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Presence of onconeural antibodies in sarcoidosis patients with parasarcoidosis syndrome

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Abstract. *Background:* Bothersome symptoms from sarcoidosis can develop in the absence of identified granulomas. These parasarcoidosis complaints can include small fiber neuropathy, diaphoresis, dysautonomia, and fatigue. Similar issues are also encountered in some cancer patients, especially those with onconeural antibodies. *Methods:* Serum was obtained for onconeural antibody testing from sarcoidosis patients with parasarcoidosis symptoms seen at the University of Cincinnati Sarcoidosis clinic during a six month period. Detection of antibodies was performed using an onconeural antibody panel. *Results:* A total of 268 patients with sarcoidosis and one or more features suggesting parasarcoidosis symptoms were enrolled in the study. Of these, 60 (22.4%) had one or more positive onconeural antibodies. In a control group of 46 non sarcoidosis patients seen in the interstitial lung disease clinic, there were only three patients with a positive antibody (Chi square=6.143, p=0.0132). A subgroup of sarcoidosis and control patients completed the small fiber neuropathy screening list. Sarcoidosis patients had a significantly higher score than the control patients (sarcoidosis: 7 [0-49] (Median [range] versus non sarcoidosis: 3 [0-31], p=0.0074). However, no significant differences were measured in the SFNL scores for sarcoidosis patients with an onconeural antibody (9 [3-36]) versus without (7 [0-49]). *Conclusion:* In patients with parasarcoidosis symptoms, approximately 30% have evidence of onconeural antibody production. This may be a potential cause for parasarcoidosis symptoms in some patients. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (4): 254-260)*

Key words: paraneoplastic, onconeural, parasarcoidosis, fatigue, small fiber neuropathy

Introduction

The etiology of the granulomatous process sarcoidosis remains elusive. The hallmark of sarcoidosis, the granuloma, can affect any organ of the body. However, many bothersome symptoms of sarcoidosis can develop in the absence of identified granulomas, and this entity has been termed parasarcoidosis (1). The symptoms of parasarcoidosis can include small fiber neuropathy, diaphoresis, dysautonomia, and fatigue (2). Because these perplexing manifestations usually do not respond well to anti-inflammatory therapies, treatment is usually targeted to relieve symptoms such as neurostimulants for fatigue (3) and pain management for small fiber neuropathy (4).

This phenomenon parasarcoidosis is very similar to the paraneoplastic syndrome which can develop in some cancer patients. Similarities exist between parasarcoidosis and paraneoplasia including the development of symptoms in the absence of identified disease activity and the types of complaints encountered including neuropathy, autonomic dysfunction,

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and other symptoms (5). Recently several onconeural antibodies have been associated with the paraneoplastic syndromes (6). In some cases, treatment with rituximab has reduced paraneoplastic symptoms, presumably by dampening antibody production (7, 8).

We investigated the frequency of onconeural antibody production in sarcoidosis patients with one or more parasarcoidosis symptoms. We also evaluated possible sarcoidosis phenotype and previous drug therapy correlations with antibody production.

Methods

Over a two year period of time, serum was collected for onconeural antibody testing from sarcoidosis patients seen at the University of Cincinnati Sarcoidosis Clinic who reported one or more parasarcoidosis symptoms including numbness, weakness, orthostasis, diaphoresis, or persistent fatigue. No patient had known malignancy or underlying neurological disease. Additional information collected included age, race, and sex. For the sarcoidosis patients, organ involvement was determined using the established WASOG criteria (9), and anti-inflammatory therapy usage for sarcoidosis was recorded. The study was approved by the University of Cincinnati Institutional Review Board.

Testing for onconeural antibodies was performed using a reference laboratory (Mayo Clinic Laboratory, Rochester, Minn, USA) and their established reference values. Using established criteria, results were classified as positive (one or more antibodies in the abnormal range), negative (no abnormal antibodies detected) or borderline (one or more antibodies detected but in the normal range). All patients with only borderline results were considered negative. Some patients with positive onconeural antibodies also had borderline levels of other antibodies detected; however, the borderline levels were considered negative.

During the two month period March to May 2019, all sarcoidosis patients were asked to complete the small fiber neuropathy screening list (SFNL) questionnaire (2) and results of onconeural antibody testing were noted. In addition, a control group of non sarcoidosis patients with either known malignancy or neurologic disease who were undergoing routine phlebotomy were asked to complete the SFNL questionnaire and undergo onconeural antibody testing. The answers to the SFNL were summed with value scale ranging from 0 (no symptoms) to 50 (symptoms all the time or severe intensity). Previously, a score of ≥11 was considered borderline positive for the presence of small fiber neuropathy.

Statistical analysis

Comparisons between groups were performed using Chi square analysis and Mann Whitney U test. A p value of less than 0.05 was considered significant.

Results

A total of 268 sarcoidosis patients with one or more features compatible with parasarcoidosis were enrolled in the study. Of these, 60 (22.4%) had one or more positive onconeural antibodies. Table 1 compares the demographic findings of the sarcoidosis patients with positive to negative onconeural antibodies. Black patients were more likely to develop positive antibodies compared to those with negative testing (Chi square=8.332, p=0.0115). There was no significant difference in gender, age, or presence of neurologic disease. Smoking history was available in approximately 75% of both sarcoidosis patients and controls.

Forty-six control patients with non-sarcoidosis diagnoses also seen in the University of Cincinnati Sarcoidosis Clinic were tested for the presence of onconeural antibodies. Table 1 analyzes the 46 control patients which were similar to the sarcoidosis patients in age, sex, and race. The underlying diagnoses of the control population included chronic obstructive pulmonary disease (COPD, 21 patients), idiopathic pulmonary fibrosis (IPF, 7 patients), non-IPF interstitial lung disease (ILD, 10 patients), and uveitis (8 patients). Three (6.5%) patients including two with ILD and with COPD also had positive onconeural antibodies. The rate of positive onconeural antibodies was significantly lower in the control group compared to the sarcoidosis group (Chi square=5.213, p=0.0224). Approximately 54% of the sarcoidosis patients with positive onconeural antibodies were lifetime non smokers; whereas, 62.6% of the patients with no detected antibodies were lifetime non smok-

Table 1. Demographics of all studied groups

	Sarcoidosis Antibody Positive	Sarcoidosis Antibody Negative	Non-sarcoidosis Controls §
Total number	60	208	46
Features			
Age, years	54 (33,77)	56 (26,80)	65 (20,88)
Female	46 (66.7%)	149 (71.6%)	29 (63.0%)
Black/white	33/24 ¶	69/137	11/35
Neurosarcoidosis	12 (20.0%) †	41 (19.7%)	0
Other diagnosis			COPD **: 21 IPF: 7 ILD: 10 Iritis: 8

*Median (range)
†Number (percent positive of group)
¶ Compared to negative, blacks were proportionally higher than whites (Chi square=11.153, p=0.0008)
§ Includes three with positive onconeural antibodies
**COPD: chronic obstructive pulmonary disease; IPF: idiopathic pulmonary fibrosis; ILD: non IPF interstitial lung disease

ers. Only 17.4% of the controls were lifetime non smokers (Chi square=28.447, p<0.0001).

The panel measures 16 antibodies with only half of the onconeural antibodies evaluated were positive in our sarcoidosis population. The clinical features of positive antibodies are summarized in Table 2. We found no sarcoidosis patients with positive antibodies to ANNA-1 (Hu), ANNA-2 (Ri), PCA1 (Yo), PCA2, PCA-Tr, Amphiphysin, CRMP5, or AGNA.

Table 3 summaries the antibody results for the sixty sarcoidosis patients with one or more positive onconeural antibodies. Patients could have more than one positive antibody. In some cases, a patient had one positive antibody and another borderline antibody. Overall, there were 57 positive or borderline positive results. Figure 1 summarizes the individual positive and borderline results reported in nmol/L for the five antibodies, A Ch Muscl, A Ch Gang, Neur V-G, N Type Ca Chan, and P/Q Type Ca Chan. Striational antibody was positive in ten patients with titers ranging from 1:120 to 1:153600. In five of these 10 patients the titers ranged from 1:480 or higher. The GAD65 antibody was detected in ten patients, and LGL-1 IgG antibody was found in only one patient.

A subset of patients completed the SFNL questionnaire. Thirty-three of the 141 (23.4%) of the sarcoidosis patients were completed the questionnaire had positive onconeural antibodies while only three of the 46 control patients (6.5%) had a positive antibody (Chi square=6.325, p=0.0119). The SFNL scores were significantly higher for the sarcoidosis patients than the non sarcoidosis patients (Figure 2, p=0.0074), However there was no difference in the SFNL scores between the sarcoidosis patients with or without onconeural antibodies (Figure 3, p=0.3492). We compared onconeural antibody results from the 95 sarcoidosis patients with a SFNL score of 10 or less to the 46 sarcoidosis patients with a SFNL score of 11 or more. Onconeural antibodies were detected in 19 of 95 (20%) of those with a SFNL of 10 or less, versus 14 of 46 (30.4%) of those with a SFNL of 11 or greater (Chi square=1.345, p=0.2461).

Table 4 lists current and past anti-inflammatory therapies for all three groups. All patients had received one or more systemic therapies for symptomatic sarcoidosis. The table also reveals the percentage of patients in each group on current individual therapies. Patients were prescribed either the anti-tumor necrosis (anti-TNF) monoclonal antibody adalimumab or infliximab. Because only two patients had received adalimumab alone, we combined the patients on adalimumab with those who received infliximab. Five patients had switched from infliximab

Table 2. Clinical features associated with onconeural antibody detection

Full Name	Abbreviation	Symptoms Associated with antibody
Acetylcholine Muscle Binding Antibody	A Ch Muscl	Fatigue, weakness, dysarthria, diplopia, and ptosis
Nicotinic Acetylcholine Receptor Ganglionic Neuronol Antibody	A Ch Gang	Bilateral numbness of the lower legs and feet, proximal lower limb muscle weakness, hypotension, ocular symptoms
Neuronol Voltage Gated Potassium Channel Antibody	Neur V-G	Confusion, sleep impairment, amnesia, seizure, fatigue, leg cramping, encephalitis, nausea/vomiting, ataxia, agitation
N-Type Calcium Channel Antibody	N Type Ca Chan	Seizures, neuralmuscular junction defect, limbic and extra-limbic autoimmune encephalopathy, neurodegenerative dementia, mild cognitive impairment, severe behavioral changes
P/Q Type Calcium Channel Antibody	P/Q Type Ca Chan	Limbic and extra-limbic autoimmune encephalopathy, neurodegenerative dementia, mild cognitive impairment, nonspecific cognitive disorder, severe behavioral changes, dysphagia, dysarthria, proximal muscle weakness, respiratory weakness, and seizure
Striational Antibody	Striational	More severe MG symptoms, high risk of arrythmias, respiratory complications, fatigue, weakness, dysarthria, diplopia, ptosis
Glutamic Acid Decarboxylase-65 antibody	GAD65	Cerebellum atrophy resulting in reduced motor and cognitive function, reduced GABA levels, leg spasms, falls, lumbar pain, muscle rigidity, dizziness, vomiting, leg spasms, cerebral ataxia,
Large Granular Lymphocyte-1 immunoglobulin Antibody	LGL1	Seizure, amnesia, behavioral disorders/changes, hyponatremia, FBDS, sleep disorder

MG: myasthenia gravis; GABA: gamma-aminobutyric acid; FBDS: faciobrachial dystonic seizures

Table 3. Summary of antibody tests for 60 onconeural positive sarcoidosis patients

	A Ch Muscl	A Ch Gang	Neur V-G	N Type Ca Chan	P/Q Type Ca Chan	Striational	GAD65	LGL1	Total*
Positive	6	13	10	11	11	10	12	1	74
Borderline	2	4	4	5	4	0	0	0	19

*Patient could have more than one antibody positive
See Table 2 for abbreviations

to adalimumab because of infliximab reactions, and two of these patients had positive onconeural antibodies while the other three were negative. There was no significant difference in onconeural antibody production for any specific treatment.

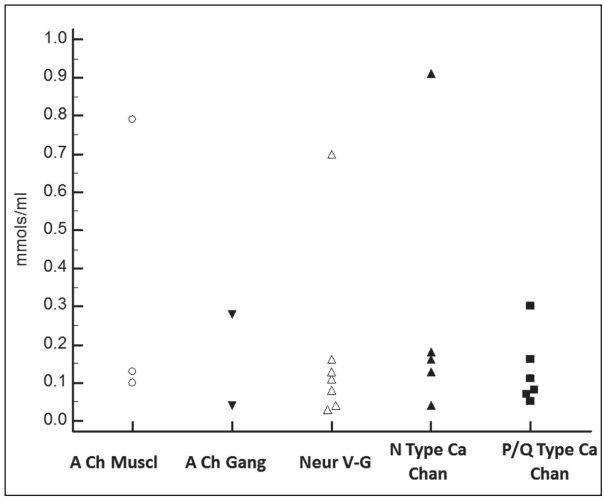

Fig. 1. The individual positive results for the five antibodies for which numeric titers were reported. A Ch Muscl: Acetylcholine Muscle Binding Antibody; A Ch Gang**:** Nicotinic Acetylcholine Receptor Ganglionic Neuronol Antibody**;** Neur V-G**:** Neuronol Voltage Gated Potassium Channel Antibody**;** N Type Ca Chan: N-Type Calcium Channel Antibody; P/Q Type Ca Chan: P/Q Type Calcium Channel Antibody

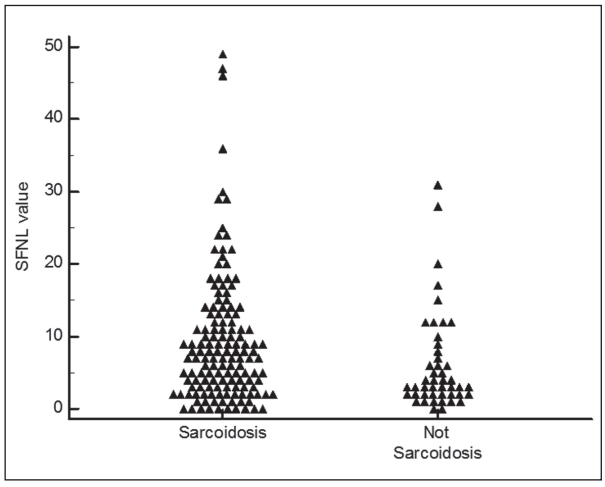

Fig. 2. The small fiber neuropathy list (SFNL) scores for the sarcoidosis patients than the non sarcoidosis patients. There was a significant difference between the two groups (p=0.0074)

Discussion

We studied sarcoidosis patients with one or more symptoms consistent with parasarcoidosis. In this group, positive onconeural antibodies were identified in 22% of sarcoidosis patients compared to only 6.5% of a control group of patients with similar symptoms but without a diagnosis of sarcoidosis. Onconeural antibodies were first described in cancer patients with paraneoplastic symptoms (6). Initially their presence was described in patients with Lambert-Eaton syndrome (10), breast cancer (5), and paraneoplastic pemphigus (11). A variety of antibodies, including calcium channel antibodies, were measured (12). With the more widespread use of onconeural antibody testing, it is clear these antibodies can be detected in non malignant settings (13, 14).

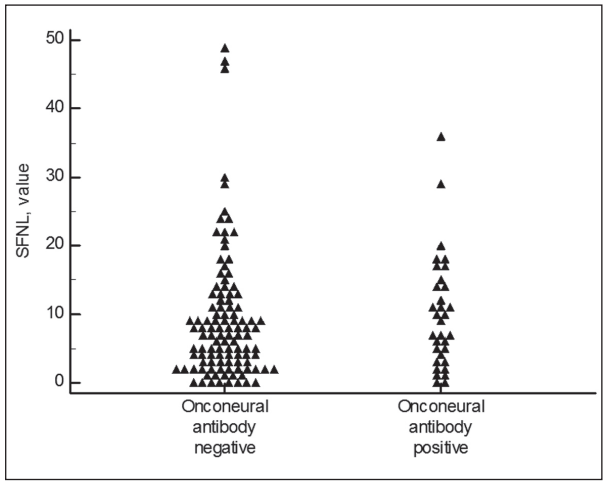

Fig. 3. The small fiber neuropathy list (SFNL) scores for sarcoidosis paitents. There was no difference in the SFNL scores between the sarcoidosis patients with or without onconeural antibodies (p=0.3492)

Controversy exists regarding the significance of positive antibodies in a non malignant setting. This is especially true for voltage-gate calcium channel antibodies (Neur V-G). In one study, over half of patients with positive antibodies were felt to have false positive testing (15). Following detailed analysis, a model was proposed to determine clinical relevance (16). Patients were viewed as having clinically relevant antibodies in the setting of movement disorders or stiff person syndrome, inflammatory CSF markers, cancer, smoking history, and concomitant hyponatremia. To date, we know of no other studies specifically looking at onconeural antibody production in the setting of sarcoidosis. Neither the sarcoidosis patients or the control group in the current study had evidence for underlying malignancy, movement disorder, or stiff person syndrome. Smoking history had no obvious impact on the results of onconeural antibody testing in our study, The majority of sarcoidosis patients in both groups were lifetime non smokers, a

Table 4. Prescribed anti-inflammatory sarcoidosis therapies

Total Number	Positive 60			Negative 208		
	Current	Past	Never	Current	Past	Never
Prednisone	37 (61.7%)*	20	3	120 (57.7%)	62	26
Methotrexate	22 (36.7%)	18	20	89 (42.8%)	45	74
Azathioprine	6 (10.0%)	4	50	21 (10.1%)	16	171
Leflunomide	7 (11.7%)	5	23	17 (8.2%)	9	182
Mycophenolate	3 (5.0%)	0	57	4 (1.9%)	1	203
Hydroxychloroquine	6 (10.0%)	7	47	32 (15.4%)	18	158
anti-TNF †	13 (21.7%)	6	41	34 (16.3%)	23	151
Rituximab	2 (3.3%)	1	57	6 (2.9%)	2	200
Repository corticotropin injection	3 (5.0%)	0	57	13 (6.3%)	2	193

*Percent on current therapy per group
anti-TNF: anti-tumor necrosis factor monoclonal antibody either infliximab or adalimumab

reflection of the fact that sarcoidosis is significantly more common in nonsmokers (17). Over 80% of the control group patients were current or ex smokers and the three control patients with positive antibodies were ex smokers.

We feel that the high titers noted in some of our patients, along with their clinical symptoms supports the concept that these antibodies may be important in patients with parasarcoidosis symptoms.

Small fiber neuropathy is one of the parasarcoidosis manifestations which led to testing for onconeural antibodies in our study. The small fiber neuropathy screening list (SFNL) has been proposed as an instrument to screen for small fiber neuropathy (2).

In a prospective study, SFNL and onconeural antibody results from 141 sarcoidosis patients and 46 non sarcoidosis patients were collected. We found that the SFNL scores were higher in the sarcoidosis patients compared to the control group.

It has been noted that patients with a SFNL of less than 11 were unlikely to have small fiber neuropathy using the temperature threshold test (TTT). However, we found more than half of our positive onconeural patients had a SFNL of ten or less. This could suggest that a positive onconeural antibody may lead to other parasarcoidosis manifestations which are not detected by the SFNL. To date, we have not been able to find a specific clinical feature that predicts a positive onconeural antibody.

Overall, 22% of sarcoidosis patients had positive onconeural antibodies. This includes a retrospective group in whom studies were performed over a two year period. Selection bias may be a study limitation, since we tested only sarcoidosis patients with parasarcoidosis symptoms. We did perform a small prospective study over a two month period and identified positive antibodies in 23% of 141 sarcoidosis patients. An additional limitation of this study was the relatively small number of non-sarcoidosis patients studied, especially for various disease subsets such as idiopathic pulmonary fibrosis. For the sarcoidosis patients, a study of a larger number of patients may identify features that are associated with a positive onconeural antibody.

We also did not study whether the presence of onconeural antibodies had any impact on response to treatment for the sarcoidosis. This could be the focus of future studies.

Anti-inflammatory therapy had no apparent impact on the presence of onconeural antibodies. Since this was a retrospective study without serial studies, we cannot be sure that some anti-inflammatory therapy may not have an impact on onconeural antibody production. However, we and others have found that most anti-inflammatory therapies have little impact on parasarcoidosis symptoms. The symptom of sarcoidosis associated fatigue rarely improves with traditional anti-inflammatory therapy (18, 19). Isolated cases have noted symptomatic improvement of small

fiber neuropathy and cognitive symptoms in some sarcoidosis patients treated with anti-TNF antibodies (20, 21). Rituximab has been reported beneficial for some onconeural antibody positive patients (8). In the current study, too few patients were treated with rituximab to comment on its effectiveness.

In conclusion, our study identified onconeural antibodies in a significant proportion of sarcoidosis patients who experienced parasarcoidosis symptoms. Our study was unable to determine whether these onconeural antibodies were the causality of the symptoms. Future studies directed at serial testing and response to specific therapy may help elucidate the role of onconeural antibodies in parasarcoidosis.

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