients was no different from the general population. A significant number of the sarcoidosis patients completing the UC/AMC questionnaire were seen at the University of Cincinnati Sarcoidosis Clinic, which is in southwestern Ohio. As of July 1, 2020, the reported cumulative rate of COVID-19 infection for this area was 5103 per 1 million (https://coronavirus.ohio.gov/wps/portal/gov/covid-19/dashboards/overview). This is similar to the rate of 5545 per 1 million for our sarcoidosis patients and 3656 per 1 million for the cancer patients. The lower rate of COVID-19 infections for sarcoidosis patient at University of Cincinnati clinic versus other patients completing the questionnaires may be due to lower overall rate of COVID-19 infection in the area and/or the more rigorous criteria for diagnosis, since at our clinic we required verification by culture. This criterion may underestimate the number of cases of COVID-19 infection (16). The hospitalization rates for COVID-19 may be more accurate, since such cases are usually confirmed by cultures.

For sarcoidosis patients, we identified five features associated with increased for COVID-19 infection. Sarcoidosis patients with a COVID-19 infected roommate had a greater than 20-fold increased risk for COVID-19 infection. In one meta-analysis, the risk of in home transmission of disease has been estimated as ten-fold (17). There was also a nearly two-fold increased risk for sarcoidosis patients who were health care workers. This increased risk has been noted for some time (12). The higher risk in Spain may be a reflection that some areas were hit sooner than other parts of the world. The widespread use of N-95 and other respiratory policies later in the pandemic and in other parts of the world may have blunted this risk factor (13;18). Comorbidities in sarcoidosis include diabetes, hypertension, heart disease, and co-existing chronic obstructive pulmonary disease (10;19). These have also been identified as risk factors for COVID-19 infection and more severe disease (2;6;8;15). However, in the current study, none of these was associated with an increased risk for infection.

In regards to immunosuppression therapy, only rituximab was associated with increased risk for COVID-19 infection for sarcoidosis patients. This is not surprising, since rituximab has been noted to have increased the risk for acquiring viral infections (20). In addition, viral infections are more severe when patients are receiving rituximab. We studied only seven patients with COVID-19 infection treated with rituximab.

Sarcoidosis is a multi-organ disease and the effect of sarcoidosis on different organs may affect the patient's ability to avoid COVID-19 infection. In this study, we found that patients with lung or neurologic involvement were at increased risk for COVID-19 infection. Chronic lung disease has been identified as a risk factor for infection and more severe disease (21).

In terms of the outcomes of COVID-19 infected sarcoidosis patients, we found that less than twenty percent required hospitalization. In a recent, retrospective study of 37 sarcoidosis patients with COVID-19 infection, the rate of hospitalization for infection was 60% and no different from the non-sarcoidosis patients seen at that center (3). However, the rate of adverse outcome as defined by requiring intubation and/or mortality was significantly higher than the non-sarcoidosis patients (3). The current study would have missed the very severe cases, at least the ones who died, as it was a study analyzing a self-reported questionnaire. In our study, the use of immunosuppressive therapy was not associated with a significant increased risk for hospitalization. The overall outcome of these patients appears more favorable than that reported in rheumatoid arthritis patients treated with immunosuppression (22-24). In one study (23), the use of $\geq$ 10 mg per day

of prednisone or its equivalent was associated with increased risk. That study analyzed 600 COVID-19 infected patients and our study may have been underpowered to detect that difference. Interestingly, that study found that anti-TNF therapy was associated with a significantly lower risk for COVID-19 infection (23).

Hydroxychloroquine has been proposed as a potential therapy for patients with COVID-19 infection and ongoing studies are evaluating this drug (25). Over 400 of our sarcoidosis patients were receiving hydroxychloroquine at time of survey. There was no change in the rate of infection or rate of hospitalization for the seven patients who developed COVID-19 infection while on hydroxychloroquine. This has also been noted in a study of patients treated with hydroxychloroquine for various rheumatologic conditions (23).

Several comorbidities have been associated with a worse clinical outcome from COVID-19 infection (7;8;21;23). We were able to analyze the outcome of 96 sarcoidosis patients with COVID-19 infection and reported comorbidities. Underlying heart disease was associated with an increased risk for hospitalization. Other comorbidities examined included diabetes, COPD, and hypertension were not felt to be significant risk factors.

There are several limitations to our study. The questionnaire did not try to quantitate severity of disease, especially significant pulmonary fibrosis. Therefore, we could not comment on impact of severe lung disease on risk or outcome of COVID-19 infection. Because of the low number of incident cases, we may have been underpowered to detect smaller, but significant risk factors including comorbidities and the impact of immunosuppression therapy. The questionnaires were completed by the patients, usually on-line. With the exception of those seen at the University of Cincinnati, there was no attempt to verify COVID-19 infection. Patients who had severe disease or even died would be unlikely to be able to complete the questionnaire, so this group was underrepresented. Also, the rate of COVID-19 infection in sarcoidosis patients varies based on local conditions and time into the pandemic. The rate of COVID-19 infection in the general population during the study period may be better understood over time, especially as serologic testing becomes more widely used. However, the rate of infection for COVID-19 infection in the general population may be higher. We compared our results to a standard reporting site which provided cumulative rates for various parts of the world. In the sub-study at University of Cincinnati, the rate of infection was not significantly different from cancer patients seen at the same time period. Future reports may provide a better understanding of the rate of COVID-19 infection in the community studied. Future rates may be affected as vaccines become available. Vaccines for other conditions have proved to be effective in preventing infections in sarcoidosis patients (26).

In summary, our data suggests an increased rate of COVID-19 infection in sarcoidosis patients. However, when compared to non-sarcoidosis patients in the same area and time of the study, the rate of COVID-19 infection was not significantly different. The most obvious risk factor for COVID-19 infection was having a roommate with COVID-19. This means that hygiene measures and distancing are extremely important at home as well as in public. To facilitate research on prevalence and risk factors of COVID-19 infection in chronic diseases, sarcoidosis specifically, it would be helpful to report in population registries not only numbers of patients with COVID-19, but also their characteristics, such as comorbidities and medication use.

Acknowledgements

We would like to thank Sarcoidose.nl, the Dutch Sarcoidosis Patient Society and the ild care foundation for preparing and supplying the Dutch version of the questionnaire. For the English version, we thank sarcoidosis patients who participated through the Foundation for Sarcoidosis Research. For the Italian questionnaire, we thank the Amici Contro la Sarcoidosi Italia ONLUS. For the Spanish questionnaire, we thank the Asociación de Enfermos de Sarcoidosis.

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SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (4); e2020020 DOI: 10.36141/svdld.v37i4.7316

Impact and prognosis of lung cancer in patients with combined pulmonary fibrosis and emphysema

Jee Youn Oh, Young Seok Lee, Kyung Hoon Min, Gyu Young Hur, Sung Yong Lee, Kyung Ho Kang, Jae Jeong Shim

Division of Pulmonary, Allergy, and Critical Care Medicine, Department of Internal Medicine, Korea University Guro Hospital, Korea University College of Medicine, Seoul, Republic of Korea

Abstract. *Background*: Combined pulmonary fibrosis and emphysema (CPFE) is frequently associated with lung cancer. However, the impact and outcomes of lung cancer in patients with CPFE are unclear. *Objective:* We investigated the impact of lung cancer in patients with CPFE in terms of acute exacerbation (AE) and mortality, and identified the mortality predictors of patients with CPFE and lung cancer. *Methods*: We retrospectively reviewed 12-year medical records of patients at the Korea University Guro Hospital. Based on computed tomography findings, we selected CPFE patients with and without lung cancer, and analyzed age, sex, smoking status and history, body mass index, past medical history, pulmonary function, the gender, age, and physiology (GAP) score, AE, and mortality. *Results*: Of 227 CPFE patients, 61 were diagnosed with lung cancer. While 10 of the 61 patients experienced AE, 41 died during the observation period. Lung cancer was a significant predictor of AE (hazard ratio [HR] 3.27, 95% confidence interval [CI]1.44–7.43, $P<0.01$) and mortality (HR 4.74, 95% CI 2.55–8.81, $P<0.01$) in CPFE patients. AE, rather than age, GAP score, or lung cancer stage, was the most significant factor associated with mortality in patients with CPFE and lung cancer (HR 9.20, 95% CI 1.13–74.70, $P=0.04$). *Conclusions*: Lung cancer has a significant impact on the outcomes of CPFE and is associated with severe complications. AE was the most important mortality predictor in patients with lung cancer combined with CPFE. Therefore, the diagnosis and treatment of lung cancer should be carefully planned in patients with CPFE. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (4): e2020020)*

Key words: combined pulmonary fibrosis and emphysema, exacerbation, lung cancer, mortality, outcome

Introduction

Lung cancer is the most frequently diagnosed cancer with an increasing prevalence and mortality rate (1). Combined pulmonary fibrosis and emphysema (CPFE) has become an individualized distinct disease consisting of upper lobe predominant emphysema and lower lobe predominant fibrosis, and is characterized by a heavy smoking history (2,3,4). Emphysema and idiopathic pulmonary fibrosis (IPF) are risk factors for lung cancer (5). CPFE, which is associated with smoking, has features of both IPF and emphysema, and could, therefore, be an independent risk factor for lung cancer (6,7). Kitaguchi et al. reported a higher risk of developing lung cancer in CPFE than in chronic obstructive pulmonary disease (COPD) (46.8% vs. 7.3%) (8). Moreover, another recent study also reported a higher incidence of lung cancer in patients with CPFE than in those with IPF (50% vs. 14.5%) (9). The development of lung cancer

Received: 2 January 2019
Accepted after revision: Jae Jeong Shim, MD, PhD
Division of Pulmonary, Allergy and Critical Care Medicine, Department of Internal Medicine, Korea University Guro Hospital, Korea University College of Medicine,
148 Gurodong-ro, Guro-gu, Seoul 08308, Republic of Korea
Tel: +82-2-2626-3029, Fax: +82-2-2626-1166
E-mail: jaejshim@kumc.or.kr

in IPF is associated with poor survival and is associated with an increased incidence of severe complications (10).

In patients with lung cancer, concomitant pulmonary fibrosis and emphysema might be related to poorer survival. It has recently been reported that acute exacerbation (AE) risk is higher in patients with CPFE and lung cancer than in those with IPF and lung cancer(11). Despite the higher incidence of lung cancer and AE risk in CPFE patients, its clinical features, outcomes, and prognosis in these patients remain unclear.

It is important to understand the impact and outcomes of lung cancer in CPFE patients to establish a diagnostic and treatment plan. Therefore, we investigated the outcomes and prognosis of lung cancer in CPFE patients regarding AE and mortality. Furthermore, we aimed to identify mortality predictors in patients with both CPFE and lung cancer.

Materials and methods

Study population and assessment

We retrospectively reviewed the medical records and computed tomography (CT) scans of patients admitted to the Korea University Guro Hospital, Seoul, Korea, from January 1, 2004 to December 30, 2016. Patients were diagnosed with CPFE when criteria for both emphysema and pulmonary fibrosis were met. Emphysema was defined as the presence of well-demarcated areas of decreased attenuation marginated by a very thin (< 1 mm) or no wall, and/or multiple bullae (> 1 cm) with upper zone predominance. Pulmonary fibrosis included reticular opacities with peripheral and basal predominance, honeycombing, architectural distortion, and/or traction bronchiectasis. Diagnosis was confirmed by multidisciplinary discussion of specialists in pulmonology, radiology, and pathology. We excluded patients who (i) did not have CT images and pulmonary function testing and (ii) had occupational interstitial lung disease.

Outcomes and variables measured

AE was defined as a sudden aggravation of dyspnea within 30 days of presentation with new bilateral lung infiltration with no evidence of pulmonary infection or other known causes of worsening respiratory function (12). Baseline clinical parameters were obtained within one month of the initial diagnosis of CPFE. Demographic and clinical data including age, gender, smoking history, body mass index (BMI), comorbidities, and pulmonary function test results, were obtained. For the pulmonary function test, we checked physiological data including the forced vital capacity volume in one second (FEV_1), forced vital capacity (FVC), FEV_1/FVC, total lung capacity (TLC), and diffusing capacity of carbon monoxide (DL_{CO}) at baseline and at one year according to the American Thoracic Society/European Respiratory Society recommendations. The results were expressed as percentages of the normal predicted values. The gender, age, and physiology (GAP) score was calculated using the method described by Ley et al.(13) Data on the histopathological type, stage of disease, treatment regimen, and outcomes were collected for patients with lung cancer. Survival time was defined as the time interval between the date of first diagnosis to death or the last follow-up. AE-free time was defined as the time interval between the date of first diagnosis to AE or the last follow-up.

Statistical analysis

Data are presented as mean ± standard deviation for continuous variables or as percentages for categorical variables. The chi-square and Fisher's exact tests were used for categorical data, and the unpaired t-test and Mann-Whitney U-test were used for continuous data. Univariate Cox proportional hazards models were used to examine the association of selected variables with AE and survival. The multivariate Cox proportional hazards model using the backward elimination method was used for variables found to be significant ($P < 0.1$) in the univariate model. P-values < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS v. 20.0 software (IBM, Chicago, Illinois, USA).

Ethics statement

The present study protocol was reviewed and approved by the Institutional Review Board of the Korea University Guro Hospital (2015GR0150).

Results

Patient baseline characteristics

During the screening period, we identified 5118 patients with emphysema and 1134 patients with pulmonary fibrosis. Of these, 458 patients had both emphysema and pulmonary fibrosis. After a final review based on chest CT findings, 227 CPFE patients were analyzed. The mean age of patients with CPFE was 69.4 years, with a mean BMI of 23.1; 96% of them were men. All patients had a history of smoking (mean: 43.2 pack-years). The mean baseline FEV_1 was 83.0%, mean FVC was 85.2%, and mean FEV_1/FVC was 69.0%. The mean DL_{CO} was 59.3% and the mean GAP score was 3.4. During the observation period, 31 (13.7%) patients experienced AE, 61 (26.9%) were diagnosed with lung cancer, and 60 (26.4%) patients died. Patients with lung cancer were significantly older than those without lung cancer. The baseline characteristics of the patients with and without lung cancer are summarized in Table 1.

Incidence of lung cancer

Lung cancer was identified in 61 patients. Figure 1 shows the cumulative incidence curve of lung cancer in patients with CPFE.

Type, staging, and treatment of lung cancer

The most common histological type of lung cancer was squamous cell carcinoma (n = 32, 52.5%). Fourteen patients had adenocarcinoma, and 12 had small cell carcinoma. Three patients had poorly differentiated carcinoma that could not be classified elsewhere. Table 2 summarizes the staging of cancer and the treatment modalities offered for the study patients.

Table 1. Demographic and clinical characteristics of patients with CPFE, with and without lung cancer

	CPFE with Lung Cancer (n = 61)	CPFE alone (n = 166)	*P*-value
Age, years	71.5±6.7	68.6±9.0	0.02
Male sex	60 (98.4)	158 (95.2)	0.28
Smoking			0.46
Ex-smoker	0 (0)	4 (2.4)	
Current smoker	32 (52.5)	82 (49.4)	
Pack-years of smoking	46.5±19.4	42.0±21.0	0.15
BMI, kg/m^2	23.3±3.3	23.1±3.1	0.70
Paraseptal emphysema	9 (14.8)	38 (22.9)	0.18
Pulmonary function test, % predicted			
FVC	84.8±15.9	84.3±18.4	0.84
FEV_1	83.7±18.1	82.8±19.0	0.74
FEV_1/FVC	68.6±12.6	69.2±13.1	0.79
TLC	95.3±16.6	99.0±17.7	0.19
DL_{CO}	60.0±18.7	59.0±18.8	0.74
GAP score	3.6±1.1	3.3±1.3	0.11
AE	10 (16.4)	21 (12.7)	< 0.01

Data are presented as mean ± standard deviation or number (percentage).
CPFE, combined pulmonary fibrosis and emphysema; BMI, body mass index; FVC, forced vital capacity; FEV_1, forced vital capacity volume in one second; TLC, total lung capacity; DL_{CO}, diffusing capacity of carbon monoxide; GAP, gender, age, and physiology; AE, acute exacerbation.

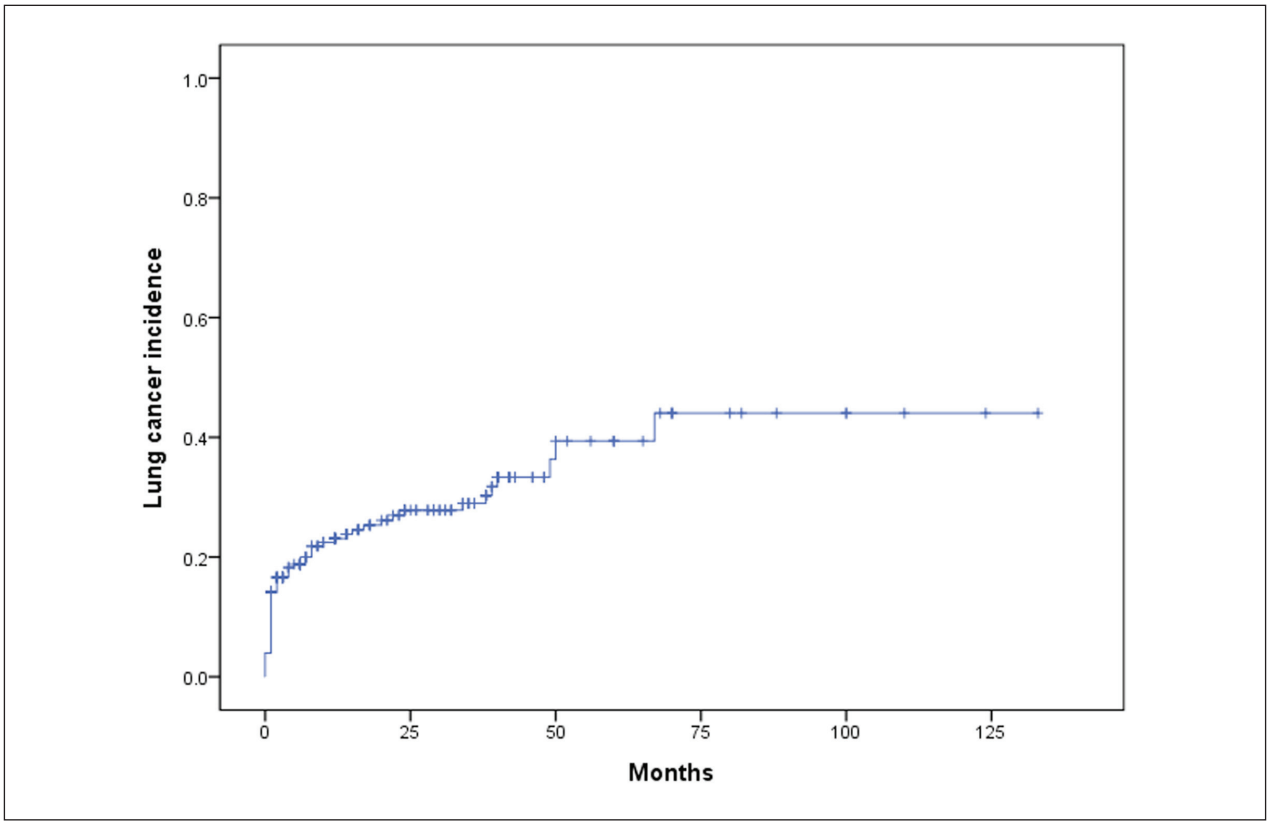

Fig. 1. Cumulative incidence curve of lung cancer in patients with combined pulmonary fibrosis and emphysema Kaplan-Meier analysis revealed that the cumulative incidence of lung cancer at 1 year and 5 years was 21.1% and 26.4%, respectively.

Incidence of AE and impact of lung cancer on AE

Of the 61 lung cancer patients, 10 experienced AE. The estimated median AE-free interval was 96.0 months [95% confidence interval (CI) 55.1–136.9] in patients without lung cancer and 26.0 months (95% CI 2.2–49.9) in patients with lung cancer.

Among the 10 patients who experienced AE, it occurred at 10 days, 4 months and 5 months after lobectomy in three patients. Another patient who underwent lobectomy had a recurrence 8 months later and experienced AE. AE occurred in a patient one month after receiving docetaxel chemotherapy, while it developed in another patient 6 weeks after chemotherapy with erlotinib. Two other patients had AE while receiving supportive care after chemotherapy. AE occurred while being evaluated for recurrence 7 years after lobectomy and adjuvant chemotherapy in one patient and after pericardiocentesis for malignant pericardial effusion in another. On multivariate analysis, lung cancer [hazard ratio (HR) 3.27, 95% CI 1.44–7.43, $P < 0.01$] was found to be a significant predictor of AE after adjusting for significant variables ($P < 0.1$) on univariate analysis (age, presence of lung cancer, FVC, and the GAP score).

Incidence and impact of lung cancer in mortality

Among the 61 patients with lung cancer, 41 died during the study period. The estimated median survival was 96.0 months (95% CI 55.1–136.9) in patients without and 26 months (95% CI 2.2–49.9) in patients with lung cancer.

The causes of death included AE in nine (22.0%) patients, respiratory infections in 20 (48.8%), cancer progression in eight (19.5%), heart failure in one (2.4%), and progression of cancer at a different site in one patient (2.4%). While one (2.4%) patient died on arrival at the hospital, another (2.4%) died after an intracranial surgery for brain metastasis from lung cancer.

Among CPFE patients, lung cancer (HR 4.74, 95% CI 2.55–8.81, $P < 0.01$) was a significant pre-

Table 2. Incidence of AE and mortality in patients with both CPFE and lung cancer according to stage and treatment

	Total (n = 61)	AE (n = 10)	Mortality (n = 41)
Stage			
I	18	3	10
II	6	1	2
III	12	3	9
IV	24	3	20
Unknown	1	0	0
Treatment			
Chemotherapy	23	5	18
Surgery	17	3	10
Radiation	2	0	1
Best supportive care	11	0	8
Surgery + adjuvant chemotherapy	6	2	3
Concurrent chemoradiation	1	0	1
Unknown	1	0	0

AE, acute exacerbation; CPFE, combined pulmonary fibrosis and emphysema.

dictor of mortality after adjusting for factors that were significant ($P < 0.1$) in univariate analysis (presence of lung cancer, FVC, GAP score, and presence of AE).

AE as a mortality predictor in patients with CPFE and lung cancer

Among patients with CPFE and lung cancer, analysis using the multivariate Cox proportional hazards model revealed that AE was a significant predictor of mortality (HR 9.20, 95% CI 1.13–74.70, $P = 0.04$) after adjusting for age, GAP score, and lung cancer stage (Table 3).

Discussion

Our findings indicate that lung cancer is the most significant predictor of poor outcomes, including AE and mortality, in patients with CPFE. AE rather than lung cancer stage was the most significant factor associated with mortality in patients with CPFE and lung cancer.

As a consequence of smoking, lung cancer is common in CPFE, especially among elderly male patients who are heavy smokers. Previous reports have shown that the most common type of lung cancer is squamous cell carcinoma (14-16). Our results are consistent with these reports. The incidence of lung cancer is reported to be 22.4–31.3% in patients with IPF and 6.8–10.8% in patients with COPD (6). Previous studies have shown that CPFE, which has features of both IPF and emphysema, is a more significant risk factor for lung cancer (35.8–46.8%) because of the "triple hit" effect of smoking, emphysema, and pulmonary fibrosis (10). There was a high incidence of lung cancer in patients with CPFE in our study (26.9%), although this is lower than previously reported. The high incidence of lung cancer in CPFE drives the need to evaluate its impact and outcomes in these patients.

Compared to emphysema or fibrosis alone, CPFE has been reported to be a worse prognostic factor for lung cancer (17,18). Usui et al. reported that among patients with lung cancer, those with CPFE had a significantly lower median overall survival and a higher incidence of acute lung injury than

Table 3. Mortality predictors in patients with CPFE and lung cancer

Parameters	HR	95% CI	*P*-value
Multivariate Cox analysis			
Age	0.97	0.73-3.94	0.22
GAP score	1.12	0.79-1.57	0.53
Lung cancer stage			
Stage I–II	ref		
Stage III–IV	1.70	0.73-3.94	0.22
AE	9.20	1.13-74.70	0.04

CPFE, combined pulmonary fibrosis and emphysema; HR, Hazard ratio; CI, confidence interval; GAP, gender, age and physiology; AE, acute exacerbation.

those with emphysema or fibrosis alone (17). CPFE was also a significant, unfavorable prognostic factor in patients with lung cancer after curative resection when compared to patients without CPFE (disease-free survival, HR 2.52; 95% CI 1.24–5.13; $P = 0.01$; overall survival, HR 4.53; 95% CI 1.91–10.70; $P < 0.01$) (18).

Lung cancer is independently associated with a poor prognosis in patients with CPFE. In our study, we found that patients with lung cancer and CPFE have a poor prognosis regarding AE and mortality. These patients had a poor survival comparable to that of lung cancer with IPF (10). Our findings are in line with those of previous studies (11,18-20). In a recent meta-analysis, lung cancer patients with CPFE had a higher 30-day mortality [OR (Odds ratio) 4.72, 95% CI 2.06–10.85, $P < 0.01$], 90-day mortality (OR 5.33; 95% CI 1.39–20.42, $P = 0.01$), and incidence of postoperative complications (OR 5.25, 95% CI 2.38–11.57, $P < 0.01$) (21). Even patients who underwent complete resection at an earlier stage of the disease and had good pulmonary function had a poor postoperative prognosis (18-20). In our study, we confirmed that patients with early stage (I–II) (AE; 16.7%, mortality; 50.0%) and advanced stage (III–IV) lung cancer showed a high incidence of AE (AE; 16.7%, mortality; 80.6%), thereby implying that lung cancer and complications from related procedures have a significant adverse impact on prognosis.

We also found that lung cancer-related mortality was influenced most by AE rather than by cancer stage or lung function. The presence of AE is a well-known risk factor for mortality in both COPD and IPF (22-25). Hata et al. reported that CPFE patients with lung cancer who undergo surgery are at risk of death due to respiratory failure caused by bacterial infection or AE (26). Otsuka et al. reported that among 23 patients with lung cancer and CPFE who underwent surgery, three developed postoperative AEs, which did not affect survival. This could be due to the small sample size of the study (27). However, in our study, we found that survival was significantly affected in patients with lung cancer who underwent procedures or chemotherapy-related AEs. Patients with CPFE often have severe dyspnea and poor cardiopulmonary reserve, and many cannot tolerate invasive procedures (6). Patients with CPFE who are diagnosed with lung cancer often undergo invasive procedures and therapies. These treatment modalities, including surgery, radiation, and chemotherapy, can result in iatrogenic complications and cause mortality. Therefore, procedures and other treatment modalities for CPFE patients with lung cancer should not be as aggressive as for lung cancer patients without CPFE.

Our study has some limitations. Being a retrospective cohort study from a single center, prospective validation is required. However, we believe this study is meaningful because it emphasizes the importance of lung cancer in CPFE and the impact of AE in lung cancer patients with CPFE.

Conclusions

The results of this study suggest that in patients with CPFE, lung cancer is the most significant predictor of poor outcomes, including AE and mortality. AE rather than lung cancer stage was the most significant factor associated with mortality in patients with CPFE and lung cancer. Therefore, the diagnosis and treatment of these patients should be approached cautiously to avoid AE.

Acknowledgments: The authors thank Hwang SY for their excellent statistical support.

Financial support: Supported by Korea University Guro Hospital "KOREA RESEARCH-DRIVEN HOSPITALS" Grant (1400071).

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SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (4); e2020014 DOI: 10.36141/svdld.v37i4.8522

Sarcoidosis: a prospective observational cohort from Northern Alberta

Dilini Vethanayagam[1], Jillian Peters[1], Emad Saad[1], Kimberley Mulchey[1], Ashley-Mae Gillson[1], Brian McNab[1], Maxine Farr-Jones[1], Tomasz Hruczkowski[2], Gregg Blevins[3], Richard Coulden[4], Gavin Oudit[2], Jeremy Beach[5]

[1]Division of Pulmonary Medicine, University of Alberta; [2]Division of Cardiology, University of Alberta; [3]Division of Neurology, University of Alberta; [4]Department of Radiology and Diagnostic Imaging, University of Alberta; [5]Emeritus, Division of Preventive Medicine, University of Alberta

Abstract. *Introduction:* Sarcoidosis is a multi-system disease reported to occur with a higher incidence in Alberta than many other health jurisdictions within and outside of Canada. The reasons for this higher incidence are currently not known. Exposure to beryllium can result in a clinically and radiologically identical disease to sarcoidosis. The purpose of our study was to identify patterns with potential occupational or environmental exposures to beryllium amongst individuals with sarcoidosis in Alberta through a tertiary referral center. *Methods:* A prospective observational study was carried out at the University of Alberta Hospital. Patients with confirmed sarcoidosis (stages 0-4) were recruited from subspecialty clinics (Respirology, Cardiology, Neurology and Occupational Health). A predetermined list of industries thought to involve potentially relevant exposures for the development of sarcoidosis was used to capture current and previous exposure history. Results were entered into a database and where possible verified by comparing with existing electronic medical records (including histories, physical examination, diagnostic imaging and physiology). *Results:* A total of 45 patients were recruited, 25 men and 20 women. Of these, 84% of participants reported working in or being exposed to an industry/environment suspected of contributing to development of sarcoidosis over their lifetime. The most frequently reported exposures were within farming and agriculture (27%), oil and gas (20%), metalworking and handling animals (18%). *Conclusions:* Amongst this cohort, a high proportion reported working with a potentially relevant exposure. Individuals being assessed for sarcoidosis should have their most responsible physician elicit a detailed work and environmental history. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (4): e2020014)*

Key words: sarcoidosis, occupational exposure, environmental exposure, beryllium, epidemiology

Introduction

Sarcoidosis is a multisystem disease that incorporates the pathologic hallmark of noncaseating granulomata. Sarcoidosis affects the lungs in about 90% of patients, but can also affect the eye, heart, nervous system, lymph nodes, skin and other organ systems (1-5). Outcomes can vary widely between patients and symptoms can resolve spontaneously, remain in remission for years, or lead to multisystem organ failure or sudden cardiac death (1-4). Alberta has a population of over 4 million individuals. According to information from the Government of Alberta, rates of this disorder appear to be rising in recent years within Alberta (6). In 2015, the incidence rate for females was 7.8 and 11.2 for males (each per 100,000; not age-standardized). For comparison, the American Thoracic Society

Received: 23 May 2020
Accepted after revision: 15 November 2020
Correspondence: Dilini Vethanayagam, MD, FRCPC
Division of Pulmonary Medicine, University of Alberta
E-mail: dilini@ualberta.ca

Statement on sarcoidosis from 1999 reported incidence rates of 5.9 (men) and 6.3 (women) per 100,000 person years in the United States. Additionally, although some studies of sarcoidosis show a higher prevalence amongst women (2,7), in Alberta rates for women have increased only 33% over a 10-year period whereas rates for men have increased 157% over the same period (6). While no one cause of sarcoidosis has been identified, occupational and/or environmental exposures have been suggested to contribute to the development of this disease. Furthermore, the spectrum of clinical phenotypes has led researchers to believe that there may be more than one exposure that may lead to immunologic sensitization (1).

There has been little success in identifying exactly which exposures may lead to sarcoidosis in which individuals, although beryllium exposure has been shown to cause a disease clinically and radiologically similar to sarcoidosis called chronic beryllium disease (CBD) (8,9). Beryllium is a metal with many useful properties when used as an alloy in a variety of industries, including aerospace, computers, and the oil and gas industry (10). Up to 16% of individuals exposed to beryllium become sensitized through the cutaneous and / or inhalational route (8,9). Inhalation of beryllium particulates can result in the development of a "sarcoid-like illness" which, when biopsied, also reveals noncaseating granulomata. Other exposures which have been suggested as potential links to sarcoidosis, or mortality due to sarcoidosis, include: agricultural dust, wood-burning (11), metalworking, health care and teaching (12,13). Given the recent rise in rates of sarcoidosis in Alberta, the purpose of this study was to identify patterns in occupational or environmental exposures among patients diagnosed with sarcoidosis in this province.

Methods

A prospective observational study was undertaken. Recruitment of patients attending relevant ambulatory clinics in two main tertiary care hospitals in the Edmonton region (University of Alberta Hospital and Royal Alexandra Hospital). The subspecialty clinics all had a pre-existing interest in sarcoidosis, and collaborated in clinical research with the provision of clinical care to patients with this disorder. As opposed to other large studies, such as the ACCESS study (A Case Control Etiologic Study of Sarcoidosis) which only recruited subjects from one subspecialty group (Respirologists) (12), our study broadly evaluated potential subjects to recruit from subspecialty clinics including Respirologists, Neurologists, Cardiologists and Occupational Medicine Specialists and their affiliated clinics. This was reflective of patterns of subspecialty referral for patients in Northern Alberta.

Recruitment was carried out over 12 months (May 2016-May 2017) and included all adult subjects (18 years of age and older) screened through our common electronic medical record. They were approached and willing to participate in the study with biopsy-confirmed sarcoidosis (all radiographic stages), except for those with Löfgren's Syndrome where only a clinical review with classic radiographic findings were sufficient (no biopsy required). Pathologic samples confirmed sarcoidosis in the appropriate clinical context and the presence of typical noncaseating granulomata. Patterns of industries and / or environmental exposures thought to be relevant to the development of sarcoidosis, were identified utilizing prior studies which reviewed similar environmental and occupational exposures (12).

Exclusion criteria comprised active malignancy and/or currently receiving chemotherapy, as well as those expected to die within the next 6 months due to any cause. Additionally, individuals with a history of lung or other solid organ transplant or hematologic transplant were excluded. Active tuberculosis was excluded in all research participants.

All patients with confirmed sarcoidosis attending the relevant clinics were identified prior to their visit through a review of patient files by their primary clinician. Individuals were informed about the study by clinic staff at the time of their attendance through the study research assistant (RA) and asked if they were willing to meet after their clinic visit. If agreed to, the RA met with the subject following their clinic visit and the study procedures discussed with them. After providing informed consent, clinical information was reviewed by the RA with the subject. Participants were then given a list of occupations and industries with potential for exposure to agents associated with an increased risk of sarcoidosis both from expert input and available literature (14). This included lifetime exposures both at the workplace and otherwise

(not limited to just at the time of recruitment). Blood was drawn and stored for biobanking at the time of recruitment for future work. Clinical information including thoracic staging along with other diagnostic imaging studies performed as part of clinical care, along with progress to date was obtained through review of the clinical record. The EQ-5D quality of life index was performed, along with screening for neurologic involvement with the 9-hole peg test (15,16) and Montreal Cognitive Assessment (MoCA) (17).

Study information was collected and entered into the REDCap (20) electronic data capture tools hosted and supported by the Faculty of Medicine and Dentistry at the University of Alberta. Verification of data entered was performed by two individuals (JP, DV) for accuracy. Data were exported from REDCap to Excel (MS Excel, 2016) for further analysis.

Institutional ethics approval was provided by the University of Alberta Research Ethics Board (Study ID: Pro00061653) and operational approval through the University of Alberta Hospital (Alberta Health Services [AHS]). The latter included access for research staff to the AHS supported electronic medical record system, e-Clinician (Epic-based system, Wisconsin).

Results

Overall, 49 patients from the various clinics were approached, one excluded due to coexisting (active) malignancy and three chose not to participate (no particular reason provided). A total of 45 subjects (25 males / 20 females) were recruited for the study. The majority were white Caucasian by self-identified ethnicity (91%). Based on thoracic diagnostic imaging, the majority of recruited subjects were females with stage I disease and males with stage II disease. There was also a significant proportion (27%) who resided in rural and remote communities - outside of urban / suburban areas mainly captured with other large sarcoidosis cohorts previously published.

Available information was gathered from the shared electronic medical record hosted by Alberta Health Services (Epic-based system, Wisconsin). All subjects also had electrocardiograms (ECGs) and echocardiograms reviewed, along with cardiac MR and 24 hour holter monitor tests when available. All subjects had neurologic imaging including computed tomography (CT) Heads and magnetic resonance imaging (MRI) Brains reviewed when available.

Subjects were reassessed at 6 and 12 months. There were no deaths or loss to follow up over the study duration. No adverse change was observed in this cohort during the 12-month time period.

The majority of subjects had sarcoidosis involvement of the respiratory tract (98% of participants). In addition, approximately 1/3 (31%) had cardiac involvement and 1/5 (18%) had neurologic involvement (Table 1). Some patients had active disease; some had dormant disease (latter primarily from the respiratory and occupational medicine clinics). The number of months between a diagnosis of sarcoidosis and study recruitment ranged from 1 month (6 subjects) to 444 months (the latter being a single outlier followed since their diagnosis in the 1970s with inactive disease but significant prior morbidity). The median number of months from diagnosis was 20 months, with 12 subjects (1/4) recruited within 4 months of diagnosis. Whereas all patients with a neurologic presentation had neurological deficits, none of the recruited subjects with other organ system presentation had evidence of neurologic compromise through clinical review and formal bedside neurologic screening tests.

The majority of subjects recruited into this cohort were radiographic stage I and II thoracic disease, as determined by computed tomography (CT) of the chest (Figure 1).

Lifetime industry/occupation and environmental exposure (Table 2) incorporated a variety of areas with *some overlap* – including farming and agriculture (27%), oil and gas (20%), metalworking (18%) and animal handling (18%) constituting the most frequently reported exposures (Table 2). Although farming and agriculture had equal representation of males and females in our cohort, all individuals in the oil and gas industry were males.

Quality of Life as determined by the EQ-5D did not show any difference between baseline and at the 6 and 12 month intervals of follow up utilized in this study (Figure 2).

Discussion

The Sarcoidosis Study evaluated a group of high-

Table 1. Baseline Demographics of Sarcoidosis Recruits

Gender (F/M)		20/25	
Age at recruitment (years)		50.6 +/- 13.6	
Body Mass Index (kg/m^2)		31.5 +/- 6.7	
			% of total
Region of residence	Urban	21	47
	Suburban	12	27
	Rural/remote	12	27
Ethnicity	White	41	91
	South Asian	2	4
	Black	1	2
	Mixed	1	2
Organ systems involved	respiratory	44	98
	cardiac	14	31
	neurological	8	18
	ocular	6	13
	sarcoidosis arthritis	5	11
	skin	4	9

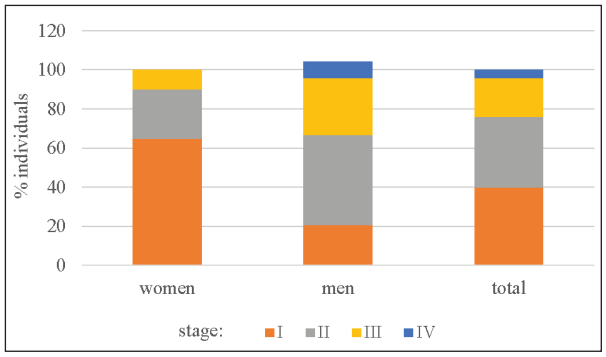

Fig. 1. Thoracic Radiographic Staging

er risk individuals with sarcoidosis in relation to occupational and environmental exposures. We found a striking proportion of individuals with potentially relevant occupational and / or environmental exposures that were missed during their initial clinical assessments. As well, new environmental exposures have more recently been elucidated (26, 27).

In comparison to other recent cohorts from Northern Europe (28, 29), our demographics in Northern Alberta constitute a unique population due to the predominant industries found in this region. The oil and gas industry captured within the list of industries queried was particularly unique in our study, as all positive respondents were male. This may explain some of the unusual provincial demographics that were previously noted through Alberta Health (6). Interestingly, recent work on sarcoidosis that was published from Ontario, Canada has also shown a relative increase in male preponderance spanning two decades (31). This Central Canadian province-wide study utilized health administrative database. It is well known that over the latter half of their study period which was during the economic boom in Alberta, many Ontarians traveled back and forth to work in Northern Alberta, often living in large camps during extended work periods spanning several weeks. Many Ontarian residents have been seen by specialist clinicians at the site of our tertiary referral center for different acute and chronic conditions including sarcoidosis (clinical observation - with only limited specialist follow up feasible). Individuals frequently traveled back to their home of residence (home province of Ontario) during their subsequent extended periods of time away from

Table 2. Lifetime Exposure History by Job Title/Category

Job Title / Exposure Category*	Females (%)	Males (%)	Total (%)
Farming/agricultural work	6 (30)	6 (24)	12 (26.7)
Oil and gas	0	9 (36)	9 (20)
Metalworking	1 (5)	7 (28)	8 (17.8)
Animal handler/veterinarian	4 (20)	4 (16)	8 (17.8)
Construction	1 (5)	6 (24)	7 (15.6)
None of the above	4 (20)	3 (12)	7 (15.6)
Health care sector	5 (25)	1 (4)	6 (13.3)
Lumber/wood products	0	5 (20)	5 (11.1)
Mining	1 (5)	2 (8)	3 (6.7)
Chemical industry	0	3 (12)	3 (6.7)
Stone, clay, glass or concrete	0	3 (12)	3 (6.7)
Metal industry	1 (5)	2 (8)	3 (6.7)
Manufacturing of heating equipment	1 (5)	2 (8)	3 (6.7)
Repairing electrical equipment	0	3 (12)	3 (6.7)
Pulp and paper industry	0	2 (8)	2 (4.4)
Manufacturing of industrial equipment	0	2 (8)	2 (4.4)
Manufacturing of automotive electrical equipment	0	2 (8)	2 (4.4)
Repairing/rebuilding automotive electrical equipment	0	2 (8)	2 (4.4)
Manufacturing/rebuilding of non-electrical vehicle parts	0	2 (8)	2 (4.4)
Armed forces	1 (5)	0	1 (2.2)
Firefighting	0	1 (4)	1 (2.2)

**Adapted from Henneberger, P., et al. (14)*

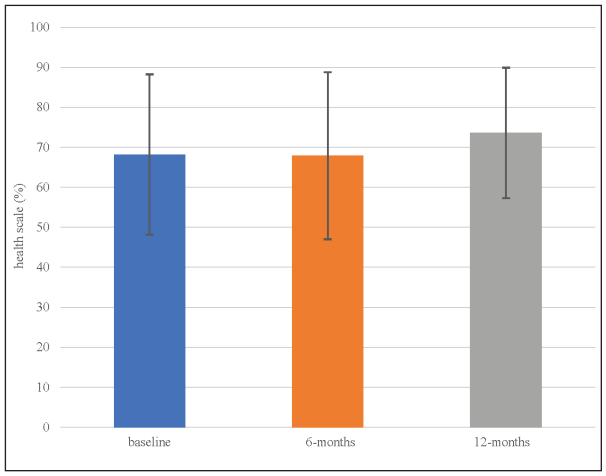

Fig. 2. Patient Quality of Life Status (EQ-5D™) at Baseline and at 6** and 12-Month* Interviews. (*In one subject, 6-month value was used for 12-month data point as no information could be obtained for final interview. **Two additional data points missing from 6-month interviews).

work. However it would have been impossible to capture this work-related information with the methodology used (this health administrative database is not linked to individuals' place of work).

Another recent study was published on the largest cohort of sarcoidosis patients from Belgium to date (31). Of the 234 subjects in this study, a similar male preponderance was also noted (60%), however the industries captured were slightly different than found in our cohort; close to half of the Belgian subjects were white-collar workers (31). The construction and chemical industries appeared to be over-represented in their cohort.

It is not at all surprising that no change in quality of life was noted in our study over the short time frame of monitoring (12 months), as sarcoidosis can present and evolve over many years.

Study limitations included reliance on subject

recall for prior occupational and environmental exposures (however, this would result in more negatives as has been noted in asbestos-related studies) specific to environmental exposures both within and external to the workplace (21-25). However, this is often the case with occupational histories pertinent to respiratory health in many similar studies (22). Inevitably using job title to identify relevant workplace exposures will have led to us missing some accidental exposures for occupations where exposure was not anticipated. However, we believe the approach taken using a standardised list with occupations and industries known to be at risk was most practical in the circumstances.

TB has been noted with noncaseating granulomata and can be difficult to distinguish from sarcoidosis. Therefore a high degree of clinical suspicion is required to exclude this infectious etiology and biopsies require culture to correlate with cytomorphology (32, 33).

Beryllium sensitization has been revealed as a common issue in certain industries, however, the regulatory standards vary significantly between different countries. Within Canada's Provinces and Territories the American Conference of Governmental Industrial Hygienists Threshold Limit Value (ACGIH TLV) is used. The threshold limit value of a chemical substance is believed to be a level to which a worker can be exposed day after day for a working lifetime without adverse effects. The primary tool to objectively assess exposure with subsequent sensitization, based on a detailed history, is utilization of the beryllium lymphocyte proliferation test (8,9,18,19) (BeLPT) either through blood and / or bronchoscopy with bronchoalveolar lavage (BAL). The technical aspects of this assay require centralization of this study to primarily one site (QA/QC) through the National Jewish Hospital (Denver). Further research on this would be supported by better availability of testing for beryllium sensitization. However, this is complex and expensive. Indeed, the collected fresh blood or BAL sample requires processing within twenty-four hours to ensure limitation of falsely negative results, which are otherwise common without strict adherence to transport protocol. An alternative, utilising testing for a genetic polymorphism known to be associated with CBD, and which has already been used in Alberta, can be a valuable diagnostic tool, but is not as specific as a BeLPT (8,9,18,19).

We plan to carry out a prospective trial to more formally evaluate beryllium sensitization when resources are available both for funding and for accurate testing. Genetic associations should be evaluated further, and individuals considered for pre-screening prior to work in high risk occupations.

Imaging techniques utilized in the past for thoracic staging have relied on plain chest radiographs, however the literature supporting this is older. Given new techniques with low radiation exposure, we chose to use low dose high resolution computed tomography of the chest (HRCT Chest) (34, 35) to stage individuals accordingly, as is done within our clinical environments.

Sarcoidosis remains a heterogeneous disease with occupational and environmental factors that need to be taken into account. How assessments are done within clinical settings, particularly within a single payer system as exists in Canada, could include more formal access to Industrial Hygienists or a dedicated nurse. This done within a more structured inter-disciplinary clinic to ensure a more comprehensive assessment of exposures can be captured as part of the patient assessments and better structured patient-oriented care, which will mitigate the many "misses" for important occupational exposures in particular as noted in other occupational lung diseases (36). We need to better understand environmental and occupational exposures related to its occurrence through further prospective clinical trials in larger cohorts. Additionally, more formal inter-disciplinary clinical case conferences to rule out unusual presentations of TB (which can occasionally present with noncaseating granulomata), and drug-induced sarcoid-like reactions which may have inadvertently have been missed, would be important to consider. Without a complete occupational and environmental history being taken for all patients with sarcoidosis, relevant exposures will continue to be missed, and with them opportunities for prevention.

Acknowledgements

Ms. Iris de Guzman for administrative support. Drs. Lisa Maier and Sebastian Straube for manuscript review

Funding Sources: Alberta Health Services, InterMune (investigator-initiated grant), In-kind support from physicians and secretarial services

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SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (4); e2020011 DOI: 10.36141/svdld.v37i4.10136

Clinical Characteristics of Sarcoidosis in Asian Population: A 14-year Single Center Retrospective Cohort Study from Thailand

Athiwat Tripipitsiriwat[1], Chulaluk Komoltri[2], Ruchira Ruangchira-urai[3], Patompong Ungprasert[2]

[1]Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; [2]Clinical Epidemiology Unit, Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; [3]Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Abstract. *Background:* Little is known about epidemiology and clinical characteristics of sarcoidosis in Asian population. *Objectives:* This study aimed to examine the epidemiology and clinical characteristics of Thai patients with sarcoidosis, using databases of a tertiary care medical center. *Methods:* Potential cases of sarcoidosis were identified from two sources, the medical record-linkage system and the pathology database of Siriraj Hospital, Mahidol University in Bangkok, Thailand. Patients with ICD-10-CM codes for sarcoidosis were identified and retrieved from the medical record-linkage system from 2005 to 2018. Patients with histopathology positive for non-caseating granuloma were identified and retrieved from the pathology database from the same time period. All potential cases underwent individual medical record review to confirm the diagnosis of sarcoidosis which required compatible clinical pictures supported by presence of non-caseating granuloma, radiographic evidence of intrathoracic sarcoidosis and exclusion of other granulomatous diseases. *Results:* From 2005 to 2018, 89 confirmed cases of sarcoidosis were identified. 80.9% of them were female and mean age at diagnosis was 46.8 years (standard deviation (SD) 13.9 years). The majority of patients had intrathoracic disease (81 cases; 91.0%) but less than half had respiratory symptoms (34 cases; 41.9%). Extrathoracic disease was common in this cohort that pulmonary sarcoidosis was accompanied by extrathoracic involvement in 53 patients (65.4%). Sarcoid uveitis was the most common extrathoracic disease (35 cases; 39.3%), followed by cutaneous sarcoidosis (24 cases; 26.9%), extrathoracic lymphadenopathy (18 cases; 22.5%) and sarcoid arthropathy (4 cases; 4.5%). *Conclusion:* The current study examined clinical characteristics of sarcoidosis in an Asian population and found high prevalence of uveitis and marked female predominance. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (4): e2020011)*

Key words: sarcoidosis, epidemiology, non-caseating granuloma, uveitis, asian

Introduction

Sarcoidosis is a chronic granulomatous disease of unknown etiology that is believed to be a result of complex interaction between host factors and environmental triggers (1, 2). It is known that epidemiology and clinical phenotype of sarcoidosis are influenced by ethnicity. For instance, the annual incidence of sarcoidosis is as high as 70 per 100,000 population among African-Americans but is as low as 1-2 per 100,000 population among Asians and Hispanics (2-7). Nonetheless, data on clinical manifestations of Asians with sarcoidosis are still relatively limited and most of the previously published studies are from East Asia (4, 5, 8-11). The current study identified cohort of patients with sarcoidosis from medi-

Received: 3 July 2020
Accepted after revision: 20 October 2020
Correspondence: Patompong Ungprasert, MD, MS
2 Wanglang Road, Bangkoknoi, Bangkok, Thailand 10700
Tel. +66 655898155
Fax +66 24113062
E-mail: p.ungprasert@gmail.com

cal record-linkage system of a tertiary care center in Thailand, a country in South East Asia.

Methods

Approval for this study was obtained from the Faculty of Medicine Siriraj Hospital, Mahidol University Institutional Review Board [IRB no. 763/2561(EC2)]. Since there was no direct contact to the patients, the need for informed consent was waived. Potential cases of sarcoidosis were identified from two sources, the medical record-linkage system and the pathology database of the hospital. Patients with International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10-CM) codes for sarcoidosis (D86 – D86.9) were identified and retrieved from the medical record-linkage system from 2005 to 2018. Patients with histopathology positive for non-necrotizing granuloma or non-caseating granuloma were identified and retrieved from the pathology database from the same time period. All potential cases retrieved from either source underwent individual medical record review to confirm the diagnosis of sarcoidosis. Diagnosis of pulmonary sarcoidosis required compatible clinical pictures supported by presence of non-caseating granuloma, radiographic evidence of intrathoracic sarcoidosis and exclusion of other granulomatous diseases, especially tuberculosis. Presence of caseous granuloma is also an acceptable alternative if extensive investigations for other causes of granulomatous inflammation, especially tuberculosis, were negative. The only exception for the requirement of histological confirmation was stage I pulmonary sarcoidosis that required only thoracic imaging evidence of symmetric bilateral hilar adenopathy. This diagnostic approach is in accordance with the guidance from the American Thoracic Society/ European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders (12). Diagnosis of extra-thoracic sarcoidosis was also based largely on compatible clinical pictures and presence of non-caseating granuloma. Exceptions included neurosarcoidosis, cardiac sarcoidosis and sarcoid uveitis because of the challenge with the inaccessibility of the affected organs. Diagnosis of neurosarcoidosis was accepted if the patient fulfilled at least probable criteria as described by Zajicek et al (13). Diagnosis of cardiac sarcoidosis was accepted if the patient fulfilled either the first or second diagnostic pathway as described by the Heart Rhythm Society in 2014 (14). Diagnosis of sarcoid uveitis was accepted if the patient fulfilled at least probable criteria as described by the International Workshop on Ocular Sarcoidosis in 2009 (15).

A standardized case record form was used to record demographics and disease characteristics Descriptive statistics (means, proportions, standard deviation etc.) were used to summarize the data. Analyses were performed using IBM SPSS version 22.0 (IBM Corp, Armonk, NY, USA).

Results

From 2005 to 2018, 89 confirmed cases of sarcoidosis were identified. There was female predominance (80.9%) with mean age at diagnosis of 46.8 years (standard deviation [SD] 13.9 years) and mean follow-up time of 5.4 years (SD 4.5 years). The majority of patients with sarcoidosis in this cohort had intrathoracic disease (81 cases; 91.0%). About half of them had stage I pulmonary sarcoidosis (43 cases; 53.1%), followed by stage II (32 cases; 39.5%), III (5 cases; 6.2%) and IV (1 case; 1.2%). However, less than half of patients with intrathoracic disease were symptomatic (34 cases; 41.9%) with dyspnea and cough being the most common symptoms (25.9% and 22.2%, respectively). The yield of intrathoracic biopsy was fair that histopathology was positive for non-caseating granuloma in 52 of 68 patients (76.5%) who underwent biopsy. There was also a case that hilar lymph node biopsy was positive for caseous granuloma.

Extrathoracic disease was common in this cohort that pulmonary sarcoidosis was accompanied by extrathoracic involvement in 53 patients (65.4%). Isolated extrathoracic disease was observed in 8 patients. Sarcoid uveitis was the most common extrathoracic disease (35 cases; 39.3%; 4 males and 31 females), followed by cutaneous sarcoidosis (24 cases; 26.9%), extrathoracic lymphadenopathy (18 cases; 22.5%) and sarcoid arthropathy (4 cases; 4.5%). Less commonly involved organs included nervous system, kidney, parotid gland, liver, spleen, heart and bone. Calcium was tested in 56 patients and hypercalcemia was seen in 9 of them (16.1%). Biopsy of extrathoracic organs appeared to have a high sensitivity with

21 of 22 skin biopsies and all 8 extrathoracic lymph node biopsies showed evidence of non-caseating granuloma.

A total of 48 patients (53.9%) received at least one systemic treatment during the course of their illness. The most commonly prescribed systemic treatment was oral prednisolone (46 cases; 51.7%) followed by methotrexate (16 cases; 17.9%), azathioprine (8 cases; 9.0%) and chloroquine (3 cases; 3.4%). Topical and inhaled corticosteroids were also frequently used (31.5% and 6.7%, respectively). Information on demographics, disease characteristics, laboratory investigations and treatment of patients with sarcoidosis in this cohort are described in table 1.

Table 1. Characteristics of patient with sarcoidosis in the current study

	Mean or proportion (N = 89)
Age at diagnosis in years (standard deviation)	46.8 (13.9)
Duration of follow-up (standard deviation)	5.4 (4.5)
Male	17 (19.1%)
Intrathoracic disease	
Intrathoracic involvement -Stage I -Stage II -Stage III -Stage IV	81 (91.0%) 43 (53.1%) 32 (39.5%) 5 (6.2%) 1 (1.2%)
Pulmonary symptoms -Dyspnea -Cough -Chest pain	34 (41.9%) 21 (25.9%) 18 (22.2%) 2 (2.5%)
Extrathoracic disease	
Uveitis	35 (39.3%)
Skin	24 (26.9%)
Joint	4 (4.5%)
Nervous system	3 (3.4%)
Kidney	3 (3.4%)
Parotid gland	3 (3.4%)
Liver	2 (2.2%)
Spleen	2 (2.2%)
Heart	2 (2.2%)
Bone	1 (1.1%)
Extrathoracic lymph node	18 (22.5%)
Hypercalcemia	9 out of 56 patients who had at least one calcium level checked (16.1%)
Biopsy (positive for non-caseous granuloma / number performed)	
Intrathoracic	53/68 (77.9%)*
Skin	21/22 (95.5%)
Extrathoracic lymph node	8/8 (100.0%)
Parotid gland	1/1 (100.0%)
Kidney	1/1 (100.0%)

(continued)

Table 1 ***(continued)*****.** Characteristics of patient with sarcoidosis in the current study

	Mean or proportion (N = 89)
Treatment	
Oral prednisolone	46 (51.7%)
Methotrexate	16 (17.9%)
Azathioprine	8 (9.0%)
Chloroquine	3 (3.4%)
Leflunomide	1 (1.1%)
Sulfasalazine	1 (1.1%)
Inhaled corticosteroids	6 (6.7%)
Topical corticosteroids	28 (31.5%)

*One case was positive for caseous granuloma

Discussion

The most prominent findings of this cohort are the high prevalence of uveitis and the marked female predominance. Prevalence of uveitis in this cohort was almost 40% which is far higher than previous reports from Europe and North America that observed the prevalence of around 10%-15% (2, 3, 6, 16). Similarly, two recent studies from China found prevalence of uveitis of only less than 6% (10, 11). Thus, the prevalence of uveitis in this cohort was strikingly high even compared with other non-Japanese Asian cohorts. This could indicate that uveitis is indeed very common among Thai patients with sarcoidosis or it could be a result of referral bias as the cohort was identified from a single tertiary care center.

There was a female predilection for sarcoid uveitis as almost 90% of patients with sarcoid uveitis in this cohort were females, which is consistent with data from other ethnic groups (2, 3, 6, 7). In fact, a study of predominantly white patients from the United States found that male sex was a significant protective factor against development of uveitis with odds ratio of 0.76 (16).

The female-to-male ratio of this cohort was about 4:1 which is much higher than slight female predominance with female-to-male ratio of less than 2:1 in cohorts of white and black patients (1, 2, 6) as well as studies from Korea and Japan (4, 5). Two studies from China showed a slightly higher female-to-male ratio of about 2.5:1 but is still lower than the current cohort (10, 11). The aforementioned high prevalence of uveitis may partially explain this as uveitis is more likely to occur in females with sarcoidosis than males with sarcoidosis.

More than 90% of patients in this cohort had intrathoracic disease although less than half had pulmonary symptoms. This phenomenon has also been observed in other ethnic groups (2, 5, 7). Therefore, it is advisable that thoracic imaging should be obtained when there is a clinical suspicion for sarcoidosis, even in the absence of respiratory symptoms.

Since histopathological confirmation is required to establish the diagnosis of sarcoidosis in most circumstances, selecting the site for biopsy is of clinical importance to physicians to maximize the yield and to avoid potential complications. The current study confirmed the relatively lower yield of transbronchial lung biopsy plus endobronchial biopsy and/or endoscopic ultrasound-guided core needle biopsy of hilar nodes with the false negative rate of about one-fourth of patients, similar to previous studies (17, 18). On the other hand, biopsy of skin rash and extrathoracic lymph node had a sensitivity to detect non-caseating granuloma of almost 100%, possibly due to the better accessibility to this tissue (19).

About half of patients in this cohort were treated with systemic corticosteroids and/or immunosuppressive agents. This is not unexpected as sarcoidosis, especially pulmonary sarcoidosis, can be asymptomatic and spontaneous regression is often observed (20). Nonetheless, the percentage of patients who received systemic therapy in this cohort is in the higher range compared with prior studies, which could be

the result of the clinical phenotype of our patients that had a high prevalence of sarcoid uveitis, an extrathoracic disease that often necessitates systemic immunosuppression.

The retrospective nature of the cohort is the main limitation. It is possible that the true prevalence of extrathoracic involvement could be higher than reported here because some subclinical diseases may not be recognized without dedicated examination and comprehensive laboratory screening. The other concern is the reliance on coding to identify cases of sarcoidosis. As coding error is common, it is possible that some patients with sarcoidosis maybe misclassified in the database (i.e., did not received the ICD-10-CM codes for sarcoidosis). Nonetheless, this study did not rely exclusively on the medical-record linkage system as cases were also identified based on histopathology which may help improving the comprehensiveness of case identification.

Conclusion

The current study described clinical characteristics of sarcoidosis in an Asian population. The most prominent findings that are different from sarcoidosis in other ethnic groups included the high prevalence of uveitis and the marked female predominance.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Credit authorship contribution statement
Athiwat Tripipitsiriwat: Methodology, data curation, formal analysis, writing – original draft, validation
Chulaluk Komoltri: Methodology, writing – original draft, validation
Ruchira Ruangchira-urai: Methodology, data curation, writing – original draft, validation
Patompong Ungprasert: Methodology, data curation, formal analysis, writing – original draft, validation

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SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (4); e2020012 DOI: 10.36141/svdld.v37i4.9966

Predictive value of platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio in patients with hypersensitivity pneumonia

Fatma Üçsular, Gülru Polat, Gülistan Karadeniz, Aysu Ayranci, Merve Keskin, Melih Buyukşirin, Filiz Güldaval, Enver Yalniz

Department of Pulmonology, Health Siences University Dr. Suat Seren Chest Diseases and Surgery Training and Research Hospital, Izmir, Turkey

Abstract. *Aim:* To evaluate Platelet-to-Lymphocyte Ratio (PLR) and Neutrophil-to-Lymphocyte Ratio (NLR) in patients with HP. *Method:* A sample of 140 total patients, 50 having chronic HP and 20 having acute HP, and a control group of 70 more patients were included in this retrospective study conducted with hospital Ethical Committee approval. *Results:* PLR and NLR values were significantly higher in all HP patients than in the control group (p <0.001). In addition, these biomarkers were significantly higher in patients with acute HP than in the chronic HP group (p = 0.017 and p = 0.044, respectively). The cutoff values for PLR and NLR were: (1) 177 (p = 0.020) and 2.76 (p <0.0001) between the HP patients and the control group, and, (2) 110 (p = 0.0054) and 2.15 (p = 0.03), between the acute and chronic HP groups. *Conclusion:* PLR and NLR values are inexpensive and easy parameters that can guide in diagnosing hypersensitivity pneumonia in combination with clinical, radiological and pathology findings.and the acute-chronic differentiation of the disease. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (4): e2020012)*

Key words: platelet-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio, acute/chronic hypersensitivity pneumonia

Introduction

Hypersensitivity pneumonia (HP) is an immune-mediated disease that ocuurs up-on exposure to particles of animal or vegetable origin and/or chemical agents in patients who are shown to be genetically sensitive to those antigens. The severity and duration of the disease and how a patient presents with symptoms in the clinic can vary. The intensity and duration of antigen exposure is dependent upon many factors, including, but not necessarily limited to, the type of antigen and patient specific host factors (1,2). The development of HP disease in patients is not yet completely understood. However, T-cell hyperreactivity and the immune complex-mediated immune response formation are believed to play a significant role. Previous research classified disease progression into three groups:

(1) acute,
(2) subacute,
(3) and chronic (3).

Current thinking is to approach the disease more practically into two groups:

(1) Acute/inflammatory/nonfibrotic HP, and,
(2) Chronic/fibrotic HP.

based on the clinical-radiological-pathological correlation(4).

Received: 31 May 2020
Accepted after revision: 15 November 2020
Correspondence: Gülru Polat
Gaziler St. No: 331 Health Siences University
Dr. Suat Seren Chest Diseases and Surgery Training and Research Hospital E Block Yenişehir/Konak/Izmir
Tel. +90 0 536 834 62 91
E-mail: gulruerbay@yahoo.com

While neutrophil and platelet levels increase in inflammation, lymphocyte levels decrease. Systemic inflammation can be determined using PLR and NLR that are easy and inexpensive to obtain. Many observational studies emphasize that PLR is an inflammatory marker of immune-mediated, metabolic, prothrombotic and neoplastic diseases. It has diagnostic and prognostic value in many diseases (5-8). In those studies, the NLR value was found to be higher in sarcoidosis patients than in the control group and in those patients without extrapulmonary involvement. Research shows NLR may be used as a biomarker in determining the progression in sarcoidosis (9). Another research also showed that increasing PLR can facilitate sarcoidosis diagnosis, and in gauging the extent of lung involvement (8). However, there is little research on the use and value of PLR and NLR ratios in diagnosing or predicting the progression of hypersensitivity pneumonia. The purpose of this study is to evaluate the diagnostic value of these inflammatory serum markers in hypersensitivity pneumonia.

Materials/Methods

Patients

This study was conducted using retrospective patient data from a tertiary teaching, high bed capacity hospital for the period between August 2013 through December 2017. This study included 70 HP patients.

All clinical and radiologic data together with a full occupational, exposure, smoking, family and drug history were collected. Lung biopsies were examined in the Department of Pathology.

70 healthy individuals who applied to our outpatient clinic were included as the control group. Patients exhibiting the following symptoms were included in the acute HP subgroup:

(1) Patients with a symptom duration of less than 6 months,
(2) upper-middle zone weighted GGO,
(3) Patients having acentrilobuler nodule, mosaic attenuation, air trapping or consolidation on HRCT

Patients exhibiting the following symptoms or pathology were included in the chronic HP subgroup:

(1) patients with symptoms longer than 6 months,
(2) patients having upper middle zone-weighted fibrosis; and,
(3) patients with honeycomb, mosaic attenuation, air trapping and centrilobular nodules.

In all patients definitive diagnosis reached by multidisciplinary diagnosis(MDD), confirmed by guidelines (10)

Patients presenting with following diseases which can affect inflammatory proocesses and bleeding were excluded from the study:

(1) rheumatoid arthritis,
(2) vasculitis,
(3) inflammatory bowel diseases,
(4) hematologic diseases,
(5) chronic respiratory diseases,
(6) malignancies,
(7) autoimmune diseases,
(8) chronic heart diseases, and,
(9) anyone having had a blood transfusion in the last 3 months.

Collection of Blood and Inflammatory-Marker Data

Complete blood counts were determined by spectrophotometry/impedance (Beckman Coulter LH

780 Analyzer; Beckman Coulter, Inc., CA, USA). Venous blood samples were collected and placed into ethylenediaminetetraacetic acid tubes. The following hematological parameters were recorded at the time of diagnosis:

(1) leukocyte count,
(2) neutrophil count and percentage,
(3) lymphocyte count and percentage,
(4) hemoglobin,
(5) Hematocrit levels,
(6) mean corpuscular volume,
(7) red cell distribution width,
(8) Platelet counts,
(9) mean platelet volume (MPV),
(10) platelet distribution width,
(11) NLR, and,
(12) PLR.

The NLR and PLR were defined as follows:

- NLR = neutrophil count divided by the lymphocyte count, and,
- PLR = platelet count divided by the lymphocyte count.

C-Reactive Protein (CRP) was determined by the turbidimetric method (Cobas c 702, cobas 8000 ISE; Hitachi High-Technologies Corporation, Tokyo, Japan). Peripheral blood was obtained at the time of diagnosis. Electron Spin Resonance (ESR) was determined by spectrophotometric method (Alaris, ALS 100, Auto ESR Analyzer, Turkey).

Pulmonary Function Tests (PFTs)

Pulmonary function data was collected by performing PFTs using a ZAN 300 device (ZAN Messgerate, Oberthulba, Germany), in the sitting position. The highest value of Forced Expiratory Volume in 1 second (FEV1) and Forced Vital Capacity (FVC), was recorded after patients performed at least three technically satisfactory maneuvers differing by less than 5%.

Statistical Analysis

Statistical analysis was performed using 'SPSS 18.0 for Windows'. The means, standard deviations, medians and the minimums and maximums were calculated. Normality was evaluated using the Kolmogorov–Smirnov test. Comparison of results between groups was performed using Analysis of Variance (ANOVA) for parametric results and the Kruskal–Wallis test for nonparametric results. For ANOVA values with $p < 0.05$, an unpaired Student's t-test was used for parametric results. The Mann–Whitney U-test for nonparametric results was used for pair-wise comparisons. Categorical comparisons were performed using the Chi-squared test. Relationship between the parameters were evaluated using the Spearman Rank Correlation test for normally distributed results. The Pearson Correlation coefficient was calculated for abnormally distributed results. The predictive significance of the NLR and PLR was analyzed by graphically plotting Receiver Operating Characteristic (ROC) curves. Sensitivity, specificity and likelihood ratios were calculated using different cutoff values.

Ethics

This study was designed and performed in accordance with The Helsinki Declaration and good clinical practices, and approved by our hospital Ethics Committee.

Results

Of the 70 HP patients, the mean age was 58.9 with a standard deviation of 13.4. Females represented 52.9% (n=37) while 47.1% (n=33) of our patients were males. Half of the patients (n=35) were smokers. History of contact with birds was determined for 39 (55.7%) patients. 20 (28.6%) patients were found to have exposure to organic antigens. 11 (15.7%) patients had other antigenic exposure. The most common symptoms were cough and dyspnea. HRCT findings in 50 (71.4%) of patients revealed predominant ground glass opacity associated with other pathologies.

Traction bronchiectasis was detected in 43 (61.4%), , honeycomb 25 (32.9%), centrilobuler nodule 17 (24.3%) and mosaic attenuation in 16 (22.9%) patients. Upper-middle-lower zone involvement was seen in 25 (35.7%), upper-middle zone involvement was 7 (10.0%), middle-lower zone involvement was 25 (35.7%) and lower zone involvement was seen in only 13 (18.6%) patients. Pathological diagnosis was used to confirm the diagnosis in 43(61%) patients. Medical thoracoscopy was used for obtaining a lung biopsy in 23 (32.8%) patients.Transbronchial lung biopsy (TBLB) was used for 15 (21.4%) patients. Video Assisted Thoracoscopic Surgery (VATS) was used for 5 (7.1%) patients

Comparison of the hemogram parameters of the 70 HP patients with the 70 patients in the control group is shown in Table 1. In comparison with the control group, PLR ($p = 0.000$) and NLR ($p = 0.000$) values were significantly higher in all HP patients. Also leucocyte, neutrophil, PLT count, MCV, MPV, RDV and PDW levels were significantly higher in patients with HP than in the control group. In the ROC analysis between patients with HP and the control group:

(1) the PLR cutoff was 177, sensitivity 32.8%, specificity 95.7%, AUC 0.61 (0.51-0.70) ($p = 0.020$), and,
(2) the NLR cutoff was 2.76, sensitivity 52.8%, specificity 82.8%, AUC 0.72 (0.63-0.80) ($p < 0.0001$) (Figure 1).

50 patients (71.4%) were diagnosed as being in the chronic HP subgroup. 20 (28.6%) were placed in the acute HP subgroup.

Comparative demographic data and laboratory findings of acute and chronic HP patients are given

Table 1. Comparison of hemogram parameters in Hypersensitivity Pneumonia patients and the patient control group

	HP(n:70)	Control(n:70)	p value
Age (years) mean (min;max)	63 (18 ; 82)	58 (24 ; 77)	0.12
Leucocyte (×10³/μl) (min;max)	9 (3.8 ; 24.1)	7 (4.4 ; 10.9)	0.000
Hb(gr/dl)	13.7 ±1.9	13.9±1.1	0.49
Hct(%)	41.2±5.4	41.6±3	0.51
MCV(fl)	84.5±6.2	86.6±4.5	0.025
RDW(fl)	14.2 (10.2 ; 20)	13.3 (11.2 ; 18.6)	0.000
PLT(×103/μl)	270 (122 ;742)	245 (149; 393)	0.016
MPV(fl)	8.5±1	9.1±1	0.001
PDW(fl)	16.7 (11.5 ;19)	16.5 (9.4 ;22)	0.012
Neutrophile (×10³/μl) (min;max)	5.5 (2.2 ;20.7)	4.1 (2.2 ;6.9)	0.000
Lymphocyte (×10³/μl) (min;max)	2.1 (0.8 ;4.5)	2.1 (1.3 ;3.6)	0.41
NLR	2.8 (0.9 ;18.8)	1.9 (0.6 ;3.9)	0.000
PLR	129 (37.8 ;573.4)	114 (50.8 ;220)	0.021

Hb: Hemoglobin;Hct:Hematocrit; MPV: Mean platelet volume; MCV: Mean corpuscular volume; NLR: Neutrophil-to-lymphocyteratio; PDW: Plateled distribution width; PLR: Platelet-to-lymphocyte ratio; PLT: Platelet; RDW: Red cell distribution width.

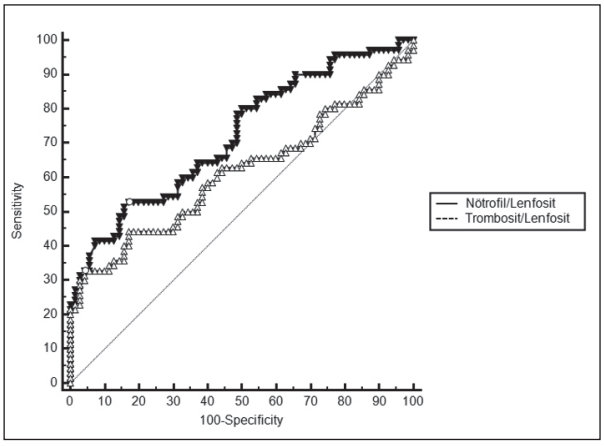

Fig. 1. ROC analysis for PLR / NLR values of all patients with hypersensitivity pneumonia and the control group.

in Table 2. When acute and chronic HP cases are compared; the mean age was significantly higher in the chronic group (p = 0.006). Steroid treatment was given to 30 of the 70 patients (42.8%). Of those 30 patients, 20 (66.7%) of the treated subjects were chronic HP and 10 (33.3%) were acute HP. In the acute HP group, PLR (p = 0.017) and NLR (p = 0.044) values were significantly higher than the chronic group. In the ROC analysis between the chronic HP and acute HP:

(1) the PLR cutoff was 110, sensitivity 44%, specificity 90%, AUC 0.68 (0.56-0.79) (p = 0.0054), and,
(2) the NLR cutoff was 2.15 sensitivity 42%, specificity 85%, AUC 0.65 (0.53-0.76) (p = 0.03) (Figure 2).

NLR was correlated with ESR, PLR, MCV, RDV and PDW, and p = 0.03, p<0.0001, p = 0.013, p = 0.017 and p = 0.028, respectively. PLR was also correlated with ESR, NLR, MCV, MPV and PDW, and p = 0.04, p<0.0001, p = 0.001, p = 0.001 and p<0.0001, respectively.

Discussion

In this study, PLR and NLR, leucocyte and neutrophile count, PDW levels were significantly higher in patients with HP than in the control group. PLR and NLR were significantly higher in patients with acute HP than those having chronic HP.

Previous studies showed that NLR and PLR increased in sarcoidosis, COPD, pulmonary embolism,

Table 2. Demographics of chronic and acute HP patients

	Chronic HP n: 50	**Acute HP n: 20**	**p value**
Age (years)(min;max)	65 (18 ;82)	52 (22 ; 71)	0.006
Gender **Male** **Female**	 29 (%58) 21 (%42)	 4 (%20) 16 (%80)	0.009
Cigarette **Non-smoker** **Smoker** **Ex-smoker**	 22 (%44) 5 (%10) 23 (%46)	 13 (%65) 2 (%10) 5 (%25)	0.242
BMI	27.3 (15.1; 41.5)	27.4 (18.3; 44.2)	0.98
FEV1% **FVC %** **FEF25-75 %**	74 (39;135) 69 (34;120) 88 (22;181)	65.5 (33;114) 65.5 (45;94) 70 (13;117)	0.08 0.25 0.024
DLCO%	44.5 (3;114)	39.5 (18;102)	0.71
ESR(mm/h)	21.5 (8;93)	27.5 (8;74)	0.19
CRP(mg/dl)	0.75 (0.05;23)	1 (0.05;3.8)	0.58
Hb(gr/dl) **Hct(%)**	14.3 (10.2 ;17.2) 42 (31;52)	13.5(7.9;16.5) 39.9 (24;48)	0.04 0.10
MCV(fl)	86 (73;96)	82 (60;92)	0.016
RDW(fl)	14 (10;17)	14.4 (13;20)	0.15
PLT(×10³/μl)	265 (122;567)	313 (217;742)	0.06
MPV(fl)	8.4 (6.4;12.6)	8.4 (6.8;10.7)	0.90
PDW(fl)	16.8 (11.5;19)	16.6 (13.5; 17.8)	0.53
Neutrophile(×10³/μl)(min;max)	5.3 (2.2;20.7)	6.1 (2.4; 16.2)	0.32
Lymphocyte(×10³/μl)(min;max)	2.2 (0.8;4.5)	1.8(1.1;3)	0.19
PLR	124 (37;573)	176 (85;352)	0.017
NLR	2.7 (0.9; 18.8)	3.5 (1.7;10.8)	0.04

CRP: C-reactive protein; DLCO: Diffusing capacity of carbon monoxide; ESR: Erythrocyte sedimentation rate; FEV1: Forced expiratory volume in 1 s; FVC: Forced vital capacity;Hb:Hemoglobin; Hct: Hematocrit; MPV: Mean platelet volume; MCV: Mean corpuscular volume; NLR: Neutrophil-to-lymphocyteratio; PDW: Platelet distribution width; PLR: Platelet-to-lymphocyte ratio; PLT: Platelet; RDW: Red cell distribution width, BMI: Body mass index.

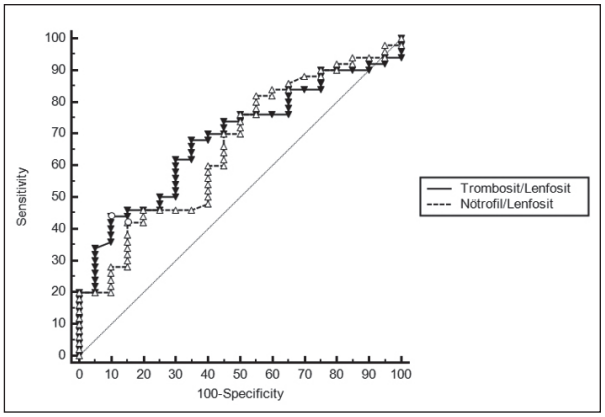

Fig. 2. ROC analysis for PLR and NLR values in patients with chronic and acute HP

lung cancer, and inflammatory / rheumatological diseases. This is associated with poor prognosis (11-14). Various cells have been investigated in serum and BAL samples in hypersensitivity pneumonia patients (15,16). Our study is the first to evaluate PLR and NLR in HP patients.

Yalnız et al. found PLR and NLR significantly higher in all sarcoidosis patients compared to the control group. They found these biomarkers significantly higher in sarcoidosis patients (stage 2,3,4) with parenchymal involvement compared to those without parenchymal involvement (stage 0,1). In predicting the diagnosis of sarcoidosis, they determined the cutoff value for PLR and NLR as 158 (wıth

57% sensıtıvıty and 93% specıfıcıty and posıtıve and negatıve predıctıve values 93% and 59% respectively), and the cut-off value for NLR as 2.4 (87% sensıtıvıty and 58% specıfıcıty wıth ppv and npv 64 and 85% respectively) (8). In our study, we determıned the cut-off for NLR as 2.76, with the sensıtıvıty of 52.8%, specıfıcıty of 82.8%, and plr cut-off was 177 with the sensıtıvıty of 32.8%, specıfıcıty of 95.7% fort he diagnosis of HP. When we compared with the sarkoidosis study, the sensıtivity of both plr and nlr was lower whereas specifity was higher for HP in our study. 'Karadeniz et al. Showed that PLR increased in COPD patients compared to the control group, and it was higher in the AE-COPD group than stable COPD. In addition, they observed a negative correlation between PLR and FEV1 (11). Sakurai et al. also found that NLR predicted the exacerbations and severity of COPD (17). In acute pulmonary embolism, it is reported that the increase in NLR and PLR is related to all-cause mortality (18).

Hypersensitivity pneumonia is an inflammatory disease that develops against an inhaled antigen. Neutrophils may also increase in inflammatory diseases. In addition, platelets also increase in many inflammations with an acute phase reactant-like effect. We also found that neutrophil and platelet counts increased significantly in HP patients compared to the control group. We believe that, as a result, PLR and NLR values have increased. In addition, studies in sarcoidosis patients have reported that a peripheral lymphopenia may develop due to lymphocyte accumulation in the granulomas, and, consequently, an increase in PLR and NLR was seen (8,19). Similar to sarcoidosis in HP, lymphocytes may decrease in peripheral blood due to lymphocytic inflammation and granulomas developing in the lung. Consequently, PLR and NLR are higher in HP patients than in the control group. In addition, as the disease progresses to chronic HP, granulomas and inflammation in the lung decrease gradually and they are replaced by fibrosis (chronic / fibrotic HP) (4). As a result, we believe that PLR and NLR decreases in the chronic period compared to the acute period.

While lymphocytic alveolitis is mostly seen in the BAL of HP patients, sometimes neutrophilic alveolitis can also be seen (15,16). According to clinical, radiological and BAL findings, differentiating between acute and chronic HP can be difficult. Therefore, we PLR and NLR biomarkers can be used as predictors both in the diagnosis of HP and in the distinction between acute and chronic HP.

Serum KL-6 and SP-D produced from type II pneumocytes are biomarkers that can be used in the differential diagnosis of ILD. T. Okomoto et al. determined serum KL-6 and SP-D levels significantly higher in acute HP and chronic HP than in patients with IPF, CVD-IP, sarcoidosis. For patients in the chronic HP group who had acute exacerbation (AE), KL-6 level was significantly higher than KL-6 level one month before AE (20).). Precipitating antibodies were used to differentiate chronic HP from other fibrotic interstitial lung diseases and it was concluded that serum precipitating antibodies have no role in the diagnostic approach to chronic HP (21). In a study in which Cathepsin-K was used as an immunohistochemical marker, Cathepsin-K was found to be a sensitive and specific marker that detects granulomatous reactions in chronic HP (22).

We found that PLR and NLR values were higher in HP patients compared to the control group, and were significantly higher in acute HP than chronic HP. PLR and NLR cutoff values between the HP and the control group 177 and 2.76, respectively. We also found the cutoff values for PLR and NLR to predict acute HP and chronic HP differential diagnosis were 110 and 2.15, respectively.

Limitations of the study

There were limitations to this study:it was retrospective,in the control group, laboratory values such as ESR and CRP showing inflammation and respiratory function test parameters could not be examined, the lack of cell analysis in the BAL fluid of all patients And the value of both ratios to diagnose HP and to differentiate between AHP and CHP is characterized by a low sensitivity which could lead to a number of false negative results.

Conclusion

Sometimes there may be difficulties in the diagnosis and differentiation of acute vs. chronic HP. Other biomarkers such as KL-6 and SP-D are difficult to obtain and are expensive. PLR and NLR values, which are inexpensive and easily accessible, can guide

us both in the diagnosis and the distinction of acute vs. chronic HP forms together with clinical, imaging, BAL findings and with the history of exposure.

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SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (4); e2020016 DOI: 10.36141/svdld.v37i4.9584

Pulmonary Low Attenuation Areas on CT in ANCA-associated Vasculitis: A quantitative and semi-quantitative analysis correlated with pulmonary function testing for obstructive airway disease

Christian W. Cox, Brian J. Bartholmai, Misbah Baqir, Jennifer R. Geske, Ulrich Specks
Department of Radiology, Mayo Clinic Rochester

Abstract. *Objective:* A subset of ANCA-associated vasculitis (AAV) patients are known to manifest obstructive airway disease. Using low attenuation areas (LAA) in the lung on HRCT as an imaging marker for obstructive airway disease, we analyze HRCT studies in AAV patients compared to a matched non-AAV group using visual semi-quantitative and automated quantitative analysis for presence and severity of LAA. Furthermore, HRCT and pulmonary function testing are compared to assess agreement between tests for airway obstruction. *Materials and Methods:* 100 randomly selected AAV patients with HRCT were compared to 100 best-fit matched control subjects. HRCT cases were visually assessed for LAA, along with additional pulmonary patterns. Automated quantitative software analyzed images for texture features and volume of attenuation values of –950 HU or less (e-950). Evidence of obstructive airway disease established by pulmonary function testing, when available, was compared to HRCT analysis for LAA. Additional clinical information, diagnostic testing and mortality data were also compared. *Results:* Both study groups were comprised of 57 females and 43 males with 35 smokers averaging 10.7 pk/yrs, with average age for the AAV and control groups being 59.4 yrs and 61.9 yrs, respectively. Visually, 46 AAV patients demonstrated LAA on HRCT compared to 25 control patients (p=0.0017) with the difference in LAA presence entirely within the non-smoking subgroup (25 to 3, respectively, p=<0.0001). Quantitatively, greater than 5% e-950 demonstrated similar significant differences between AAV (36/100) and controls (19/100) (p=0.0065), predominantly in non-smokers (p=0.006). Obstruction on PFTs was significantly increased in AAV (p=0.002) with moderate agreement of obstructive disease with visual LAA on CT (Kappa 0.509). Of the obstructive disease metrics, visual LAA on CT correlated best with mortality (p=0.0085). *Conclusion:* Visual LAA and automated quantitative analysis for e-950 on HRCT demonstrate statistically significant increases in AAV patients compared to age, gender and smoking matched controls, with differences primarily seen in the non-smoking subset. AAV revealed statistically significant greater obstructive pulmonary disease on PFTs *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (4): e2020016)*

Key words: ANCA-associated vasculitis, computed tomography, emphysema, air-trapping, fibrosis

Abbreviations:
AAV- Anti-Neutrophilic Cytoplasmic Autoantibody (ANCA)-associated vasculitis
MPO- Myeloperoxidase
PR3- Proteinase 3
MPA- Microscopic polyangiitis
EGPA- Eosinophilic Granulomatosis with polyangiitis
V-LAA- Visual low attenuation areas
Q-LAA- Quantitative low attenuation areas
UIP- Usual Interstitial Pneumonia
CALIPER- Computer-Aided Lung Informatics for Pathology Evaluation and Rating
PFT- Pulmonary Function Test

Received: 20 April 2020
Accepted after revision: 2 December 2020
Correspondence: Christian W. Cox MD
*200 First Street SW, Rochester MN 55905
E-mail: cox.christian@mayo.edu

Introduction

Anti-Neutrophilic Cytoplasmic Autoantibody (ANCA)-associated vasculitis (AAV) is an umbrella term used for a group of systemic autoimmune syndromes characterized by necrotizing inflammation of small vessels and the presence of ANCAs targeting either myeloperoxidase (MPO) or proteinase 3 (PR3) (1). AAV comprises microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA) and eosinophilic granulomatosis with polyangiitis (EGPA or Churg-Strauss) (2). A recent International Consensus Statement supports immunoassay testing for PR3-ANCA and MPO-ANCA as a primary screening tool for MPA and GPA (3).

Respiratory tract manifestations are a common clinical presentation of AAV; the most common in GPA patients and present in up to a third of MPA patients (4). While upper respiratory involvement with sinusitis is most common, lower respiratory tract manifestations of AAV range from subglottic stenosis to diffuse alveolar hemorrhage. When strictly examining thoracic computed tomography (CT) manifestations of AAV, imaging findings most often include pulmonary nodules/masses, consolidation, cavitation, tracheobronchial wall thickening and bronchiectasis (5,6), although this varies by subtype. More recent imaging based studies have identified an association of MPA and pulmonary fibrosis (7-12).

In our practice, we have recognized sporadic non-smoking AAV patients with CT evidence of multifocal hyperlucent changes of the lung, commonly tracking along the bronchovascular bundles, with features suggestive of emphysema, cysts and/or mosaic attenuation. (Figure 1) Particular types of small vessel vasculitides other than GPA or MPA have a well-recognized association with small airway obstruction. Asthma is a disease-defining feature of EGPA, and hypocomplimentemic urticarial vasculitis can be associated with basilar predominant panacinar emphysema similar to alpha-1 antitrypsin deficiency disease along with the urticarial skin manifestations (13). In the setting of GPA and MPA, obstructive lung disease and associated imaging changes have been described in a few isolated case reports, small case series and general imaging studies (9,13-16). However, we are unaware of any literature specifically studying hyperlucent or low-attenuation area (LAA) changes on HRCT in patients with GPA or MPA. Therefore, the aim of this retrospective study was to compare a cohort of AAV patients to a sex, age and smoking matched control group for the presence of LAA on HRCT using both visual semi-quantitative and automated quantitative methods. Pulmonary function tests, when available in AAV patients, were assessed for obstructive airway disease and compared to HRCT results.

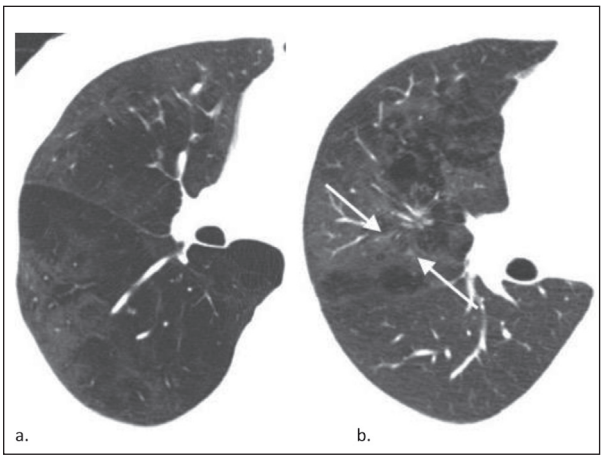

Fig. 1. 42-year-old male (a) and 26-year-old male (b), both non-smokers with PR3 positive ANCA associated vasculitis. Select axial HRCT images in lung window demonstrate low attenuation areas tracking along bronchovascular bundles. Additional ground-glass surrounds regions of LAA in the 26-year-old patient (arrows).

Methods

Study Subject Selection

Following approval from our Institutional Review Board (#15-008086) as minimal risk research allowing waiver of informed consent, a total of 751 patients with an AAV diagnosis were identified from the Pulmonary Vasculitis Clinic at the Mayo Clinic Rochester between 2008 and 2015. Filtering for confirmed presence of PR3-ANCA or MPO-ANCA and acquisition of a HRCT between 2008 and 2015, 273 ANCA positive vasculitis patients met study criteria. After screening for subject research authorization approval, the first 100 AAV patients were selected following random number assignment. Study power was determined by estimating a 20% increase in LAA presence on CT for AAV patients compared to control.

For the control cohort, 100 patients from the COPDGene study were selected by 1:1 best fit matching for sex, smoking history and age with weighting based on that order. Asymptomatic non-smokers, asymptomatic smokers and symptomatic smokers with variable degrees obstructive lung disease matched from the COPDGene study composed the control cohort. Regan et al. further outlined the COPDGene study design to include subject selection in 2010 (17).

Imaging Criteria

For subject study inclusion, a volumetric high-resolution chest CT, as defined by contiguous <2mm slice thickness without intravenous contrast reconstructed with high spatial resolution algorithm images, must have been acquired between 2008 and 2015. For the AAV group, all studies were performed at our institution on multidetector scanners for clinically indicated reasons with similar supine full inspiratory imaging acquisition and reconstruction parameters. Specifically, Siemens scans (91 cases) were reconstructed with 1.5mm image thickness at 1.0mm interval using B46f or Bv49 kernel. GE scans (9 cases) were reconstructed with 1.25mm images at 1.0 mm internal using BONE kernel. Eighty-nine were scanned at routine clinical dose, with eleven patients receiving a reduced dose protocol. In the control population, high-resolution CT studies were all performed following COPDGene protocol (17).

Semi-quantitative Visual Analysis

Two subspecialty thoracic radiologists (CWC and BJB) with 10 and 18 years experience, respectively, performed consensus review of all 200 randomly sorted anonymized CT studies blinded to clinical data. Imaging review was performed at our institution PACS workstation on diagnostic grade monitors (EIZO RadiForce RX340) using 4.1v Centricity GE radiology viewing software. Studies were evaluated for presence or absence of visual low attenuation areas (V-LAA) with estimated percent lung involved to the nearest 5%, dominant LAA pattern and distribution. No strict lung density cut-off was applied for the visual assessment, but cumulative changes to include relative decreased density, vascular attenuation, and architectural distortion all contributed to the interpretation of LAA presence. "Average Percent LAA" represents the mean of percent lung involvement by LAA across the entire population. "Severity Percent LAA" was calculated by averaging the percent of lung involved only in patients with V-LAA present LAA. In the setting of minimal V-LAA, such as a few focal cysts, the distribution was characterized as "scattered". Three or less apical blebs were excluded from V-LAA consideration due to known incidental occurrence in the normal population. V-LAA was subcategorized by the predominant pattern as emphysema (absence of lung parenchyma without definable wall), obstructive (distended lung parenchyma and/or attenuated vasculature with associated relative decrease in lung density), or cystic (absence of lung parenchyma with evident thin wall). CT studies were also evaluated for alternative dominant pulmonary parenchymal patterns of disease. When appropriate, additional characteristics were noted such as 2017 Fleischner Society UIP CT pattern categorization of pulmonary fibrosis (18). Additional acquisitions, such as expiratory imaging, and multi-planar reformatting were not included to maintain consistency across the study groups.

Quantitative Analysis

Using Computer-Aided Lung Informatics for Pathology Evaluation and Rating (CALIPER) automated analysis as previously described (19), all HRCT imaging was analyzed for multiple quantifiable characteristics, to include but not limited to Total Lung Volume, E-950, and texture/density features including quantitative low attenuation areas (Q-LAA), honeycombing, reticulation, ground-glass opacities and pulmonary vascular related structures (PVRS). Quantified absolute values were then converted into percent values relative to the total lung volume. Fibrosing Interstitial Pneumonia (FIP) was calculated by combining the CALIPER values for "Honeycombing" and "Reticulation". The extent of CALIPER-detected "Moderate Q-LAA" and "Severe Q-LAA" were combined to calculate the "Total Q-LAA" volume. To determine the "presence" or "absence" from the quantitative data, a threshold value equal to or greater than 5% was used for the percent E-950 and Total Q-LAA volume, and value equal to or greater than 1% for FIP.

Additional Diagnostic Testing and Mortality Data

In the study cohort, 88 out of 100 AAV patients underwent prior pulmonary function testing (Table 1). In contrast, all 100 control patients underwent pulmonary function testing as part of the COPDGene study (20). Pulmonary function calculations of obstruction were based on the Third National Health and Nutrition Examination Survey reference values with all patients demonstrating obstruction divided into categories of mild, moderate and severe. Chart review was performed on all AAV subjects with alpha-1 antitrypsin deficiency results recorded. Patient mortality through 12/31/2017 was recorded from the institution electronic medical record for AAV subjects and reported from the COPDGene study.

Statistical Analysis

Descriptive statistics are presented as n (%) or mean (standard deviation). The rates or presence of categorical variables between matched AAV patients and controls were compared using McNemar tests. Quantitative variables were compared using paired t-tests or paired Wilcoxon rank sum tests. Agreement is described with kappas for present/absence, or intraclass correlations (ICCs) for quantitative agreement. Mortality risk was compared using univariate and multivariable Cox Proportional Hazard models. Age, gender, LAA, smoking status and packs per year were considered as confounders. Analyses were conducted using SAS (version 9.4; Cary, NC).

Results

Study Subject Selection

Table 1 summarizes the demographic data of both AAV and control cohorts. Ages ranged from 45 to 79 years old in COPDGene study, resulting in 18 AAV subjects younger than 45 years old and 9 AAV subjects greater than 79 years old.

Semi-quantitative Visual Analysis

On semi-quantitative visual inspection of high-resolution chest CT, 46% (46/100) AAV patients demonstrate visual low attenuation areas (V-LAA) compared to 25% (25/100) control patients (p=0.0017) although collectively, AAV patients averaged a similar average percent of lung involvement by V-LAA compared to the control cohort (10.0% and 9.1%, respectively, p=0.7518). When considering semi-quantitative evaluation of V-LAA severity, severity of V-LAA is statistically significantly greater in controls compared to AAV patients (p=0.0331). With respect to subtypes, V-LAA appeared predominantly obstructive (n=24, 52%) in AAV, where V-LAA in the control cohort appeared predominantly emphysematous (n=20, 80%). Figure 2 provides an example of a young non-smoker with substantial V-LAA characterized a predominantly obstructive. A summary of the V-LAA scoring and quantitative analysis is provided in Table 2. V-LAA in both groups was most often upper lung predominant or diffuse. When categorized as "emphysema", AAV patients tended to demonstrate a centrilobular pattern. Dividing the AAV patients by PR3 and MPO subgroups, no statistical difference was present with respect to V-LAA with 46% (29/63) PR3-ANCA patients and 46% (17/37) MPO-ANCA patients demonstrating presence of V-LAA (p=0.9934).

Considering only the 65 non-smoking AAV patients, semi-quantitative visual inspection reveals

Table 1. Study Subject Demographics

	AAV Cohort (n=100)	Control Cohort (n=100)
Mean Age in Years (Range, SD)	59.4 (17-86, 16.7)	61.9 (45-79, 9.7)
Gender		
Female	43*†	43
Male	57	57
Prior/Current Smokers	35	35
Mean Packs/Year (±SD)	10.7 (±16.9)	10.7 (±16.9)
AAV Subtype		
PR3 Positive	63	N/A
MPO Positive	37	N/A
PFT Performed	88	100

*Unless noted as a mean with standard deviation (SD), all values are absolute
†Absolute values are equal to percent, given cohort populations of 100
AAV=ANCA-Associated Vasculitis, PFT=Pulmonary Function Test

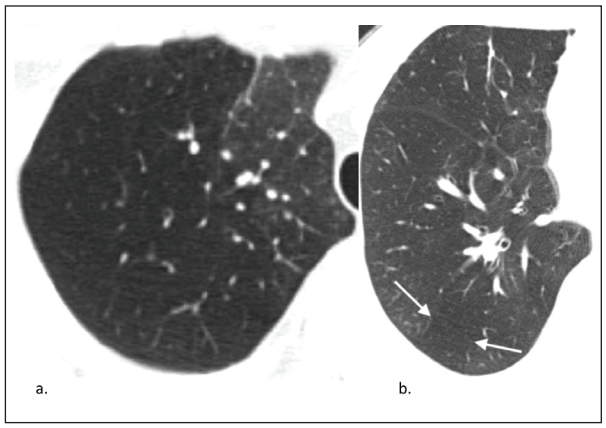

Fig. 2. 36-year-old PR3 positive AAV male (a) and 60-year-old MPO positive female (b), both non-smokers. Select axial HRCT images in lung window reveal low attenuation areas categorized as "obstructive" involving the majority of the right upper lobe in 36-year-old male. More regional areas of obstruction (arrows) are present in the 60-year-old female.

a greater statistically significant difference in AAV patients with V-LAA (n=25, 38%) compared to the non-smoking control patients (n=3, 5%) (p<0.0001). A predominantly mosaic appearance of the V-LAA persisted in the non-smoking AAV patients (n=18, 72%), where all 3 non-smoking control patients demonstrated a cystic appearance.

Table 3 summarizes alternative pulmonary parenchymal findings to low-attenuation changes, such as pulmonary fibrosis and nodularity. Visual assessment reveals statistically significant presence of pulmonary fibrosis in AAV patients compared to control (n=34 and 5, respectively, p<0.0001), with 8 vasculitis patients demonstrating a "consistent with" or "probable" UIP CT pattern by 2017 Fleischner Society Criteria, compared to none in the control group. With respect to PR3/MPO status, 49% (18/37) MPO positive patients demonstrate fibrosis with 25% (16/63) PR3 patients demonstrating fibrosis. The presence of "nodules", "groundglass" and "consolidation" were also statistically significantly greater in AAV patients compared to control (p=0.0027, p=0.0001, p<0.0001, respectively). Alternatively, visual HRCT findings of an "airway pattern" to include bronchial wall thickening and/or centrilobular groundglass nodularity was greater in the control group compared to vasculitis patients (43 and 28, respectively, p=0.0431) with centrilobular groundglass nodules perceived in 23 non-smoking control patients.

Quantitative Analysis

Quantitative presence of low attenuation areas as measured by >5% e-950 also demonstrates statistically significant higher rates in AAV patients compared to control, 36% (36/100) AAV to 19% (19/100) control patients (p=0.0065). The difference in average percent of e-950 in AAV versus control is similar, with statistically significant greater average e-950 in non-smoking AAV patients compared to the non-smoking control patients (p=0.0018). CALIPER textural analysis of quantitative low attenuation areas (Q-LAA) results in no significant difference between AAV and control patients, although percent involvement by Q-LAA in controls was statistically greater than in AAV patients (p=0.0053) (Table 2). With regards to fibrotic interstitial lung disease (ILD) as assessed by combining honeycombing and reticulation textural patterns, quantitative analysis also reveals statistically significant greater presence and average percent involvement in AAV compared to controls (both p<0.0001) (Table 3). Of note, presence and average percent vessel volume also demonstrate statistically significant greater values in AAV (p<0.0001 and p=0.0032, respectively). An example CALIPER 3D reconstruction and glyph is provided of a select AAV patient with substantial LAA changes. (Figure 2)

When comparing agreement between obstructive disease CT metrics, e-950 and visual LAA resulted in 68.0% agreement (kappa=0.264, 0.125 to 0.403 95% CI).

Pulmonary Function and Laboratory Testing

Similar to visual and automated e-950 analysis for LAA, pulmonary function tests (PFT) also demonstrate statistically significant greater obstructive lung disease in AAV compared to controls (p=0.002), attributable entirely to the difference between non-smoking subgroups (Table 4). In the non-smoking subgroup, PFT values in AAV demonstrate significant obstruction in 35.7% (20/56) of patients. In the smoking subgroup, evidence of obstruction and severity are slightly greater in control patients relative to AAV patients. When comparing agreement between visual presence of LAA, quantitative presence of >5% e-950, and PFT obstruction, greatest agreement is present between visual LAA and PFT obstruction

Table 2. Visual Semi-quantitative and Automated Quantitative HRCT Analysis Results for Low Attenuation Changes; AAV versus Control further divided by smoking status

	Smoking			Nonsmoking			Total		
	AAV	**Controls**		**AAV**	**Controls**		**AAV**	**Controls**	
	n=35	n=35	p-value	n=65	n=65	p-value	n=100	n=100	p-value
Visual Analysis (V-LAA)									
Presence	21 (60.0%)	22 (62.9%)	0.8084	25 (38.5%)	3 (4.6%)	<0.0001	46 (46.0%)	25 (25.0%)	0.0017
Avg %	11.9% (19.8%)	25.7% (30.6%)	0.0213	8.8% (16.0%)	0.2% (0.9%)	<0.0001	10.0% (17.4%)	9.1% (21.7%)	0.7518
Severity %	19.9% (22.3%)	40.9% (29.4%)	0.0119	23.1% (18.5%)	4.2% (1.4%)	<0.0001	21.6% (20.1%)	36.5% (30.1%)	0.0331
Subtype V-LAA									
Emphysema	11 (52.4%)	20 (90.9%)	0.0068	4 (16.0%)	0	1	15 (32.6%)	20 (80.0%)	0.0001
Obstructive	6 (28.6%)	2 (9.1%)	0.1324	18 (72.0%)	0	0.0366	24 (52.2%)	2 (8.0%)	0.0002
Cysts	5 (23.8%)	2 (9.1%)	0.2404	5 (20.0%)	3 (100.0)	0.0171	10 (21.7%)	5 (20.0%)	0.8639
Automated Analysis									
Avg e-950	408.0 (656.8)	471.5 (641.5)	0.6198	195.5 (221.8)	118.9 (148.8)	0.0253	269.9 (436.3)	242.3 (429.2)	0.5787
Avg % e-950 TV	6.5% (8.7%)	6.6% (8.4%)	0.9429	3.9% (3.8%)	2.0% (2.4%)	0.0018	4.8% (6.1%)	3.7% (5.8%)	0.0994
>5% e-950	15 (42.9%)	12 (34.3%)	0.4054	21 (32.3%)	7 (10.8%)	0.006	36 (36.0%)	19 (19.0%)	0.0065
Avg Total Q-LAA	543.2 (1022.6)	1159.4 (1650.6)	0.0621	239.4 (409.7)	453.3 (818.8)	0.0376	345.7 (699.2)	700.4 (1218.0)	0.0053
Avg % Q-LAA TV	8.3% (13.9%)	16.0% (21.8%)	0.055	4.6% (7.1%)	7.3% (12.7%)	0.1472	5.9% (10.1%)	10.3% (16.9%)	0.0158
Q-LAA Presence	22 (62.9%)	27 (77.1%)	0.1317	31 (52.3%)	39 (60.0^%	0.3532	56 (56.0%)	66 (66.0%)	0.1138

AAV= ANCA-associated Vasculitis, V-LAA= Visual Low attenuation area, Avg= Average, TV= Total Volume, Q-LAA= Quantitative Low attenuation area

Table 3. Visual Semi-quantitative and Automated Quantitative HRCT Analysis Results for Alternative Pulmonary Changes; AAV versus Control further divided by smoking status

	Smoking			Nonsmoking			Total		
	AAV	**Controls**		**AAV**	**Controls**		**AAV**	**Controls**	
	n=35	n=35	p-value	n=65	n=65	p-value	n=100	n=100	p-value
Visual Analysis									
Fibrosis	10 (28.6%)	4 (11.4%)	0.0578	24 (36.9%)	1 (1.5%)	<0.0001	34 (34.0%)	5 (5.0%)	<0.0001
Airway	8 (22.9%)	14 (40.0%)	0.1336	20 (3.08%)	29 (44.6%)	0.1495	28 (28.0%)	43 (43.0%)	0.0431
Nodularity	0	0	-	1 (1.5%)	0	-	1 (1.0%)	0	-
Nodules	4 (11.4%)	1 (2.9%)	0.1797	11 (16.9%)	2 (3.1%)	0.0067	15 (15.0%)	3 (3.0%)	0.0027
Cavities	1 (2.9%)	0	-	2 (3.1%)	0	-	3 (3.0%)	0	-
Groundglass	4 (11.4%)	2 (5.7%)	0.4142	18 (27.7%)	1 (1.5%)	<0.0001	22 (22.0%)	3 (3.0%)	0.0001
Consolidation	2 (5.7%)	0	-	9 (13.8%)	0	0.9924	11 (11.0%)	0	0.9916
Septal Thick	0	0	-	1 (1.5%)	0	-	1 (1.0%)	0	-
DPO	0	0	-	1 (1.5%)	0	-	1 (1.0%)	0	-
Automated Analysis									
HC Presence	2 (5.7%)	0	-	5 (7.7%)	0	-	7 (7.0%)	0	-
Avg FIP % TV	4.5% (8.0%)	1.0% (2.3%)	0.02	6.5% (10.8%)	0.8% (1.8%)	0.0001	5.8% (9.9%)	0.9% (2.0%)	<0.0001
FIP Presence	19 (54.3%)	7 (20.0%)	0.003	35 (53.9%)	11 (16.9%)	<0.0001	54 (54.0%)	18 (18.0%)	<0.0001
Avg Vess Vol	122.5 (58.7)	106.2 (25.2)	0.1229	107.6 (42.7)	92.7 (20.7)	0.0083	112.8 (49.1)	97.4 (23.2)	0.0032
APVV	2.59% (1.67%)	1.82% (0.5%)	0.0135	2.56% (1.25%)	1.78% (0.34%)	<0.0001	2.57% (1.40%)	1.79% (0.38%)	<0.0001
>3% APVV	6 (17.1%)	1 (2.8%)	0.0379	15 (23.1%)	0	<0.0001	21 (21.0%)	1 (1.0%)	<0.0001

AAV= ANCA-associated Vasculitis, DPO= Diffuse Pulmonary Ossification, TV= Total Volume, HC= Honeycombing, FIP= Fibrosing Interstitial Pneumonia, Vess Vol= Vessel Volume, APVV= Average Percent Vessel Volume

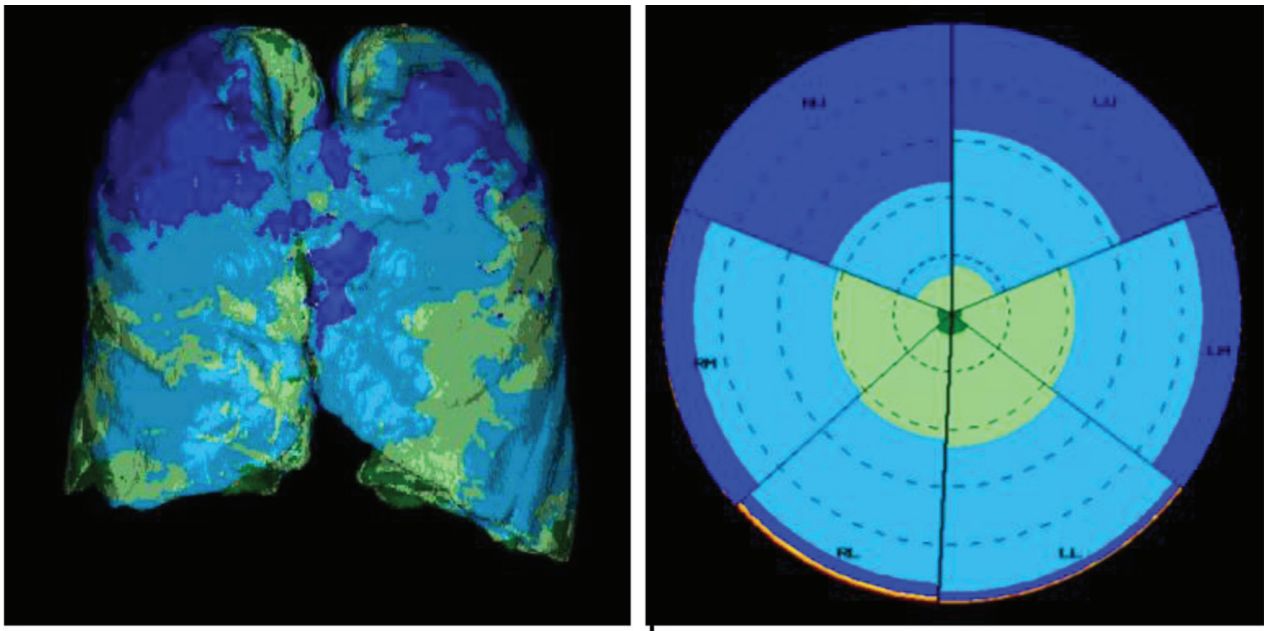

Fig. 3. 75-year-old male smoker with PR3 positive ANCA-associated vasculitis. CALIPER surface rendering (a) and glyph (b) demonstrate significant moderate (light blue) and severe (dark blue) textural low attenuation areas

Table 4. Pulmonary Function Test (PFT) Results, ANCA-associated Vasculitis (AAV) versus Control further divided by smoking status

	Smoking			Nonsmoking			Total		
	AAV	**Controls**		**AAV**	**Controls**		**AAV**	**Controls**	
	n=35	n=35	p-value	n=65	n=65	p-value	n=100	n=100	p-value
PFT Results	31	35		56	65		87	100	
Obstruction	12 (38.7%)	17 (48.6%)	0.593	20 (35.7%)	0	<0.0001	32 (36.8%)	17 (17.0%)	0.002
Severity			0.1302			-			0.1744
Mild	1 (8.3%)	5 (29.4%)		3 (15.8%)	0		4 (12.9%)	5 (29.4%)	
Moderate	7 (58.3%)	4 (33.3%)		8 (42.1%)	0		15 (48.4%)	4 (23.5%)	
Severe	4 (33.3%)	8 (47.1%)		8 (42.1%)	0		12 (38.7%)	8 (47.1%)	

(Kappa 0.509, 0.377 to 0.641 95% CI and 79.1% agreement). Alpha-1 Antitrypsin Deficiency testing was performed on 6 AAV patients, all negative.

Mortality

All-cause mortality between AAV and control patients was not statistically different, 12 compared to 8, respectively (HR (95% CI): 2.33 (0.92, 5.90), p=0.0729). Age and gender were also significantly associated with mortality risk after adjusting for AAV (males HR (95% CI): 2.93 (1.12, 7.69, p=0.029; age HR (95% CI): 1.10 (1.05, 1.16), p=0.0002). Across the entire study population, V-LAA demonstrated the greatest association with mortality (p=0.0085), but was not significant after adjusting for age, gender, and AAV. Total population mortality results are summarized in Table 5.

Table 5. All-Cause Mortality Comparisons and Cox Proportional Hazard Model results

	Mortality		HR (95% CI)	p-value
	Yes (n=20)	No (n=180)		
Study Group				
AAV Subjects	12 (12.0%)	88 (88.0%)	1.97 (0.80, 4.87)	0.1402
Control Subjects	8 (8.0%)	92 (92.0%)		
Gender				
Male	14 (16.3%)	72 (83.7%)	3.19 (1.23, 8.31)	0.0174
Female	6 (5.3%)	108 (94.7%)		
Visual Assessment				
V-LAA- yes	13 (18.3%)	58 (81.7%)	3.43 (1.37, 8.61)	0.0085
V-LAA- no	7 (5.4%)	122 (94.6%)		
Quantitative Assessment				
Adj e950 >5%	7 (12.7%)	48 (87.3%)	1.56 (0.62, 3.91)	0.3440
Adj e950 <5%	13 (9.0%)	132 (91.0%)		
Pulmonary Function Test				
Obstruction	7 (14.6%)	41 (85.4%)	2.24 (0.88, 5.68)	0.0891
No obstruction	11 (7.9%)	128 (92.1%)		
Multivariable Cox PH Model	**HR**	**95% CI**		**Adjusted p-value**
Age	1.10	1.05, 1.16		0.0002
Gender (M)	2.93	1.12, 7.69		0.0290
AAV	2.33	0.92, 5.90		0.0729

AAV= ANCA-associated Vasculitis, V-LAA= Visual Low attenuation areas

Discussion

Overall, visual semi-quantitative analysis of high-resolution chest CT for pulmonary low attenuation areas (V-LAA), automated quantitative analysis for e-950, and obstruction on pulmonary function testing are statistically significantly greater in ANCA-associated vasculitis (AAV) patients when compared to age, gender and smoking matched controls. V-LAA in AAV subjects compared to the control group demonstrated the greatest difference between the populations. With respect to a unique pattern of low attenuation area for AAV patients, only one AAV patient and no control patients demonstrated the described emphysematous changes tracking along bronchovascular bundles, suggesting that this is a unique pattern to AAV, albeit rare.

While association does not prove causality, there are potential inflammatory mechanisms for development of low attenuation areas on CT imaging and/or functional obstructive lung disease in the setting of ANCA-associated vasculitis. Complex inflammatory pathways play a primary role in the development of chronic obstructive pulmonary disease, to include but not limited to an initial trigger, such as smoking, leading to a diverse cellular response with subsequent tissue damage, release of inflammatory mediators and cellular activation for a mixed cellular inflammatory milieu. Final inflammatory cascades result in protease/antiprotease imbalance, increased oxidative

stress and altered lung cell death resulting in COPD (21). Likewise, pulmonary AAV inflammatory cascades overlap with these known COPD pathways, particularly neutrophil and T cell activation causing direct injury to the lung (22,23).

A correlated alternative pathologic process in AAV, such as alpha-1 antitrypsin deficiency (AATD), may be causing the difference in low attenuation areas on chest CT and physiologic obstructive lung disease. Presence of AATD genetic alleles is known to increase in the setting of AAV (24). Despite only 6 patients out of 100 AAV patients being tested for AATD, all AAV patients tested were negative for AATD.

When examining the study subgroups, a few specific differences stand out. First, the difference in V-LAA between AAV and control groups was entirely found in the nonsmoking subgroup. Another interesting difference is evident when comparing smokers, where AAV subjects demonstrated a slight decrease in V-LAA presence and a statistically significant decrease in V-LAA severity compared to control subjects who smoke. Mild V-LAA was present in 3% of control non-smokers, all of which were over the age of 70 years old, similar to other study findings of senescent changes of the lung (25).

Studies using CALIPER for quantitative CT analysis have demonstrated better correlation between textural quantitative low attenuation area measurements and obstructive physiology on pulmonary function testing when compared to visual assessment (26) as well as comparable performance of CALIPER measurements to physiologic testing (27). In our study, CALIPER analysis of textural quantitative low attenuation areas (Q-LAA) modeled on emphysema measured greater areas of moderate and severe Q-LAA in controls as opposed to AAV, which runs counter to the other CT and physiologic obstruction metrics. Agreement between V-LAA, e-950 and obstructive lung disease on PFTs favors the textural analysis for Q-LAA as the outlier. Since most subjects have mild V-LAA and some have additional airway features such as bronchial wall thickening or centrilobular nodules that increase density, the combination of minimal lucency and some small high-density features in the VOI likely result in CALIPER under-detecting the subtle features which are captured by pixel-counting and expert visual review. This may also explain the divergence of Q-LAA performance in our study on vasculitis when compared to the findings of Jacob et al. in the setting of hypersensitivity pneumonitis (26). Furthermore, the pattern of AAV low attenuation areas is visually different than typical smoking-related emphysema, with many subtyped as obstructive pattern. As CALIPER was trained with pathologically proven emphysema datasets, the under-detection of the AAV low attenuation areas is not entirely surprising.

The prevalence of obstructive disease in the setting of AAV varies in the literature depending on the metric. A recent evaluation of HRCT findings in patients from the Remission Induction Therapy in Japanese Patients With ANCA-Associated Vasculitis and Rapidly Progressive Glomerulonephritis Study, Suzuki et al. found emphysema in 22% of patients with another 7% demonstrating mosaic attenuation (16). Greenan et al found a relatively similar rate of 25.9% of chest CT revealing emphysema in AAV patients (9). When examining pulmonary function testing in AAV, Newall et al. found obstructive airway disease in 12 out of 30 AAV patients (40%) (28). In our study, prevalence of finding suggesting obstructive airway disease on visual HRCT analysis, quantitative HRCT analysis and pulmonary function tests ranged from 36% to 46%, which likely reflects the more general assessment for LAA identifying mosaic attenuation as a subset of obstruction in addition to emphysema on CT and better correlating with reported obstructive disease on PFT. Additional contributors may include selection bias related to increased disease severity, comorbidities or treatment differences.

Treatment of airway obstruction in AAV patients is based on clinical symptoms paired with PFT abnormalities following guidelines for COPD management. The radiographic observations in our study may in part explain clinical symptoms and PFT abnormalities in patients with ANCA-associated vasculitis that cannot be explained based on smoking history.

Outside of obstructive changes, pulmonary fibrosis was also increased in AAV patients on both visual and automated analysis compared to controls. In keeping with multiple prior studies and reports, MPO/MPA AAV patients demonstrated greater prevalence of fibrosis, although proportionally were higher at 51%. PR3/GPA patients also demonstrated increased prevalence of pulmonary fibrosis at 24%.

Automated quantitative analysis measured even greater differences of pulmonary fibrosis in AAV compared to controls to include smokers and nonsmokers. Pulmonary fibrosis may confound evaluation of obstructive airway disease in both HRCT and PFT.

Beyond retrospective design, there were a few known limitations to the study. The AAV study population was selected from a quaternary referral center, which may represent a more complicated or atypical subpopulation. Studying AAV patients with prior chest CT introduces selection bias for those having clinical indications for CT such as respiratory symptoms. Not all AAV patients underwent pulmonary function testing could artificially increase the percent demonstrating obstruction. Mortality rates were generally low, and therefore comparisons of potential mortality risks were underpowered. Control subjects were collected as part of a multicenter trial across the country and may not reflect an ideal equivalent control population. In particular, the COPDGene study design included a distribution of two-thirds Non-Hispanic white and one-third African American, which is quite different than the more homogenously non-Hispanic white cohort of AAV subjects studied.

Pulmonary low attenuation areas on visual and quantitative HRCT analysis, as opposed to narrower imaging definitions of emphysema, in AAV patient better correlates with prevalence of obstruction on pulmonary function testing. In our study, visual low attenuation areas correlated best with obstructive lung disease (79.1% agreement), although establishing quantitative measures of LAA on HRCT in the setting of AAV provides a standard to judge disease severity and track progression. These advances in pulmonary imaging assessment in AAV patients may improve diagnostic categories and may even assist in guiding treatment. Future studies focused on obstructive lung disease in the setting of AAV should consider temporal evolution of disease relative to disease onset, type and severity, development and response of obstructive disease CT findings related to treatment, and further classification of LAA HRCT findings by histopathology correlation.

Acknowledgements

This study was approved as an ancillary study (ANC243) to the COPDGene study.

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COPDGene Phase 3

Grant Support and Disclaimer

The project described was supported by Award Number U01 HL089897 and Award Number U01 HL089856 from the National Heart, Lung, and Blood Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Lung, and Blood Institute or the National Institutes of Health.

COPD Foundation Funding

The COPDGene® project is also supported by the COPD Foundation through contributions made to an Industry Advisory Board comprised of AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Novartis, Pfizer, Siemens and Sunovion.

COPDGene® Investigators – Core Units

Administrative Center: James D. Crapo, MD (PI); Edwin K. Silverman, MD, PhD (PI); Barry J. Make, MD; Elizabeth A. Regan, MD, PhD

Genetic Analysis Center: Terri Beaty, PhD; Ferdouse Begum, PhD; Peter J. Castaldi, MD, MSc; Michael Cho, MD; Dawn L. DeMeo, MD, MPH; Adel R. Boueiz, MD; Marilyn G. Foreman, MD, MS; Eitan Halper-Stromberg; Lystra P. Hayden, MD, MMSc; Craig P. Hersh, MD, MPH; Jacqueline Hetmanski, MS, MPH; Brian D. Hobbs, MD; John E. Hokanson, MPH, PhD; Nan Laird, PhD; Christoph Lange, PhD; Sharon M. Lutz, PhD; Merry-Lynn McDonald, PhD; Margaret M. Parker, PhD; Dandi Qiao, PhD; Elizabeth A. Regan, MD, PhD; Edwin K. Silverman, MD, PhD; Emily S. Wan, MD; Sungho Won, Ph.D.; Phuwanat Sakornsakolpat, M.D.; Dmitry Prokopenko, Ph.D.

Imaging Center: Mustafa Al Qaisi, MD; Harvey O. Coxson, PhD; Teresa Gray; MeiLan K. Han, MD, MS; Eric A. Hoffman, PhD; Stephen Humphries, PhD; Francine L. Jacobson, MD, MPH; Philip F. Judy, PhD; Ella A. Kazerooni, MD; Alex Kluiber; David A. Lynch, MB; John D. Newell, Jr., MD; Elizabeth A. Regan, MD, PhD; James C. Ross, PhD; Raul San Jose Estepar, PhD; Joyce Schroeder, MD; Jered Sieren; Douglas Stinson; Berend C. Stoel, PhD; Juerg Tschirren, PhD; Edwin Van Beek, MD, PhD; Bram van Ginneken, PhD; Eva van Rikxoort, PhD; George Washko, MD; Carla G. Wilson, MS;

PFT QA Center, Salt Lake City, UT: Robert Jensen, PhD

Data Coordinating Center and Biostatistics, *National Jewish Health, Denver, CO*: Douglas Everett, PhD; Jim Crooks, PhD; Camille Moore, PhD; Matt Strand, PhD; Carla G. Wilson, MS

Epidemiology Core, *University of Colorado Anschutz Medical Campus, Aurora, CO*: John E. Hokanson, MPH, PhD; John Hughes, PhD; Gregory Kinney, MPH, PhD; Sharon M. Lutz, PhD; Katherine Pratte, MSPH; Kendra A. Young, PhD

Mortality Adjudication Core: Surya Bhatt, MD; Jessica Bon, MD; MeiLan K. Han, MD, MS; Barry Make, MD; Carlos Martinez, MD, MS; Susan Murray, ScD; Elizabeth Regan, MD; Xavier Soler, MD; Carla G. Wilson, MS

Biomarker Core: Russell P. Bowler, MD, PhD; Katerina Kechris, PhD; Farnoush Banaei-Kashani, Ph.D

COPDGene® Investigators – Clinical Centers

Ann Arbor VA: Jeffrey L. Curtis, MD; Carlos H. Martinez, MD, MPH; Perry G. Pernicano, MD

Baylor College of Medicine, Houston, TX: Nicola Hanania, MD, MS; Philip Alapat, MD; Mustafa Atik, MD; Venkata Bandi, MD; Aladin Boriek, PhD; Kalpatha Guntupalli, MD; Elizabeth Guy, MD; Arun Nachiappan, MD; Amit Parulekar, MD;

Brigham and Women's Hospital, Boston, MA: Dawn L. DeMeo, MD, MPH; Craig Hersh, MD, MPH; Francine L. Jacobson, MD, MPH; George Washko, MD

Columbia University, New York, NY: R. Graham Barr, MD, DrPH; John Austin, MD; Belinda D'Souza, MD; Gregory D.N. Pearson, MD; Anna Rozenshtein, MD, MPH, FACR; Byron Thomashow, MD

Duke University Medical Center, Durham, NC: Neil MacIntyre, Jr., MD; H. Page McAdams, MD; Lacey Washington, MD

HealthPartners Research Institute, Minneapolis, MN: Charlene McEvoy, MD, MPH; Joseph Tashjian, MD

Johns Hopkins University, Baltimore, MD: Robert Wise, MD; Robert Brown, MD; Nadia N. Hansel, MD, MPH; Karen Horton, MD; Allison Lambert, MD, MHS; Nirupama Putcha, MD, MHS

Los Angeles Biomedical Research Institute at Harbor UCLA Medical Center, Torrance, CA: Richard Casaburi, PhD, MD; Alessandra Adami, PhD; Matthew Budoff, MD; Hans Fischer, MD; Janos Porszasz, MD, PhD; Harry Rossiter, PhD; William Stringer, MD

Michael E. DeBakey VAMC, Houston, *TX*: Amir Sharafkhaneh, MD, PhD; Charlie Lan, DO

Minneapolis VA: Christine Wendt, MD; Brian Bell, MD

Morehouse School of Medicine, Atlanta, GA: Marilyn G. Foreman, MD, MS; Eugene Berkowitz, MD, PhD; Gloria Westney, MD, MS

National Jewish Health, Denver, CO: Russell Bowler, MD, PhD; David A. Lynch, MB

Reliant Medical Group, Worcester, MA: Richard Rosiello, MD; David Pace, MD

Temple University, Philadelphia, PA: Gerard Criner, MD; David Ciccolella, MD; Francis Cordova, MD; Chandra Dass, MD; Gilbert D'Alonzo, DO; Parag Desai, MD; Michael Jacobs, PharmD; Steven Kelsen, MD, PhD; Victor Kim, MD; A. James Mamary, MD; Nathaniel Marchetti, DO; Aditi Satti, MD; Kartik Shenoy, MD; Robert M. Steiner, MD; Alex Swift, MD; Irene Swift, MD; Maria Elena Vega-Sanchez, MD

University of Alabama, Birmingham, AL: Mark Dransfield, MD; William Bailey, MD; Surya Bhatt, MD; Anand Iyer, MD; Hrudaya Nath, MD; J. Michael Wells, MD

University of California, San Diego, CA: Joe Ramsdell, MD; Paul Friedman, MD; Xavier Soler, MD, PhD; Andrew Yen, MD

University of Iowa, Iowa City, IA: Alejandro P. Comellas, MD; Karin F. Hoth, PhD; John Newell, Jr., MD; Brad Thompson, MD

University of Michigan, Ann Arbor, MI: MeiLan K. Han, MD, MS; Ella Kazerooni, MD; Carlos H. Martinez, MD, MPH

University of Minnesota, Minneapolis, MN: Joanne Billings, MD; Abbie Begnaud, MD; Tadashi Allen, MD

University of Pittsburgh, Pittsburgh, PA: Frank Sciurba, MD; Jessica Bon, MD; Divay Chandra, MD, MSc; Carl Fuhrman, MD; Joel Weissfeld, MD, MPH

University of Texas Health Science Center at San Antonio, San Antonio, TX: Antonio Anzueto, MD; Sandra Adams, MD; Diego Maselli-Caceres, MD; Mario E. Ruiz, MD

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (4); e2020019 DOI: 10.36141/svdld.v37i4.9877

A rare mutation on alpha-1 antitrypsin deficit and lung fibrosis: case report

Miguel Afonso[1], Clara Silva[2], Inês Pinho[3], Artur Vale[1], Ana Fernandes[1]

[1] Department of Pulmonology at Centro Hospitalar de Trás-os-Montes e Alto Douro, Vila Real, Portugal; [2] Department of Internal Medicine at Centro Hospitalar Universitário de São João, Porto, Portugal; [3] Liver Unit, Department of Internal Medicine at Centro Hospitalar de Trás-os-Montes e Alto Douro, Vila Real, Portugal

Abstract. Alpha1-antitrypsin deficiency (AATD) is an autosomal codominant disease, and different genetic variants are known, some of which very rare. Usual pulmonary manifestations include emphysema, bronchiectasis and asthma. Pulmonary fibrosis is uncommon. We describe a case of a 64 year old man with an inaugural diagnosis of cirrhosis and lung fibrosis, without emphysema or bronchiectasis, associated with AATD. Further investigation identified a rare variant in heterozigosity ($MM_{Palermo}$), usually associated with liver disease. Concomitantly, he had a secondary iron overload, and in the course of the investigation, a type 2 diabetes mellitus installed. The association between AATD and pulmonary fibrosis is rare, however it has been identified in a few studies and case reports, questioning the role of AAT in pulmonary fibrosis. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (4): e2020019)*

Key words: Alpha1-antitrypsin deficiency, pulmonary fibrosis, liver diseases

Introduction

Alpha-1 antitrypsin (AAT) deficiency is a rare, underdiagnosed inherited autosomal codominant condition, caused by mutations in the *SERPINA1* gene, resulting in a reduction and/or dysfunction of circulating AAT. Several variants are known. Normal individuals are described as MM, and disease is most frequently seen when S and/or Z alleles are present (1).

Alpha-1 antitrypsin deficiency (AATD) increases the risk of lung and liver disease, especially when the Z allele occurs. The first is consequential to low AAT and usually manifests as early onset chronic obstructive lung disease (COPD), panlobar emphysema and bronchiectasis (1). Pulmonary fibrosis is uncommon. The latter is caused by the accumulation of AAT polymers, and not by its scarcity (1). Therefore only some variants may promote liver disease, such as the Z, S_{Iiyama}, M_{Duarte} and M_{Malton} but also S, $M_{Palermo}$, $M_{Nichinan}$ and $M_{Wurzburg}$.

Clinical case

We present a case of a 64 year old male, former smoker of 15 pack-years with past medical history of malaria and obesity, admitted at outpatient clinic for suspicion of chronic liver disease and hemochromatosis. A diagnosis of cirrhosis (Child-Pugh B7 and MELD-Na 14, and signs of hypertensive gastropathy) was established.

Although there was a known alcohol consumption of approximately 90gr/alcohol/day, an extended etiological study was preformed, identifying an AAT value of 70,9 mg/dL (nephelometry), and an

Received: 20 May 2020
Accepted after revision: 22 November 2020
Correspondence: Miguel Afonso
Centro Hospitalar de Trás-os-Montes e Alto Douro,
Vila Real, Portugal
E-mail: mfb.afonso@gmail.com

iron overload (total iron [Fe] of 204ug/dL; ferritin of 1078ng/ml and transferrin saturation of 99%), without primary hemochromatosis (heterozigosity for the H63D gene). Immune, copper and virology tests were negative.

Simultaneously, the patient complained of fatigue, cough and exertion dyspnoea, without relevant findings on physical examination. Further investigation revealed a pattern of probable UIP and no emphysema in high-resolution computed tomography (HRCT) (Figure 1). Bronchoscopy was unremarkable, with no microbiological agents identified, and a bronchoalveolar lavage (BAL) showing increased cellularity, with 94,0% macrophages, 5,0% lymphocytes, 1,0% neutrophils, and 0% mast cells and eosinophils. Lung function tests (LFT) were normal [post-BD FEV1/FVC 87,8; post-BD FEV1 98,4%pred; post-BD FVC 87,1%pred; TLC 89,7%pred and RV 113,9%pred], except for a moderately decreased diffusing capacity for CO (DLCO) [49% predicted]. Arterial blood gas showed hypoxemia of 68mmHg and the six-minute walking test showed a 10% decrease in peripheral oxygen saturation. No occupational risk exposures were described, and autoantibodies screening and rheumatologic evaluation were negative.

Multiplex polymerase chain reaction combined with restriction fragment length polymorphism (2) detected a heterozygous $M_{Palermo}$ allele ($M1M_{Palermo}$).

During the investigation, the patient presented hyperglycaemias above 200mg/dL and a glycated haemoglobin of 13.6%, hence diagnosing type-2 diabetes mellitus.

Thus the final diagnoses of concomitant type-2 diabetes, alcoholic and AATD cirrhosis, secondary iron overload, and idiopathic pulmonary fibrosis (IPF) were established. For the first, blood glucose control was achieved with long-acting insulin, for the second, alcohol abstinence was implemented, and for the latter pirfenidone was started as well as portable oxygen concentrator.

The patient's two daughters were tested, showing normal AAT levels.

At 6-months the patient remains stable, without hospital or emergency admissions.

Discussion

We report a case of liver cirrhosis and lung fibrosis associated with AATD, the latter representing a rare finding in this disease. Regarding the pulmonary fibrosis, AATD increases neutrophil elastase activity, promoting the destruction of lung structures such as matrix components and alveoli (3). Emphysema and bronchiectasis are the usual pulmonary disorders associated with AATD. Our patient presented pulmonary fibrosis with a radiologic pattern of probable

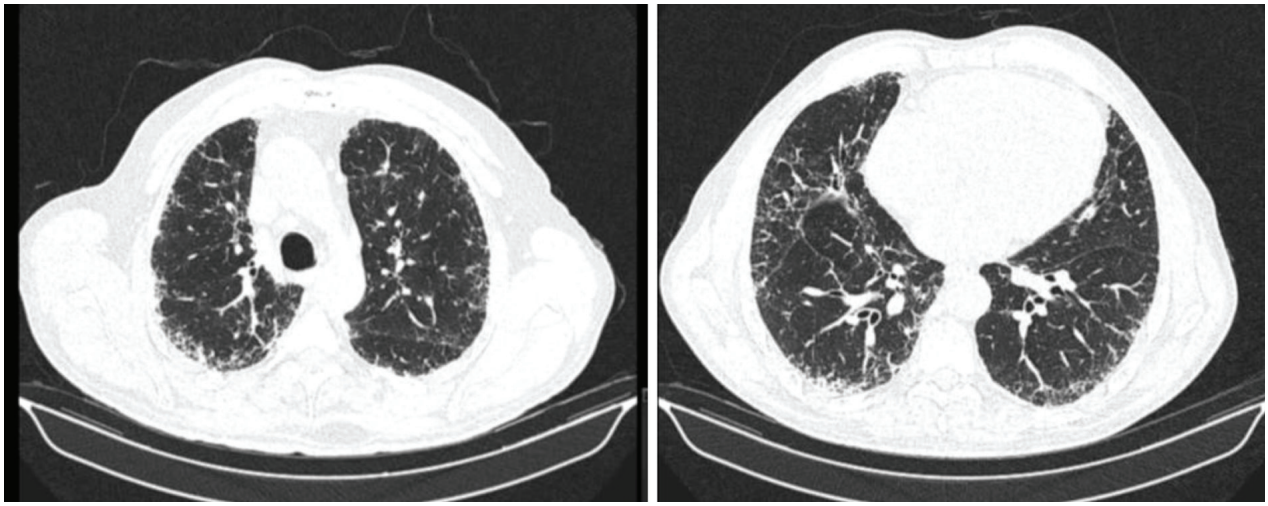

Fig. 1. Chest high resolution computed tomography, showing subpleural reticulation, scarce traction bronchiectasis, without predominantly basal distribution. Defined as a probable usual interstitial pneumonia (UIP) pattern.

UIP.

Our patient also presented a mutation of the H63D gene. Sangiuolo et al (4). compared the BAL of IPF patients with non-IPF patients, and demonstrated that iron-dependent oxygen radical generation was increased in IPF. Interestingly they also showed that the H63D HFE allele was significantly more frequent in IPF patients than in controls. Further studies are necessary to validate the hypothesis of iron-dependent oxidant generation association with the carriage of HFE allelic variants in IPF.

We performed a literature review to understand the rarity of this variant, its clinical significance, to understand whether AATD is associated with lung fibrosis and if the concomitant diagnosis of both conditions is usual, or not.

The patient's AATD was associated to a heterozygote $M_{Palermo}$ allele ($MM_{Palermo}$), with a p.Phe52del mutation (NM_000295.5(*SERPINA1*):c.221_223TCT[2] (p.Phe76del)). This allele is uncommon and shares similar characteristics to the M_{Malton} allele, both characterized by a p.Phe52del (NM_000295.5(*SERPINA1*):c.221_223TCT[2] (p.Phe76del)) mutation, however $M_{Palermo}$ is linked to M1Val213 allele, and the other to M2 basic allele (5).

Both variants share similar pathologic mechanisms, producing a poorly formed protein with loop-sheet polymers, which becomes sequestered in the endoplasmic reticulum of hepatocytes, resulting in low AAT serum concentration (6). The intracellular accumulation and reduced secretion of the protein increases the risk of hepatic disease, even in heterozigosity (5). This, associated with alcohol intake, may explain our patient's severe liver disease.

A Portuguese study on rare AAT variants, published by a national reference laboratory identified, between 2009 and 2013, twelve different rare variants among 51 patients, of which $M_{Palermo}$ was present in eight (15.7%) (5).

Ferrarotti et al. published an analysis of the Italian Registry for Severe AATD database over a 98-month span. From a total of 2922 subjects, rare alleles were identified in 37 patients with intermediate to severe AATD, of which 21 were heterozygote with a normal M allele. These showed a mean AAT level of 61 mg/dL, similar to our patient (7).

Calabró et al. reported a case of a ZZ patient with AATD and a pulmonary presentation of UIP pattern, without emphysema or bronchiectasis, and without liver involvement. It was also described how certain molecules (TNF-α, IL-1, metalloproteinases and cathepsins) overexpressed in IPF patients' BAL are also affected by AAT (3).

Michalski et al. conducted a study to determine whether AAT phenotypes were related to pulmonary fibrosis in rheumatoid arthritis (RA) and systemic sclerosis (SSc) patients. They concluded that RA patients with M_1M_2 phenotype were more likely to develop pulmonary fibrosis. The same was not true for SSc patients (8).

In AATD, neutrophil elastase degradation is markedly reduced, leading to an increase of its activity against lung matrix components and alveolar structures, resulting in the usual panlobar emphysema presentation (3). Neutrophil elastase activity is also increased in IPF, which is not surprising considering the usual high BAL neutrophil count in IPF patients (9,10).

Certain mediators overexpressed in IPF patients' BAL (TNF-α, IL-1, metalloproteinases and cathepsins) are also affected by AAT (3), and AAT is responsible for the elimination of reactive oxygen species produced by neutrophils via the NADPH oxidase enzyme as well (3). MUC5B promoter polymorphisms, a known genetic risk factor for IPF, lead to mucus hypersecretion and mucociliary dysfunction. This and other mucin genes are also upregulated by neutrophil elastase (11). Neutrophil elastase may also induce fibroblast proliferation and myofibroblast differentiation (12). All these mechanisms may indicate an increased tissue remodelling, which is characteristic of diseases such as IPF.

On a similar fashion, in AATD, the reduced AAT serum levels and consequential low inhibition of neutrophil elastase may result in similar lung damage, such as fibrosis.

Finally, an expert panel from Italy recently published a commentary on the ERS AATD statement, about respiratory disorders other than emphysema caused by AATD. The evidence is greater in bronchiectasis and asthma; however a brief reference to pulmonary fibrosis states its rarity in this context (13).

Regarding our patient's cirrhosis, he presented only a heterozygote mutation of H63D therefore not fulfilling diagnostic criteria for primary hemochromatosis. He presented a non-inherited form of the disease, defined as secondary iron overload or secondary hemochromatosis, where hepcidin deficiency

is consequential to conditions such as erythropoiesis disorders, chronic liver disease, metabolic syndrome or excessive alcohol intake (14). The clinical significance of H63D heterozigosity is controversial (15), since it occurs in about 13,6% of European population (4). Some authors claim it increases susceptibility to cirrhosis in alcohol liver disease and in viral hepatitis (4).

Cirrhosis aetiology, in our patient, was most likely the result of both moderate alcohol consumption and a rare AAT variant causing its accumulation in the hepatocytes.

Conclusion

We present a case of cirrhosis and pulmonary fibrosis, in a patient with past history of smoking, excessive alcohol intake and a rare genomic variant resulting in an AATD. This rare variant is associated with liver disease, and although in heterozigosity, combined with the alcohol consumption, is the likely cause of cirrhosis. Pulmonary fibrosis is a rare pattern for AATD, with few documented cases. More studies are required to understand whether AATD plays a role in pulmonary fibrosis physiopathology, or if the development of an independent lung disease such as IPF is coincidental.

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SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (4); e2020013 DOI: 10.36141/svdld.v37i4.9934

Transbronchial lung cryobiopsy in smoking-related interstitial lung diseases

Margarida Barata[1], Patrícia Caetano Mota[2,3], Natália Melo[2], Hélder Novais Bastos[2,3], Susana Guimarães[3,4], Conceição Souto Moura[3,4], José Miguel Pereira[3,5], António Morais[2,3]

[1] Pulmonology Department, Hospital Garcia de Orta, Almada, Portugal; [2] Pulmonology Department, Centro Hospitalar Universitário de São João, Porto, Portugal; [3] Faculty of Medicine of Porto University, Porto, Portugal [4] Pathology Department, Centro Hospitalar Universitário de São João, Porto, Portugal; [5] Radiology Department, Centro Hospitalar Universitário de São João, Porto, Portugal.

Abstract. *Background:* Transbronchial lung cryobiopsy (TBLC) is an emerging technique in the diagnostic approach to diffuse parenchymal lung diseases. However, the role of TBLC in smoking-related Interstitial Lung Diseases (ILDs) is still under discussion. *Objectives:* The aim of the present study was to describe our experience with TBLC in diagnostic work-up of patients with smoking-related ILDs. *Method:* We retrospectively reviewed data of patients evaluated in a tertiary hospital ILDs outpatient clinic, who underwent TBLC, from September 2014 to December 2019. TBLC was performed in accordance with the 2018 expert statement from the Cryobiopsy Working Group. *Results:* Forty-five patients (25 men [55.6%]) with a mean age of 53.9 years [SD, 9.1] were included. The most frequent radiological pattern was ground glass opacity (42 patients). TBLC was performed in different segments of the same lobe in 38 patients and in two lobes in 7 patients. The mean maximal diameter of the samples was 5.2 mm (range, 3–16 mm [SD 2.0]). Pneumothorax occurred in seven patients (15%) and moderate bleeding occurred in one patient. A specific pathological diagnosis was achieved in 43 of 45 patients. The most frequent histopathologic pattern found was desquamative interstitial pneumonia (33 patients), followed by smoking-related interstitial fibrosis (7 patients), respiratory bronchiolitis - ILD (1 patient) and pulmonary Langerhans cell histiocytosis (1 patient). Two patients had alternative diagnosis (Pneumoconiosis and Interstitial Pneumonia with unspecific features) and one patient had normal lung parenchyma. A definitive multidisciplinary team (MDT) diagnosis was reached in 95.5% (43 of 45 cases). Two patients were submitted to additional diagnostic techniques. *Conclusions:* The results from this series support TBLC as a safe procedure with a meaningful diagnostic value in the context of a MDT approach of smoking-related ILDs. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (4): e2020013)*

Key words: transbronchial lung cryobiopsy, smoking-related interstitial lung diseases, diagnostic yield, complications, diffuse lung diseases, surgical lung biopsy

Introduction

Cigarette smoking is implicated in a heterogeneous spectrum of diffuse parenchymal lung diseases (DPLD) referred to as smoking-related interstitial lung diseases (ILDs) (1-3). Lung diseases that have a causal association with tobacco exposure include respiratory bronchiolitis - interstitial lung disease (RB-ILD), desquamative interstitial pneumonia (DIP), pulmonary Langerhans cell histiocytosis (PLCH) and acute eosinophilic pneumonia (AEP) (3). Smoking-related interstitial fibrosis (SRIF) is a relatively newly appreciated entity, with distinct histopathologic features that has gained prominence. Its clinical

Received: 28 May 2020
Accepted after revision: 2 November 2020
Correspondence: Margarida Baptista Duarte Barata
Department of Pulmonology, Hospital Garcia de Orta
Av. Torrado da Silva. City, State,
Almada, 2801-951, Portugal
E-mail: margarida.barata7@gmail.com

ramifications and radiologic features are still unclear, however it should be considered in differential diagnosis of RB-ILD and DIP (4-5).

With exception of AEP that has an acute onset, on clinical presentation, patients usually complain about insidious dyspnea and cough over the course of weeks to months (1). On imaging, RB-ILD shows upper lung predominance of the findings, characterized by the evidence of low attenuation centriacinar nodules and ground-glass opacities (GGOs). The radiological findings in DIP are lower lobe predominant and characterized by GGOs and reticular opacities interposed with relatively normal lung zones. PLCH presents with a mix of nodules and bizarre shaped cysts on upper lobes. Imaging findings of AEP are predominant in the lower lungs, showing diffuse consolidations, GGOs and ill-defined centrilobular nodules (1,3,6).

The current approach of smoking-related ILDs includes a multidisciplinary team (MDT) diagnosis combining clinical, radiological, and pathologic features (7-9).

In smoking-related ILDs, namely in RB-ILD, PLCH and AEP, in active smokers with suggestive clinical, radiological and bronchoalveolar lavage (BAL) features, lung biopsy is usually not required (3). Attempts at obtaining tissue should be reserved for ambiguity in diagnosis.

Surgical lung biopsy (SLB) has been considered as the definitive mean of obtaining adequate biopsy specimens. However, side effects of SLB such as prolonged air leakage, infections, acute exacerbation and death, are not negligible (10-12).

Transbronchial Lung Cryobiopsy (TBLC) have been introduced since 2009 by Babiak et al. as an alternative to SLB in the diagnostic approach to DPLD (13). A growing body of evidence suggests the utility of TBLC in the diagnostic algorithm of ILD as it allows, compared to transbronchial lung biopsy with conventional forceps, a better identification of complex histological patterns and can provide information which seems to have a clinical impact on the MDT discussion similar to that provided by SLB (14-22). Additionally, if performed correctly, it appears to have a better safety profile than SLB (15, 16, 23-25).

Although several series evaluating TBLC diagnostic yield contain patients with smoking-related ILDs, the role of TBLC in smoking-related ILDs is still a topic under discussion.

Concerning the regular use of TBLC in MDT diagnostic evaluation, the aim of this study was to find its diagnostic accuracy and safety in patients with suspected smoking-related ILDs, based on clinical, radiological and BAL features.

Materials and Methods

Design

We conducted a retrospective review of the medical records of patients undergoing TBLC from September 2014 to December 2019 at the bronchoscopy unit of the pulmonology department at Centro Hospitalar São João (Porto, Portugal). The ethics committee approved this study.

Patients

All the patients had a prior evaluation at the ILD outpatient clinic where a detailed history, complete physical examination, in addition to laboratory tests, lung function, thoracic HRCT were taken. The patients' electronic medical records were assessed retrospectively, and the following data were collected: demographic data, drug and occupational history, thoracic HRCT, BAL results, procedure details and complications and pathology reports.

The diagnosis of smoking-related ILDs was based on a multimodality approach that combined clinical, radiological and BAL features. Over the period examined, all patients with suspected smoking-related ILDs were discussed on a MDT meeting, composed by clinicians, radiologists and pathologists with a long time experience on ILD. They were proposed for TBLC only when clinical, radiological findings and BAL features did not conclude a final diagnosis and a biopsy was deemed useful for a diagnosis. After TBLC procedure, there were a second MDT meeting where clinical information, radiological features and biopsy results were then reviewed and a multidisciplinary diagnosis was made, with cryobiopsy considered diagnostic if additional evaluation was considered to be unnecessary.

Procedure

The TBLC procedure was performed according to 2018 expert statement (26). All TBLC were

performed by a senior bronchoscopist. As previously described by Almeida et al. (24), it was used a combination of rigid bronchoscopy (tracheoscope 14mm, Karl Storz, Germany) and flexible bronchoscopy (Olympus BF-XT40, Europe) under general anesthesia with manual jet ventilation (working pressure of approximately 2 bar). A 2.4 mm cryoprobe (ERBE, Germany) was introduced through the working channel of the flexible bronchoscope. Biopsies were taken under fluoroscopic guidance from an optimal distance between the probe and the thoracic wall of 10 mm. Biopsy sites were selected based on HRCT abnormalities evaluated in MDT discussion. Once brought into position, the probe was cooled to –85°C with nitrogen oxide for approximately 5–6s, thus freezing the lung tissue in contact with the probe. The frozen specimen attached to the tip of the probe was removed by pulling out the cryoprobe together with the bronchoscope. In all procedures, a Fogarty balloon was always routinely used to prevent severe bleeding. When bleeding occurred, the Fogarty balloon was deflated only after cessation of bleeding and before any additional biopsies were performed. The samples, still attached to the probe, were first inserted in saline and then in formalin. Following the procedure, patients were extubated and kept under observation. After 3h, a chest X-ray was performed to exclude pneumothorax.

Written informed consent was obtained before TBLC from all patients.

Complications

Pneumothorax was described according to observation measures or chest tube insertion requirements. Endobronchial bleeding was defined using the British Thoracic Society system as mild bleeding (requiring suction to clear but no other endoscopic procedures), moderate bleeding (requiring endoscopic procedures like bronchial occlusion-collapse and/or instillation of ice-cold saline), and severe bleeding (causing hemodynamic or respiratory instability, requiring tamponade or other surgical interventions, transfusions or admission to the intensive care unit).

Clinical, Radiological, and Functional Assessment and BAL

The HRCT pattern was classified as micronodular, ground-glass opacity, reticulation and emphysema according to the glossary of the Fleischner Society (27). Pulmonary function tests were performed in accordance with the standard recommendations of the American Thoracic Society (ATS) and European Respiratory Society (ERS) and findings categorized as normal, obstructive, restrictive, or mixed abnormalities (28). BAL was performed following the recommendations of the ERS (29). BAL cellular patterns were classified as lymphocytic (>15%), neutrophilic (>3%), and eosinophilic (>1%).

Pathologic assessment

The sample tissue was formalin-fixed for at least 6h and at most 24h before paraffin embedding. Sections measuring 3μm were stained with hematoxylin–eosin. In all cases serial cuts were made at three levels, with additional use of special stains or immunohistochemical stains when necessary. All samples were measured under the optical microscope and the area of each fragment evaluated. The presence of pleural tissue was recorded. The artifact areas were included in the measurement, however when the fragments were only pleura or adipose tissue they were not measured.

The biopsy was considered adequate if at least one of the fragments consisted of alveolated lung parenchyma. The diagnosis was made based on the same criteria used for SLB.

Statistical Analysis

Descriptive statistics were used to analyze patient characteristics. Normally distributed continuous data were described as means and SD. The categorical variables were reported in percentages of total subjects. The data were analyzed using SPSS software (version 25; IB, Armonk, NY USA).

Results

Forty-five patients were included in the study cohort with a mean age of 53.9 years (range, 31-74 years [SD, 9.1]). Of these, 25 were men (55.6%) and all had a history of smoking. Pulmonary function testing revealed ventilatory abnormalities in 36.6% of subjects. Diffuse lung capacity for carbon monoxide (DLCO) was impaired in 87.8% of subjects, in

whom the mean percentage of DLCO was 52% of predicted (SD, 10). Mean PaO2 was 79 mmHg (SD 10). The most frequent HRCT pattern was ground glass opacity present in 42 patients. A BAL with total and differential cell counts was performed in 37 patients (82.2%) and eosinophilia was the most frequent feature (table 1).

TBLC was performed in different segments of the same lobe in 38 patients and in two lobes in 7 cases. The median number of samples per procedure

Table. 1. Demographic and clinical characteristics of patients.

Variable (n = 45)	No.
Age at diagnosis, mean (SD), y	53.9 (9.1)
Sex, male (%)	25 (55.6)
Smoking habits, %	
Smoker	94.8
Ex-Smoker	15.2
Lung function pattern, %	
Normal	63.4
Obstructive	29.3
Restrictive	7.3
Diffuse lung capacity for carbon monoxide	
Decrease, %	87.8
Percentage of predicted, mean (SD)	52 ± 10
Normal, %	12.2
Percentage of predicted, mean (SD)	82 ± 13
PaO_2, mean (SD), mmHg	79 (10)
Bronchoalveolar lavage cellular pattern, (n = 37), No., %	
Normal	9 (24.3)
Eosinophilic	14 (37.8)
Eosinophilic and Neutrophilic	10 (27.1)
Neutrophilic	4 (10.8)
Predominant high-resolution CT pattern, (n = 45), No., %	
Ground-glass	27 (60.0)
Ground-glass + Emphysema	9 (20.0)
Ground-glass + Micronodularity	4 (8.9)
Micronodularity	2 (4.5)
Cystic	1 (2.2)
Ground-glass + Cystic	1 (2.2)
Ground-glass + Reticulation	1 (2.2)
No. of specimens per procedure (n = 45), median (range, SD)	3 (1-5, 1)
Diameter (n = 45), mean (range, SD) mm	5.2 (3-16, 2.0)
Area (n = 45), mean (range, SD) mm^2	17.5 (5-35, 7.3)
No. of pleura per patient (%)	16 (36)
No. of Pneumothorax (%)	7 (15)
No. of Moderate Bleeding (%)	1 (2)

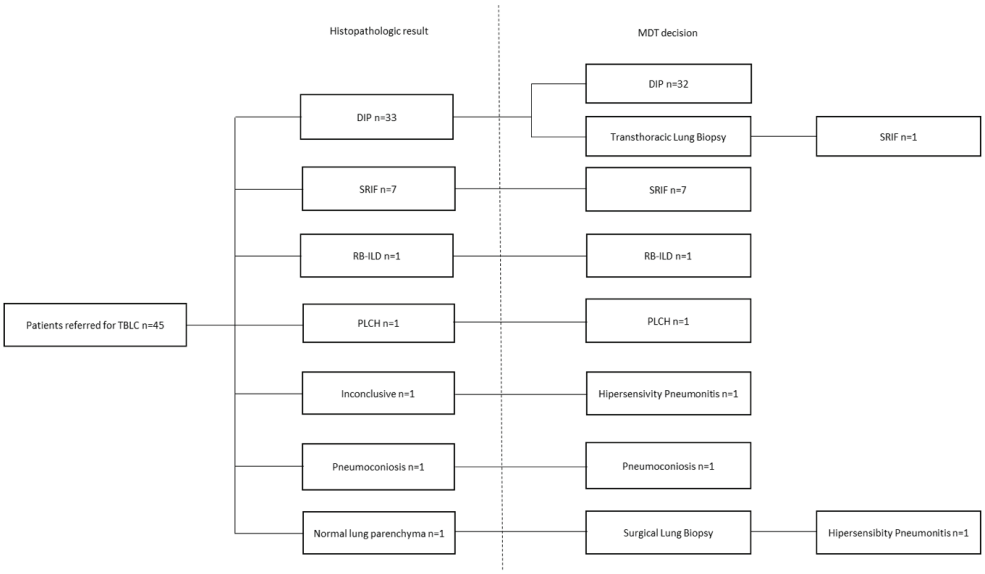

Figure 1. Diagrammatic representation of the diagnostic performance of patients with suspected Smoking Related ILD. DIP (Desquamative Interstitial Pneumonia), ILD (Insterstitial Lung Diseases), MDT (Multidisciplinary Team), PLCH (Pulmonary Langerhans Cell Histiocytosis), RB-ILD (Respiratory Bronchiolitis - Interstitial Lung Disease), SRIF (Smoking-Related Interstitial Fibrosis), TBLC (Transbronchial Lung Cryobiopsy).

was three (range, 1-5). All the samples were considered adequate. The mean sample length was 5.2 mm (range, 3–16 mm [SD, 2.0]), and the mean area was 17.5 mm^2 (range, 5-35 mm [SD, 7.3]). Histologic slides were available for review by two lung pathologists before the MDT discussion. The presence of pleura was observed in 16 patients (36%).

The most frequent histopathologic pattern found was DIP (33 patients), followed by SRIF (7 patients), RB-ILD (1 patient) and PLCH (1 patient). In one patient the histopathologic pattern was suggestive of interstitial pneumonia with unspecific features, and in another it was suggestive of pneumoconiosis. One patient had normal lung parenchyma in cryobiopsy (Figure 1).

In the MDT, correlation of clinical, radiological and histopathologic findings yielded a definite diagnosis in 43 patients. Forty-one patients had a diagnosis of smoking-related ILD. One patient had a working diagnosis of Hypersensitivity Pneumonitis and another one the diagnosis of Pneumoconiosis.

There was a patient whose thoracic HRCT showed images of diffuse ground-glass, with centrilobular nodules and cystic lesions. The cryobiopsy histopathologic features were suggestive of DIP. Due to the discrepancy between radiological and histopathologic features, a CT transthoracic lung biopsy was performed that showed SRIF features, which was in accordance with CT imaging.

The patient which cryobiopsy revealed normal lung parenchyma was submitted to surgical lung biopsy that was suggestive of Hypersensitivity Pneumonitis and was treated adequately.

Of the patients with a diagnosis of smoking related ILD, 10 were lost for follow up, since they had been followed in a different institution. Of the 32 patients in our ILD's outpatient clinic, all of them were advised to participate in smoking cessation counselling and 10 patients initiated corticosteroid therapy. The mean time of follow up were 30 months (range, 9 - 69). To the date of submission, no patients were referred to lung transplant.

Complications

Pneumothorax was the most common complication, observed in 7 patients (15%). Of these, 5 patients had pleura in the histologic sample. None of them had concomitant emphysema. In 4 cases it was necessary to insert a chest drain; the remaining cases improved spontaneously, without intervention. Mean prolonged hospital stay due to this complication was about 2.8 ± 2 days. There were several cases of mild bleeding, but these were not documented as we considered them to be standard events for this procedure. One patient had moderate bleeding, that motivated a hospital stay of 2 days. None of these events was reported as life-threatening. No acute exacerbation of the underlying condition was observed.

Discussion/Conclusion

We found that TBLC proved to be an accurate and safe procedure for the diagnosis of smoking-related ILDs. A specific pathological diagnosis was achieved in 43 of the 45 patients and a definitive MDT diagnosis was confirmed in 43 of 45 patients (95.5%). The concordance between pathologists' impression and MDT diagnosis was of 93.3%. Only two patients were submitted to other diagnostic techniques (transthoracic lung biopsy and SLB) after TBLC, due to discrepancy between radiological and histopathologic findings.

A consensus clinical diagnosis reached by a MDT discussion is currently the gold standard when establishing a diagnosis of DPLD (7-9). This approach has significantly improved diagnostic accuracy, overall agreement, and diagnostic confidence (7, 8). When smoking-related ILDs are suspected, the information recorded (clinical scenario, lung function, BAL data and HRCT scan features) must be thought-fully reviewed and analyzed (2, 3). More invasive procedures are considered only when these investigations are considered to be insufficient to provide a confident diagnosis (2). The role of SLB is well established in DPLD, and in most cases, a confident diagnosis was reached in more than 90% of patients (29-31). However, numerous studies have reported the risks of a SLB in ILDs. More recent reviews, albeit heterogeneous in their study composition, report a 30-day mortality of around 2% (10-12). To the best of our knowledge, there aren't any studies specifically dedicated to the SLB on smoking-related ILDs.

The introduction of TBLC as a promising and safer alternative to SLB is generating considerable interest in the pulmonary community. Indications for TBLC in diagnostic algorithm of DPLD are currently under evaluation, and a standardization of this technique is imminent. Most published data on clinical usefulness of TBLC to date are on fibrosing DPLD, namely in Usual Interstitial Pneumonia (UIP) / Idiopathic Pulmonary Fibrosis (IPF). In this context, this technique has been reported to be significantly accurate (14, 16). Less high-quality evidence is available for other patterns such as Non-Specific Interstitial Pneumonia or DIP. However, the combination of morphological information provided by TBLC and BAL profiles (e.g., an increase of "smoker" macrophages and eosinophils in patients with DIP or the presence of granulomatous-like nodules composed mainly of histiocytes with a positive stain for CD1a antigen in patients with PLCH) along with clinical profile and HRCT features might be diagnostic and avoid SLB (25-26, 33).

Few data are available on the specific role of TBLC for the diagnosis of smoking-related ILDs. There is a small case series reporting only five cases of DIP diagnosed through TBLC (27). The largest series of patients (699 patients) with suspected DPLD that were submitted to TBLC reported 36 cases of DIP/RB-ILD and 7 cases of PLCH, after an MDT discussion (28). To the best of our knowledge, this is the first study dedicated specifically to the role of TBLC in patients with suspected smoking-related ILDs.

In our study, the diagnosis based on MDT discussion was reliable with high confidence in 43 of 45 patients (95.5%). Only two patients were submitted to other diagnostic techniques. One patient with a suspected SRIF/PLCH (a history of cigarette smoke with a HRCT revealing ground glass and thin-walled cysts) with a histology of DIP and CD1a stain negative was submitted to Transthoracic Lung Biopsy, due to discrepancy between radiological and histopathologic diagnosis. Other patient with a suspected DIP (smoking history and GGOs on HRCT) with a cryobiopsy with normal lung parenchyma, was submitted to SLB that was suggestive of Hypersensitivity Pneumonia. The diagnostic yield of TBLC in our study is higher than that previously reported in overall DPLD series. Despite methodological differences, diagnostic yield in previous studies, varied from 51% to 98%, with a pooled estimate of 79% (95% CI, 65-93) (32-33). One possible explanation is that in contrast with UIP, DIP and SRIF have a uniform involvement (3-6). On the other hand, in case of PLCH a specific histological diagnosis can be made instead of an identification of a pattern of injury (e.g., UIP), increasing the diagnostic yield of a TBLC sample (3).

Complications of TBLC were detected in 17% of patients, which is consistent with the literature. Pneumothorax was the most common complication observed in our series, occurred in 15% of cases (in 4 cases it was necessary to insert a chest drain). Reported incidence rates vary considerably (0 to 30%), possibly due to differences in procedure or in types of disorders (16, 32-33). This complication rate could be related to our approach, as we usually take sam-

ples from a distance of 10mm from the pleura, or it might also be explained by the presence of associated emphysema in smoking-related ILDs. Bleeding is a relatively frequent event in TBLC, but the use of prophylactic placement of a bronchial blocker allows for immediate tamponade without further positioning maneuver (25, 33). Only one patient (2%) experienced moderate bleeding, which was managed by standard flexible bronchoscope techniques (e.g., scope tamponade, iced saline). No severe bleeding was observed in our cohort. Bleeding rates for moderate/severe bleeding associated with TBLC vary considerably in the literature, with rates ranging from 0% to 78%. This wide range can probably be explained by the use of different approaches and classification systems (32, 33). None of the events that occurred in our cohort were considered life-threatening.

Our study is limited by its retrospective design. Moreover, it is the result of the experience of only one centre, so data inevitably reflects its specific clinical and technical methodology. On the other hand, it contains a small number of patients, due to the fact that smoking-related ILDs encloses a group of rare diseases and a significant number of cases are diagnosed without histology requirement. Additionally, some of these patients are heavy smokers with poor lung function and other comorbidities that contraindicates invasive procedures. However, the availability of TBLC with lower morbidity compared to SLB may extend the indications for lung biopsy.

As a conclusion, our single-center cohort demonstrated that TBLC has a meaningful diagnostic value (95.5%) in the context of an MDT approach of smoking-related ILDs and should be considered a reliable diagnostic tool in this particular scenario.

Statement of Ethics: The ethics committee of Centro Hospitalar Universitário de São João in Porto approved this study.
Written informed consent was obtained before TBLC from all patients.

Disclosure Statement: The authors have no conflicts of interest to declare.

Funding Sources: The authors declare that no funding was received for this paper.

Author Contributions: M. B. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. M. B. contributed to the conception and design; collection, analysis, and interpretation of data; drafting and critical revision of the article. H. N. B. contributed to the analysis and interpretation of data. S. G. contributed to the analysis and interpretation of data. P. C. M. contributed to the analysis and interpretation of data. N. M. contributed to the analysis and interpretation of data. C. S. M. contributed to the analysis and interpretation of data. J. M. P. contributed to the analysis and interpretation of data. A. M. Substantial contribution to conception and design, analysis and interpretation of data, drafting the article, and finalizing the version to be published. All authors approved the final article.

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SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (4); e2020010 DOI: 10.36141/svdld.v37i4.10188

Subcutaneous sarcoidosis on the digits

Natsumi Norikawa, Toshiyuki Yamamoto
Department of Dermatology, Fukushima Medical University

Case Report

Subcutaneous sarcoidosis is a relatively rare type of specific lesion of cutaneous sarcoidosis, mainly affecting the lower extremities. We describe a case of subcutaneous sarcoidosis arising on the digits as an initial manifestation.

A 47-year-old female visited our department complaining of subcutaneous nodules on the digits without tenderness that appeared and increased in number one year previously. She had been working as a truck driver. She had type 2 diabetes and cervical cancer operated 5 years previously. Physical examination showed that multiple subcutaneous nodules were found on the first to third digits of her dominant hand (right hand) (Figure 1). Histological examination showed non-caseating epithelioid cell granulomas in the lower dermis and subcutis with multinucleated giant cells, and mononuclear cell infiltration (Figure 2,3). Detailed physical examination revealed subcutaneous nodules on the bilateral forearms, and scar sarcoidosis on the knees. Laboratory tests showed increased levels of angiotensin-converting enzyme (34.2 U/l, normal 7 to 25), and soluble Interleukin-2 receptor (883 U/ml, normal 121 to 613). Blood chemistry data showed increased levels of serum aspartate aminotransferase (44 U/L, normal 13 to 30), alanine transaminase (37 U/L, normal 7 to 23), and normal renal function. Chest X-ray showed bilateral hilar lymphadenopathy (BHL). CT scan showed enlarged bilateral hilar lymph nodes and mediastinum adenopathy. Bronchoscopic biopsy revealed non-caseating epithelioid granulomas with mononuclear cell infiltration and Zeihl-Neelsen stain was negative. Detailed examination including electrocardiogram and echocardiography excluded cardiac sarcoidosis, and ophthalmological examination did not reveal ocular sarcoidosis.

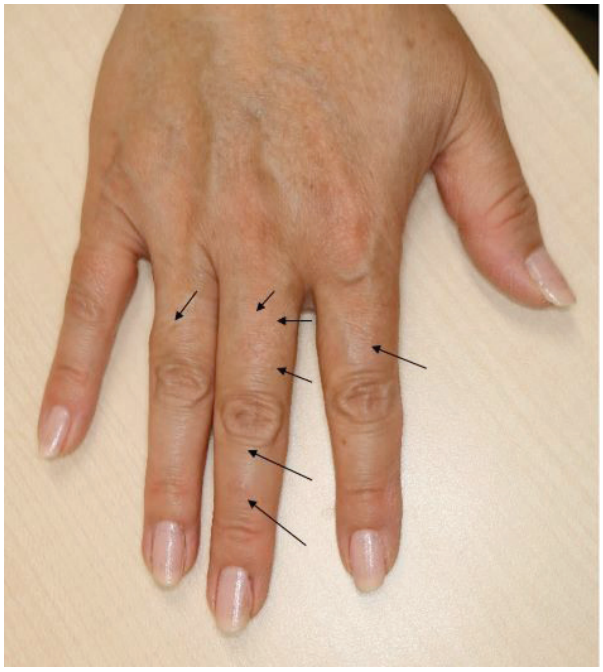

Fig. 1. Multiple subcutaneous nodules on the dorsum of the fingers.

Received: 9 July 2020
Accepted after revision: 26 October 2020
Correspondence: Natsumi Norikawa
Department of Dermatology, Fukushima Medical University,
Hikarigaoka 1, Fukushima 960-1295, Japan
E-mail: nnatsumi@fmu.ac.jp

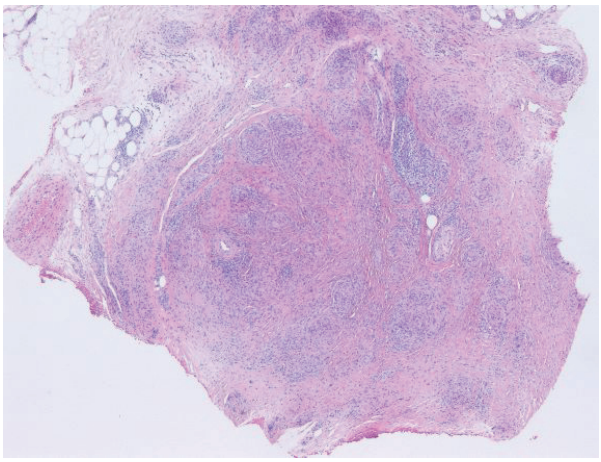

Fig. 2. A biopsy specimen from the right index finger shows non-caseating epithelioid cell granulomas with multinucleated giant cells in the lower dermis and subcutis. (H&E stain, original magnification ×200, and ×400)

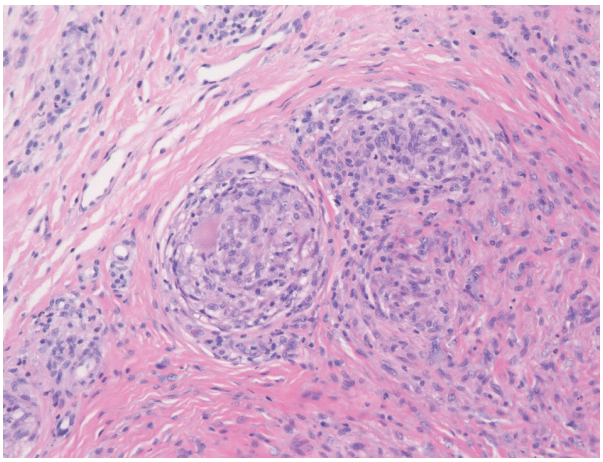

Fig. 3. A biopsy specimen from the right index finger shows non-caseating epithelioid cell granulomas with multinucleated giant cells in the lower dermis and subcutis. (H&E stain, original magnification ×200, and ×400)

The frequency of subcutaneous sarcoidosis has been estimated between 1.4 and 6% of the patients with systemic sarcoidosis (1,2). By contrast, a recent study has shown that subcutaneous sarcoidosis was observed in 11.8% (10/85) of specific cutaneous sarcoidosis cases (3). Therefore, cases of subcutaneous sarcoidosis may exist at higher frequency than was expected. According to a previous report on subcutaneous sarcoidosis, there is a female predominance and extremities are frequently involved (4). By contrast, cases of subcutaneous sarcoidosis occurring on the acral sites are rare, and only a few cases of digital occurrence have been reported to date (5-7). The previously reported cases of acral subcutaneous sarcoidosis is shown in Table 1. All cases were in around middle-aged adults, and there was no gender predominance. Dactylitis of the fingers was accompanied except for the present case. Bilateral hilar lymphadenopathy and mediastinal lymphadenopathy were observed in all cases except for 1 unknown case, suggesting that acral subcutaneous sarcoidosis is closely related to lung sarcoidosis. Other organ involvement, *i.e.* ophthalmological and cardiac sarcoidosis, were not observed, respectively.

The present case developed subcutaneous nodules on the digits as an initial manifestation, and detailed examination revealed similar subcutaneous nodules on the forearms, as well as pulmonary sarcoidosis. Our case suggests the need of histological examination when diagnosing patients presenting with acral subcutaneous nodules. Finally, our patient was a truck driver for a long time, and engaged in loading and unloading of heavy loads, and thus frequently used her hands. The present case developed subcutaneous nodules on her dominant hand, which may suggest Köbner phenomenon in sarcoidosis (8).

Table 1. Reported cases of acral subcutaneous sarcoidosis

Source	Age	Sex	Duration	Location of Lesions	Systemic Sarcoidosis
Curco N et al (5)	60	F	3 months	second finger on both hands, legs and forearms	BHL and mediastinal adenopathy
Morganroth PA et al (6)	52	M	No data	left second finger and right fourth finger, trunk and arms	BHL and mediastinal adenopathy
González-Cantero Á et al (7)	35	M	6 months	right second finger	No data
Present Case	47	F	1 year	first to third fingers, both forearms	BHL and mediastinal adenopathy

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Investigation of sarcoidosis patients during COVID-19 pandemic

Arda Kiani[1], Fatemeh Razavi[2], Negar Bandegani[2], Mehrdad Farahani[3], Atefeh Abedini[2]

[1]Tracheal Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran; [2]Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran; [3]Departemant of Interventional Pulmunology, Tracheal Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran

To the Editor,

Coronavirus disease 2019 (COVID-19), is a globally spread contagion caused by a viral pathogen from the coronavirus family called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)(1). The epidemic originated in Wuhan, China, and was first detected in December 2019, and announced as a global pandemic by the World Health Organization's (WHO) in March 2020 (2).

Sarcoidosis is a multiorgan, inflammatory, granulomatous disease that can affect different tissues, but the lungs are involved in more than 90% of the cases (3).

All human beings are prone to Sars-CoV 2 virus, which can cause respiratory tract infection. Notably, in most cases of sarcoidosis patients, the respiratory system is already damaged and inflamed. Additionally, most of the sarcoidosis cases are treated with immunosuppressive therapies, which make them more susceptible to infections, including coronavirus infections. Hence, we were expecting a high number of sarcoidosis patients contracting COVID-19.

Surprisingly, at Masih Daneshvari Hospital, despite the massive load of COVID-19 patients from 15.02.2020 until 10.04.2020 (300 patients with Covid-19), we have only encountered 10 cases of COVID-19 positive patients among all registered cases in the Sarcoidosis clinic of The National Research Institute of Tuberculosis and Lung Diseases (NRITLD) with approximately 1000 followed-up patients (Table 1). Our findings are in line with Conticini et al, cohort study in which 859 patients with different rheumatic diseases who were treated with disease-modifying antirheumatic drugs (DMARDs) were studied in Italy. Among those two patients were diagnosed with COViD-19 (4).

It is worth to mention a patient of ours with sarcoidosis that has been under the treatment regimen, including methylprednisolone 5mg daily and 7.5 mg Methotrexate (MTX), for years. Her husband passed away due to COVID-19, that is while her test results were repeatedly negative for SARS-COV-2.

There are several hypotheses in this regard.

• Firstly, Angiotensin-converting enzyme-2 (ACE2) is a type of integral membrane glycoprotein. ACE2 that is expressed in the lungs and serves as the main entry point into cells for some coronaviruses (5) is involved in the progression of pulmonary sarcoidosis (6). That is while the presence of the ACE2 enzyme protects lung cells from damage caused by the virus by increasing the level of the angiotensin-1-7 (7).

• Secondly, sarcoidosis patients apply more self-protection due to the history of chronic disease. It is possible that their extra precautions reduce the risk of being infected.

Received: 21 April 2020
Accepted after revision: 22 November 2020
Correspondence: Atefeh Abedini
ORCID ID: 0000-0002-3821-7016
Chronic Respiratory Disease Research Center, Masih Daneshvari Hospital, Shaheed Bahonar Ave, Tehran, Iran
PO Box: 19569-44413
E-mail: dr.abedini110@gmail.com

• Moreover, their regimen consists of combinations of multiple medicaments including: methylprednisolone, Methotrexate (MTX), hydroxychloroquine, ect. Although it is not currently known how exactly these medicaments affect the COVID-19 infection, some of these medications suppress signs and symptoms of the disease; therefore, sarcoidosis patients taking them might be asymptomatic carriers of COVID-19 and pose a threat to their family and the society.

• And finally, the sarcoidosis treatment regimen may have protective effects against COVID-19.

In order to investigate all probable hypotheses in this regard, we are already conducting a study in which we follow up on our sarcoidosis patients regarding all their signs and symptoms that have developed recently. Herewith, we hope to find a new perspective on this disease.

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Table 1. Data of the 10 patients with sarcoidosis and positive polymerase chain reaction COVID-19 test

Gender	Age	Duration Of Disease (sarcoidosis) (year)	Positive Covid-19 test In Family	Hospitalization	Prednisolone	Methotrexate	Hydroxychloroquine	Chills	Fever	Myalgia	Loss Of Appetite	Olfactory Disorder	Taste Disorder	Chronic Disease	Death
Male	31	2	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	No	No	No	No
Female	46	2	Yes	No	Yes	No	No	No	Yes	Yes	Yes	No	No	No	No
Female	50	1	No	No	Yes	Yes	Yes	No	No	Yes	Yes	No	No	DM	No
Female	53	10	Yes	No	Yes	No	No	Yes	Yes	No	No	No	No	No	No
Male	43	8	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No	IHD	No
Female	34	10	No	No	Yes	Yes	Yes	No	No	No	No	No	No	No	No
Female	53	6	Yes	No	Yes	No	No	Yes	Yes	Yes	No	No	No	HTN	No
Female	51	8	Yes	No	Yes	No	No	Yes	Yes	Yes	No	No	No	Hypothyroidism	No
Male	48	3	Yes	No	Yes	No	Yes	No	No	Yes	Yes	Yes	No	No	No
Male	42	3	Yes	No	Yes	No	No	No	Yes	Yes	Yes	No	No	No	No

DM: diabetes mellitus type 2; IHD: ischasmic heart-disease; HTN: hypertension