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The Relationship Between the Neuromyelitis Optica Spectrum Disorder and Sjögren's Syndrome: Central Nervous System Extraglandular Disease or Unrelated, Co-occurring Autoimmunity?

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Abstract

Objective—Sjögren's syndrome (SS) patients may be affected by the neuromyelitis optica spectrum disorder (NMOSD), a severe demyelinating syndrome associated with anti-aquaporin 4 antibodies (anti-AQP4 antibodies). The relationship between SS and NMOSD has been a sustained focus of investigation. Among SS patients, anti-AQP4 antibodies have been detected exclusively in those with NMOSD. It has therefore been speculated that NMOSD is not a neurological complication of SS. However, such studies evaluated small numbers of SS patients, often admixed with other inflammatory disorders.

Methods—We compared frequencies of anti-AQP4 and SS-associated antibodies in 109 SS patients, including 11 with NMOSD, 8 with non-NMOSD demyelinating syndromes, and 90 without demyelinating syndromes.

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Results—When assessed using a fluorescence-activated cell sorting (FACS) assay, anti-AQP4 antibodies were seen exclusively in those SS patients with NMOSD (72.7%), but not in SS patients without NMOSD ($p<0.01$). In contrast, anti-Ro52, anti-Ro60 and other autoantibodies were not more prevalent in SS patients with NMOSD versus those without. Anti-AQP4 antibodies were detected more frequently among NMOSD patients by FACS assay than with a commercial immunohistochemical (IHC) assay (72.7% versus 54.5%), despite assessment after a more prolonged period of immunosuppressive therapy (median 38 versus 5 months, $p<0.01$).

Conclusion—The syndrome-specificity of anti-AQP4 antibodies, along with an otherwise similar antibody profile in SS NMOSD patients, indicates that NMOSD is not a direct central nervous system manifestation of SS. Anti-AQP4 antibodies can persist and be refractory to prolonged immunosuppressive therapy.

The demyelinating syndromes which occur in Sjögren's syndrome (SS), such as myelitis and optic neuritis, are thought to potentially reflect "multiple-sclerosis-type" (MS-type) disease (1). This concept that central nervous system (CNS) demyelinating disease in SS may present with MS-type manifestations has been a sustained focus of the literature over the past decades (2). However, it is now recognized that demyelinating syndromes encompass disorders which are distinct from MS. In particular, the neuromyelitis optica spectrum disorder (NMOSD) may present with optic neuritis, myelitis which is frequently longitudinally extensive (i.e. spanning ≥3 vertebral segments on MRI imaging), along with characteristic lesions which may affect the brainstem and hypothalamus (3). In contrast to MS, NMOSD is associated with an antibody that targets aquaporin-4 (AQP4), the primary CNS water channel protein, which is prominently expressed on astrocytic foot processes. These antibodies are highly sensitive (>80%), distinguish NMOSD from MS with specificity approaching 100% (4), and cause damage due to blockade of water flux, disruption of glutamate homeostasis, and complement activation (4).

There is increased expression of AQP4 protein in salivary glands and other organs targeted in SS (kidney and lungs) (5). Given that anti-AQP4 antibodies are generated outside of the CNS (4), AQP4 proteins could be peripherally targeted in the salivary glands and other SS end-organs. Studies to date have found that anti-AQP4 antibodies are seen exclusively in SS patients with NMOSD, suggesting that NMOSD is not a direct and specific CNS manifestation of SS (6–7). However, these studies were limited by the small number of SS patients (6–7), admixture of SS with other inflammatory diseases, or inclusion of blinded serological studies of patients evaluated at different institutions (7).

To address these limitations and further define the relationship between SS and NMOSD, this study was performed on a large single-center cohort of SS patients with demyelinating disease. We report herein the demographic and clinical characteristics, and the frequencies of antibodies against AQP4, Ro52 and Ro60 in SS patients with NMOSD, with non-NMOSD demyelinating syndromes, and without demyelinating syndromes. We also developed a sensitive fluorescence-activated cell sorting (FACS) assay to detect anti-AQP4 antibodies.

PATIENTS AND METHODS

Study type

This was a three-year, cross-sectional study in which SS patients were referred to the Johns Hopkins Jerome L. Greene Sjögren's Syndrome Center from 2009–2011. This center includes a neuro-rheumatology clinic, which is dedicated to SS patients affected by neurological complications of the disease. This includes patients with peripheral nervous system (PNS) disease as well as demyelinating syndromes. Patients with demyelinating syndromes were evaluated in this outpatient setting by one of the study authors, who is board-certified as a neurologist as well as a rheumatologist (J.B.). Patients could be referred by neurologists or rheumatologists after outpatient management, as well as after hospitalizations for acute attacks of demyelinating disease. The remaining SS patients without demyelinating syndromes and other PNS disease were consecutively evaluated by J.B or another study rheumatologist (A.N.B.).

Inclusion criteria

All SS patients fulfilled the 2002 American-European Consensus Group classification criteria (8). NMOSD was categorized based on the revised 2015 diagnostic criteria (3).

Patient cohort

We studied 109 SS patients, 11 with NMOSD, 8 with non-NMOSD demyelinating syndromes, and 90 without any demyelinating syndrome. All patients were evaluated in our Center between the years 2009 and 2011, but some had been managed in other clinics at our institution and thus had commercial serologic data prior to 2009. Sera were routinely prepared from blood within a few hours of collection, aliquoted into small working volumes (30 microliters) and stored at −80°C.

Autoantibody assays

Serum from each of the 109 patients was tested in the Johns Hopkins Rheumatic Disease Research Core Center laboratory for the presence of anti-AQP4, anti-Ro52, and anti-Ro60 antibodies. Each of the antibody assays was performed on the same patient serum sample.

Anti-Ro52 (SSA), anti-Ro60, and La (SSB) antibody testing

Antibodies against Ro52 and La were assayed using commercially available ELISA kits, per the manufacturer's protocol (QUANTA Lite, Inova Diagnostics). Anti-Ro60 antibodies were determined by immunoprecipitation of ^{35}S-methionine-labeled Ro60 generated by *in vitro* transcription and translation, as previously described (9).

Anti-AQP4 antibody FACS assay

Anti-AQP4 antibodies were tested in all 109 SS patients using a FACS assay. HEK 293 cells were transiently transfected with AQP4 cDNA (C-terminal FLAG tag) or empty vector (negative control) cDNA using Lipofectamine 2000, per the manufacturer's protocol (Invitrogen). DNA encoding the M23 isoform was used, based on earlier observations that it binds antibodies with higher affinity than M1 AQP4 (10). Incubations with patient sera

(diluted 1:320 in PBS/1% FBS) were performed at 4°C for 30 minutes, followed by phycoerythrin (PE)-conjugated anti-human IgG (1:300, Sigma; 4°C, 15 minutes). For each serum, HEK 293 cells transfected with an empty vector were used to define the negative/background value. Cells with a fluorescence intensity higher than the negative control were identified as the positive population. To evaluate the expression of M23 AQP4, transfected cells were permeabilized (Intracellular Fixation and Permeabilization Buffers, eBioscience) and stained with an anti-FLAG antibody (Sigma, 1:1000; 4°C, 15 min) followed by PE-conjugated anti-mouse IgG antibody (1:500, Sigma; 4°C, 30 min). HEK 293 cells stained with the secondary alone antibody were used to define the negative/background value. Cells with a fluorescence intensity higher than the negative control were identified as the positive population. Data were collected using a BD FACSAriaI SORP Cell Sorter (BD Biosciences) and analyzed using FCS Express 4 (De Novo Software). The assay was validated using sera from 10 healthy donors, and 4 treatment-naïve NMOSD patients known to be seropositive for anti-AQP4 antibodies by the Mayo immunohistochemical (IHC) assay (see below). The former were all negative using this assay, whereas the latter set gave robust positive signals.

Commercial anti-AQP4 antibody testing

Anti-AQP4 antibodies were tested by the Mayo Neuroimmunology Laboratory during the course of routine clinical care of SS patients in whom NMOSD was suspected. At the time the sera from these patients were tested [2006–2011] this assay was performed with IHC staining of a composite substrate of adult mouse cerebellum, gut, and kidney (4). In two patients who had the Mayo anti-AQP4 assay performed on multiple occasions (patients 6 and 7, Table 1), we defined anti-AQP4 antibody status using the Mayo assay result closest to that of the serum assayed by FACS.

Analysis of data

The primary binary outcome was SS NMOSD versus SS without demyelinating disease, and the secondary binary outcome was SS NMOSD versus SS non-NMOSD demyelinating disease. These comparator groups were evaluated for associations with demographic features, anti-AQP4 antibodies, anti-Ro52 and anti-Ro60 antibody specificities, and other antibodies and markers of B-cell activation. The association of these outcomes with these covariates was evaluated by Wilcoxon-signed rank status or t-test for continuous variables, and by Fisher’s exact test or chi-squared analysis for categorical variables. In addition, among SS NMOSD patients, we compared (i) the frequency and sensitivity of anti-AQP4 antibody status reported by the commercial IHC assay versus the FACS approach, and (ii) the duration of immunosuppressive therapy at the time of serum collection for FACS versus IHC studies using the Wilcoxon’s paired signed-rank test. For all analyses, a p-value <0.05 (2-tailed) was considered statistically significant. The data analysis was performed using the STATA 11.0 statistical program (StataCorp, College Station, TX) (11).

RESULTS

Characteristics of demyelinating disease

Patient groups—The 19 SS patients with demyelinating syndromes included 11 with NMOSD, and eight with non-NMOSD demyelinating syndromes.

The neurologic findings of the 11 NMOSD patients are detailed in Table 1. There was a total of seven patients presenting with recurrent longitudinally-extensive transverse myelitis (LETM) (2–7 attacks), which was associated with unilateral, monophasic optic neuritis in one patient, and with monophasic bilateral optic neuritis in one patient. Two patients presented with monophasic LETM, one patient presented with monophasic optic neuritis, and one patient presented with recurrent bilateral optic neuritis. There were two patients with brainstem lesions, and one patient with a hypothalamic lesion not associated with an endocrinopathy.

The eight patients with non-NMOSD demyelinating syndromes could be subcategorized as follows: 1) three satisfied criteria for MS (12); 2) three had a clinically isolated syndrome (CIS) lasting more than 24 hours, suggestive but not diagnostic of MS (12); and 3) two patients had myelopathies not consistent with CIS or MS, including one patient with recurrent complete transverse myelitis without brain lesions, and one patient with longitudinally-extensive transverse myelitis without criteria consistent with NMOSD. Five of the six patients in groups 1 and 2 had characteristic MS (or MS-type) presentations of a “partial” myelitis: presenting with asymmetric sensory or sensory>motor deficits (4 patients and 1 patient, respectively), spinal-cord MRI lesions which spanned <3 vertebral segments, and with such lesions limited to or predominantly affecting the dorsal horn (3 patients and 2 patients respectively). The sixth patient had acute onset of painful dysesthesias in the right arm and leg that persisted for one week, had normal examination and MRI of the cervical spine, but with multi-focal brain lesions suggestive of MS (>3 ovoid periventricular lesions, Dawson’s fingers [lesions radiating perpendicularly from ventricular surface], corpus callosum lesions, T1 hypointense lesions [i.e. black holes]) (12).

NMOSD demyelinating syndrome and anti-AQP4 antibodies—Table 1 lists clinical and radiographic features of the 11 patients with NMOSD together with anti-AQP4 antibody status (all 11 sera were assayed for these antibodies by both IHC and FACS), and the interval between immunosuppressive therapy and collection of sera.

All 11 NMOSD patients had anti-AQP4 antibodies at some time point as defined by FACS and/or IHC assays. Two patients (numbers 6 and 7) were initially anti-AQP4 antibody positive by IHC, but were negative in subsequent IHC assays. Patient 6 was seropositive for anti-AQP4 antibodies by IHC, but was subsequently seronegative when anti-AQP4 status was measured two years later (by IHC) and four years later (by IHC and by FACS) and received the most aggressive immunosuppressive therapy among all NMOSD patients in this study (including mycophenolate mofetil, cyclophosphamide, and rituximab). Patient 7 initially had anti-AQP4 antibodies (by IHC); these were not detected after one month (by IHC) and seven months (by FACS) following plasma exchange and mycophenolate mofetil treatment.

As evident in Table 1, the FACS assay identified anti-AQP4 antibodies more frequently than the IHC assay when results on serum samples drawn in temporal proximity were compared. The median interval between these temporally-paired FACS and IHC studies was 13 months (range 0–58 months). Anti-AQP4 antibodies were detected in 73% (8/11) of NMOSD

patients evaluated by FACS, and in 55% (6/11) of patients evaluated by IHC from temporally-paired sera.

This increased frequency of anti-AQP4 antibodies detected by FACS was notable, given that the sera assayed by FACS were collected after longer exposure to immunosuppressive therapy (this reflects the fact that the FACS assay was only performed on research serum samples collected during the period of 2009–2011, whereas some patients had anti-AQP4 antibodies tested prior to 2009 during the course of routine clinical care). There was a statistically significant difference in the interval between immunosuppressive therapy and the collection of sera for anti-AQP4 antibody status as assessed by FACS (median 38 months [range 1–127 months]) versus IHC assay (median 5 months [range 0 (treatment naive)-120 months], $p<0.01$).

Using the FACS assay, we evaluated the anti-AQP4 frequency in SS subgroups. Anti-AQP4 antibodies were detected in 73% (8/11) of NMOSD patients, but were not detected in any of the non-NMOSD demyelinating syndrome patients (0/8), and in 0/90 SS patients without demyelinating disease ($p<0.01$).

Demographic and immunological features of SS patients with NMOSD, non-NMOSD demyelinating disorders, and without demyelinating disorders

Table 2 compares demographic features and antibody specificities in 11 SS patients with NMOSD, eight SS patients with non-NMOSD demyelinating disorders, and 90 SS patients without demyelinating syndromes. In all three groups, the median age of sicca symptom onset was in the fifth decade, and ~90% of SS patients were female. Interestingly, non-Caucasian ethnicities comprised 55% (6/11) of NMOSD patients versus 13% (12/90) of patients without demyelinating disorders ($p<0.01$). There was an increased frequency of non-Caucasian ethnicities comparing the eight SS patients with non-NMOSD demyelinating syndromes versus 90 SS patients without demyelinating syndromes (50% [4/8] versus 13% [12/90], $p=0.02$).

The frequencies of antinuclear, anti-Ro52, anti-Ro60, and anti-La/SS-B antibodies, rheumatoid factor, and polyclonal gammopathy were not increased in the two groups of SS patients with demyelinating disease when compared to the SS patients without demyelinating disease. The frequency of these antibodies, rheumatoid factor, and polyclonal gammopathy was not increased in patients with NMOSD versus non-NMOSD demyelinating syndromes.

Table 2 describes other neurological syndromes identified in SS subgroups. None of the ten patients referred for PNS disorders had co-occurring demyelinating syndromes. There was a decreased frequency of headaches comparing patients with NMOSD versus non-NMOSD demyelinating syndromes (0% [0/11] versus 50% [4/8], $p=0.02$). There were otherwise no differences between subgroups with regard to other “diffuse” neurological syndromes, including depression and cognitive complaints. Strokes and seizures were infrequent, and seen only in patients without demyelinating syndromes (strokes [5/90] and seizures [3/90]).

DISCUSSION

We sought to clarify the relationship of NMOSD with SS by comparing demographic features and the frequency of anti-AQP4 and other antibody specificities (including anti-Ro52 and anti-Ro60) in SS patients with NMOSD versus SS patients without demyelinating syndromes. We demonstrate that anti-AQP4 antibodies are seen exclusively in SS NMOSD patients. While the syndrome-specificity of anti-AQP4 antibodies for NMOSD has been reported previously, our findings are significant given that these prior studies included smaller numbers of SS patients, evaluated SS patients as part of heterogeneous cohorts admixed with other inflammatory diseases (6–7), or included blinded serological studies in which SS patients were evaluated at different institutions (7).

Our findings have implications for reframing how SS CNS disease is defined in disease activity indices, and also for how SS patients with demyelinating syndromes are enrolled in ongoing clinical trials. Current SS activity indices indiscriminately describe all SS patients with any demyelinating disease as having highly active CNS disease (1). In this regard, all 11 of our SS NMOSD patients would have been mistakenly classified as having active CNS disease due to SS. Our findings suggest that such activity indices should be revised, and that NMOSD should be an exclusionary component of these indices. These distinctions also have therapeutic significance, given that clinical trials are investigating different and novel agents for SS versus NMOSD (i.e. anti-BAFF therapy for SS and eculizumab for NMOSD) (4, 13). Therefore, our findings suggest that enrollment in such clinical trials targeting SS extraglandular disease should specifically exclude SS patients with NMOSD if recruited for neurological disease, with assessment of anti-AQP4 antibodies constituting an important exclusionary criterion.

We also identified demographic characteristics which are consistent with NMOSD not being a direct CNS manifestation of SS. Since non-Caucasian ethnicities are seen with disproportionately increased frequency in idiopathic NMOSD (4), and given that ~85% of SS patients are Caucasian, the statistically significant increased frequency of non-Caucasians in SS NMOSD further supports a coincidental relationship between NMOSD and SS. Studies performed on larger cohorts will be needed to confirm this. This shared frequency of SS-associated antibodies and markers of B-cell activation complements the syndrome-specificity of anti-AQP4 antibodies, and further reinforces NMOSD as a separate entity from SS CNS disease.

In our study, we used a FACS assay to detect serum anti-AQP4 antibodies that was specific and sensitive. The increased sensitivity of the FACS assay was important for several reasons. First, three patients presented with recurrent myelitis or optic neuritis, did not have more disseminated CNS disease (patients 9–11, Table 1), and only had anti-AQP4 antibodies detected by FACS but not by IHC. In the absence of anti-AQP4 antibody detection by FACS, these patients would have improperly had CNS disease attributed to SS and not NMOSD. Second, we detected anti-AQP4 antibodies more frequently in FACS versus IHC despite a more prolonged period of immunosuppressive therapy. The persistence of anti-AQP4 antibodies despite aggressive immunosuppressive therapy is important given that anti-AQP4

antibodies are likely pathogenic and portend a more severe course (4), suggesting that prolonged and uninterrupted therapy is warranted.

The frequency of NMOSD in SS cannot be ascertained in this study, given that patients with demyelinating syndromes constituted a select cohort recruited to a specialized neuro-rheumatology clinic. However, such referral bias allowed us to establish that NMOSD is coincidental to and not attributable to SS. Interestingly, analogous studies in systemic lupus erythematosus (SLE) also identified that anti-AQP-4 antibodies were exclusively found in SLE NMOSD patients (6–7), and similar to our study identifies NMOSD as a CNS syndrome coincidental to both SLE and SS.

Given that six patients with non-NMOSD demyelinating disease had MS or MS-type disease (i.e. CIS), our study provides the opportunity to define the relationship between MS and SS. Findings suggesting that these patients had MS or MS-type disease that was coincidental to SS (i.e. and not a CNS manifestation of SS), include the occurrence of myelopathies in patterns classical for MS (i.e. partial myelitis) (12), the infrequency of MS relative to NMOSD in our cohort, and the background knowledge that MS is otherwise not characterized by antibodies and markers of humoral autoimmunity which are characteristic for SS. This comparatively decreased frequency of MS or MS-type disease relative to NMOSD is especially notable, given that MS is 100-fold more common than NMOSD (4). While we also demonstrated that NMOSD is coincidental to SS, the increased frequency of NMOSD (relative to MS) in SS patients likely reflects two syndromes driven by robust albeit unrelated disorders of humoral autoimmunity (7). The two remaining non-NMOSD patients presented with a complete transverse myelitis and a longitudinally-extensive transverse myelitis, which are myelopathies not characteristic of MS and as suggested can be attributable to SS (14).

Limitations to our study include the relatively small number of SS NMOSD patients evaluated by FACS. However, the validity of our assay is supported by the restriction of anti-AQP4 antibodies to patients with high-risk clinical features for NMOSD. We also compared anti-AQP4 assays by FACS to IHC and not to other assays. We did this because IHC was the only commercial assay employed at the time that anti-AQP4 antibodies were evaluated for clinical purposes. In an extensive meta-analysis, cell-based assays such as FACS have demonstrated superior sensitivity and specificity as compared to other assays (15).

In conclusion, our data are consistent with the fact that NMOSD is not a CNS complication of SS based on the syndrome-specificity of anti-AQP4 antibodies for NMOSD, the disproportionately increased frequency of non-Caucasian ethnicity in SS NMOSD patients, and the otherwise similar profile of antibodies. The use of FACS assay was important in identifying anti-AQP4 antibodies and permitting the diagnosis of NMOSD in patients who otherwise may have been improperly considered as having SS CNS disease. The persistence of anti-AQP4 antibodies suggest that NMOSD, likely an antibody-mediated disease, requires ongoing immunosuppressive therapy.

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SIGNIFICANCE AND INNOVATIONS

- Anti-aquaporin-4 (AQP4) antibodies are exclusively detected in Sjögren's syndrome (SS) patients with the demyelinating syndrome of the neuromyelitis optica spectrum disorder (NMOSD), and not in SS patients without this central nervous system syndrome.
- We used a fluorescence-activated cell sorting (FACS) assay which detected anti-AQP4 antibodies more frequently than the commercial immunohistochemical assay (IHC).
- Anti-AQP4 antibodies were detected in patients receiving prolonged immunosuppressive therapy, indicating that these antibodies can persist despite this therapy.
- NMOSD is not a direct neurological complication of SS, and therefore should not be misinterpreted as an indicator of high SS disease activity.

Table 1

Clinical and radiographic characteristics, anti-AQP4 antibody status, type and duration and immunosuppressive therapy in SS NMOSD patients.

Patient	Clinical and Radiographic Findings	Anti-AQP4 Ever	Months Between FACS & IHC	Paired Anti-AQP4 Results[a]		Prior Therapy	Duration of immunosuppressive therapy prior to sera collection, FACS (months)	Duration of immunosuppressive therapy prior to sera collection, IHC (months)
				FACS	IHC			
1	Monophasic LETM, associated with brainstem and hypothalamic lesions	Yes	26	Yes	Yes	Methotrexate	68	42
2	Recurrent LETM × 2	Yes	58	Yes	Yes[c]	Azathioprine	42	0
3	Monophasic, unilateral ON with altitudinal deficit	Yes	21	Yes	Yes[c]	Methotrexate	15	0
4	Recurrent LETM × 4, unilateral monophasic ON, brainstem lesion	Yes	7	Yes	Yes	Azathioprine	127	120
5	Recurrent LETM × 5	Yes	30	Yes	Yes	Methotrexate	80	50
6	Recurrent LETM × 7, monophasic bilateral ON, brainstem lesion	Yes[b]	0	No	No	Mycophenolate, Cyclophosphamide, Rituximab	95	95
7	Monophasic LETM	Yes[b]	6	No	No	Plasma Exchange, Mycophenolate	7	1
8	Recurrent LETM × 3	Yes	16	No	Yes	Mycophenolate	21	5
9	Recurrent LETM × 2	Yes	2	Yes	No[c]	Mycophenolate	1	0
10	Recurrent LETM × 5	Yes	13	Yes	No	Mycophenolate	13	0
11	Recurrent bilateral ON × 2	Yes	0	Yes	No	Mycophenolate	38	38

Legend: FACS = Fluorescence Activated Cell Sorting; IHC = Immunohistochemistry; LETM = Longitudinally Extensive Transverse Myelitis; NMOSD = Neuromyelitis Optica Spectrum Disorder; ON = Optic Neuritis; Prior Therapy = Immunosuppressive therapy excluding prednisone; 0 months = patient is treatment naïve

[a] Refers to paired results of anti-AQP4 assays, the one by IHC being performed closest in time to that by FACS.

[b] Patient initially had anti-AQP4 antibodies by IHC, but did not have detectable anti-AQP4 antibodies by IHC performed closest in time to FACS assay.

[c] In patients 2, 3, and 9, despite detection of anti-AQP4 antibodies by IHC, the decision by outside treating physicians was not to initiate immunosuppressive therapy even when patients were treatment-naïve. In these patients, the duration between onset of immunosuppressive therapy and sera collection for FACS assay could be less than the interval between paired sera collection.

Table 2

Demographic, clinical and immunological characteristics in Sjögren's Syndrome (SS) Patients

	SS NMOSD (N=11)	SS non-NMOSD demyelinating (N=8)	SS non-demyelinating (N=90)	p-values (SS NMOSD vs. SS non-demyelinating)	p-values (NMOSD vs. non-NMOSD demyelinating)
Demographic Features					
Age at onset of sicca symptoms, years, Median (range)	49.5 (25–63)	41.5 (30–60)	46 (14–72)	0.28	0.37
Gender, Female % (N)	90.9 (10)	87.5 (7)	88.9 (80)	1.00	1.00
Ethnicity, non-Caucasian % (N)	54.5 (6)	50 (4)	13.3 (12)	0.65	< 0.01
African-American % (N)	27.3 (3)	50 (4)	7.8 (7)		
Hispanic % (N)	9.1 (1)	0 (0)	0.0 (0)		
Asian or Pacific Islander% (N)	18.2 (2)	0 (0)	3.3 (3)		
Other % (N)	0.0 (0)	0 (0)	2.2 (2)		
Clinical, Antibody, and Immunological Features					
Dry eyes % (N)	90.9 (10)	87.5 (7)	92.2 (83)	1.00	1.00
Dry mouth % (N)	100 (11)	75 (6)	96.7 (87)	1.00	0.16
Anti-nuclear antibodies, % (N)	81.8 (9)	50 (4)	70.0 (63)	0.51	0.32
Anti-Ro52 antibodies % (N)	72.7 (8)	75 (6)	63.3 (57)	0.74	1.00
Anti-Ro60 antibodies % (N)	90.9 (10)	75 (6)	62.2 (56)	0.09	0.55
Anti-La/SS-B antibodies % (N)	45.5 (5)	25 (2)	40.0 (36)	0.73	0.63
Rheumatoid Factor % (N)	20.0 (2/10)	25 (2)	45.6 (41)	0.18	1.00
Polyclonal Gammopathy % (#/N)	40.0 (4/10)	50 (4/8)	36.0 (31/86)	1.00	1.00
Other Neurological Syndromes					
PNS Syndromes % (N)	0 (0)	0 (0)	8 (9)	N/A[a]	N/A[a]
Headache % (N)	0 (0)	50 (4)	23 (21)	0.06	0.02
Cognitive Complaints % (N)	0 (0)	13 (1)	11 (10)	0.60	0.42
Depression % (N)	27 (3)	13 (1)	17 (15)	0.45	0.60
Stroke % (N)	0 (0)	0 (0)	6 (5)	1.00	1.00

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	SS NMOSD (N=11)	SS non-NMOSD demyelinating (N=8)	SS non-demyelinating (N=90)	p-values (SS NMOSD vs. SS non-demyelinating)	p-values (NMOSD vs. non-NMOSD demyelinating)
Seizure % (N)	0 (0)	0 (0)	3 (3)	1.00	1.00

Legend: FACS = Fluorescent Activated Cell Sorting; NMOSD = Neuromyelitis Optica Spectrum Disorder;

[a]The frequency of PNS syndromes could not be determined in an unselected cohort of SS patients, given that such PNS syndromes constituted a reason for referral to our SS Center. Subtypes of PNS syndromes includes six patients with axonal sensorimotor polyneuropathies, three patients with sensory neuronopathies, and one patient with small-fiber neuropathy.