Acta Neuropsychiatrica 2017

DOI: 10.1017/neu.2016.49

ACTA NEUROPSYCHIATRICA

Frequency of autoimmune disorders and autoantibodies in patients with neuromyelitis optica

Pereira WL de CJ, Reiche EMV, Kallaur AP, Oliveira SR, Simão ANC, Lozovoy MAB, Schiavão LJV, Rodrigues PR do VP, Alfieri DF, Flauzino T, Kaimen-Maciel DR. Frequency of autoimmune disorders and autoantibodies in patients with neuromyelitis optica.

Objective: The aim of this study was to report the frequency of autoimmune disorders and autoantibodies in 22 patients with neuromyelitis optica (NMO), as well as whether the seropositivity for autoantibodies differs between anti-aquaporin 4 (AQP4) positive and AQP4 negative NMO patients.
Methods: Demographic, medical records, and a profile of autoantibodies were evaluated in 22 NMO patients, including AQP4, anti-thyroid-stimulating hormone receptor, antinuclear antibodies (ANA), anti-thyroperoxidase (anti-TPO), anti-thyroglobulin (anti-Tg), anti-double-stranded DNA, anti-neutrophil cytoplasmic, anti-cyclic citrullinate peptide, rheumatoid factor, anti-SSA/Ro, anti-SSB/La, anti-Smith antibodies (anti-Sm), anti-ribonucleoprotein, anti-nucleosome, and anti-Scl70. Thyroid-stimulating hormone and free thyroxin were measured.
Results: The frequency of women was higher than men (95.5% vs. 4.5%) and 68.2% were Afro-Brazilians. Six (27.3%) patients presented other autoimmune disorders, such as Hashimoto thyroiditis ($n = 2$), Graves' disease ($n = 1$), juvenile idiopathic arthritis ($n = 1$), systemic lupus erythematosus and systemic sclerosis ($n = 1$), and Raynaud's phenomenon ($n = 1$). The most frequent autoantibodies were anti-AQP4 (54.5%), anti-nucleosome (31.8%), ANA (27.3%), anti-TPO (22.7%), and anti-Tg (22.7%). Difference was not observed in the frequency of autoimmune disorders when the patients were compared according to their anti-AQP4 status.
Conclusion: The results of the present study underscored that the NMO patients present high frequency of autoantibodies against cellular antigens and the presence of autoimmune disorders. Further studies with large number of NMO patients may contribute to advances in the understanding of NMO disease mechanisms.

Wildéa Lice de Carvalho Jennings Pereira[1], Edna Maria Vissoci Reiche[2], Ana Paula Kallaur[1], Sayonara Rangel Oliveira[1], Andréa Name Colado Simão[2], Marcell Alysson Batisti Lozovoy[2], Lucas José Vaz Schiavão[3], Paula Raquel do Vale Pascoal Rodrigues[3], Daniela Frizon Alfieri[1], Tamires Flauzino[1], Damacio Ramón Kaimen-Maciel[3,4]

[1]Health Sciences Postgraduate Program, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil; [2]Department of Pathology, Clinical Analysis and Toxicology, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil; [3]Neurology Outpatient Department of the Outpatient Specialties of University Hospital, State University of Londrina, Londrina, Paraná, Brazil; and [4]Department of Clinical Medicine, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil

Keywords: anti-aquaporin 4; autoimmune thyroiditis; autoimmunity; juvenile idiopathic arthritis; neuromyelitis optica

Edna Maria Vissoci Reiche, Department of Pathology, Clinical Analysis and Toxicology, Health Sciences Center, Londrina State University, Av. Robert Koch, 60, 86.038-350, Londrina, Paraná, Brazil.
Tel: +55 43 3371 2619;
Fax: +55 43 3371 2619;
E-mail: reiche@sercomtel.com.br

Accepted for publication August 10, 2016

First published online October 3, 2016

Significant outcomes

- Autoimmune thyroiditis was the most frequent autoimmune disorders in patients with neuromyelitis optica (NMO).
- Systemic lupus erythematosus (SLE) with systemic sclerosis and juvenile idiopathic arthritis (JIA) were also presented in patients with NMO.
- Epidemiological, clinical, and laboratory features of the NMO patients did not differ according to the anti-aquaporin 4 (AQP4) status, presence of autoimmune disorders, and seropositivity of autoantibodies.

Limitations

- The cohort of patients with NMO is small in order to make significant comparison with previous reports.

Introduction

NMO is an inflammatory demyelinating autoimmune disease of the central nervous system (CNS) that most commonly affects the optic nerves and spinal cord causing blindness and paralysis but usually spares the brain, unlike multiple sclerosis (MS) (1). The disease affects primarily young women, accounting for roughly 85% of cases. Relapsing NMO is more frequent in female, but both sex can develop monophasic NMO. The median age at presentation is 39 years and rarely occurs in adolescents (2–6). The prevalence of NMO is lower than that of MS and is higher in non-Caucasians. Within demyelinating disorders, NMO can affect up to 48% of patients of East Asia, and its prevalence decreases among African-Brazilians (15%) and Europeans (1.5%) (6,7). The serum IgG autoantibody against the major water channel of the CNS localised in the astrocyte foot processes, named AQP4, was implicated in the pathogenesis of NMO as the main aetiologic agent of this autoimmune disease (8).

There is a strong recognised association between NMO and both non-organ-specific and organ-specific autoimmune diseases, including rheumatoid arthritis, SLE, Sjögren syndrome, antiphospholipid antibody syndrome, myasthenia gravis, anti-neutrophil cytoplasmic autoantibodies (ANCA)-associated diseases, hypothyroidism, type 1 diabetes mellitus, coeliac disease, pernicious anaemia, ulcerative colitis, idiopathic thrombocytopenic purpura, primary sclerosing cholangitis, and sarcoidosis (9–19). Some mechanisms could explain the association between NMO and other autoimmune conditions, such as environmental and genetic factors that predispose to autoimmunity and the immunopathological mechanisms of vasculopathy of the systemic rheumatologic diseases that could facilitate the pathogenesis of NMO (16). Concurrent incidence of NMO and other autoimmune diseases has been reported in different populations worldwide, mostly among the individuals from North Hemisphere (13,20–22) and has not been extensively investigated in South Hemisphere populations (14).

Aims of the study

The aim of this study was to report the frequency of autoimmune disorders and the seropositivity for autoantibodies, as well as whether the seropositivity for autoantibodies differs between anti-AQP4 positive and anti-AQP4 negative patients with NMO from Southern Brazilian population.

Materials and methods

Study design

The protocol was approved by the Institutional Research Ethic Committee of the State University of Londrina (CEP/UEL 165/2013). The enrolled individuals voluntarily agreed to participate in the study, and a written informed consent was obtained from all of them. In total, 22 patients with NMO diagnosed according to the 2006 revised Wingerchuk criteria (23) were included by consecutive evaluation in the Neurology Outpatient Department of the Outpatient Specialties of University Hospital, State University of Londrina, South Brazil. This cohort corresponded to the entire population with NMO attended at this clinic and no exclusion criteria was included. Non-organ-specific and organ-specific autoimmune diseases were diagnosed by neurologists, rheumatologists, and endocrinologists of the University Hospital, State University of Londrina, according to the specific criteria and guidelines (24–26). The diagnosis of JIA was made according to the International League of Associations for Rheumatology criteria (27).

Demographic and clinical data

Demographic and clinical data were obtained using a standard questionnaire and from medical records. The disability was evaluated using the Expanded Disability Status Scale (EDSS) (28). All the 22 patients were treated with prednisone in combination with other therapy, such as azathioprine ($n = 21$) or mycophenolate mofetil ($n = 1$), and none of them presented acute attacks during the study.

Autoantibodies

The autoantibodies were evaluated from serum samples using standardised methods and were performed according to the manufacturers' instructions and reference values. Anti-AQP 4 were detected using the indirect immunofluorescence assay (IFA; anti-aquaporin 4 IFT; Euroimmun, Lübeck, Germany), antinuclear antibodies (ANA) were detected using IFA with HEp2 cells as substrate fixed in slides (ANA Hep2 Test System; MBL Bion Enterprises Ltd, Des Plaines, IL, USA); the title and fluorescent pattern were reported and positive result was considered when the titre was $\geq 1:160$. Anti-double-stranded DNA (anti-dsDNA) antibodies were

https://doi.org/10.1017/neu.2016.49 Published online by Cambridge University Press

detected using two methods: IFA with *Crithidia luciliae* as substrate fixed in slide (anti-DNA; Imuno-CON, WAMA Diagnóstica, São Carlos, SP, Brazil) and positive result was considered when the titre was ≥1 : 10; and enzyme linked-immune sorbent assay (ELISA) and positive result was considered when the titre was ≥20 IU/ml. Anti-nucleosome antibodies were evaluated using ELISA and values ≥20 U/ml were considered positive. Anti-thyroperoxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) were detected using quantitative chemiluminescence assay (Architech™; Abbott Laboratory, Abbott Park, IL, USA), and values ≥5.6 IU/ml and ≥4.0 IU/ml, respectively, were considered significant. Antibodies against thyroid-stimulating hormone receptor (TRAb) were quantitatively detected using electrochemiluminescence assay and positive values were considered when >1.75 IU/l. Rheumatoid factor (RF) was determined using nephelometry (Nephelometer II™; Dade Behring-Siemens Healthcare Diagnostics Inc, Deerfield, IL, USA) and values ≥10 IU/ml were considered significant. Anti-Sjögren's Syndrome antigen A or Ro antigen (anti-SSA/Ro), anti-Sjögren's Syndrome antigen B or La antigen (anti-SSB/La), anti-Sm, anti-ribonucleoprotein antigen (anti-RNP), and anti-Scl70 were determined using ELISA (Orgentec Diagnostica, GmBH, Germany) and values ≥25 U/ml were considered positive. ANCA was determined using IFA with neutrophils fixed with ethanol as substrate (IFA Anti-cANCA, Human Granulocyte IgG assay; SCIMED, Denville, NY, USA) and values ≥1 : 20 were considered significant. Anti-cyclic citrulinatte peptide (anti-CCP) was detected using chemiluminescent assay and values ≥5.0 U/ml were considered significant.

Hormones measurements

Thyroid-stimulating hormone (TSH) and free thyroxin (FT4) were measured by quantitative chemiluminescence assay using the reference values of the manufacturer for TSH ranging from 0.35 to 4.94 μIU/ml, and FT4 ranging from 0.70 to 1.48 ng/dl.

Statistical analysis

Analyses of contingency tables (χ^2 test) were employed to check the associations between categorical variables according to the anti-AQP4 status. Categorical variables were expressed as absolute number (n) and percentage (%) and continuous variables were expressed as mean ± standard deviation. We assessed the differences in continuous variables between groups using Student's t-test. The Shapiro–Wilk test was used to assess normality of distribution. Logarithmic transformation of continuous data was used in the analyses when the variables were not normally distributed or when there was heterogeneity of variance (as assessed with the Levene test). The Pearson's rank correlation test was performed to investigate the relationship between the EDSS values and the seropositivity for anti-AQP4. Odds ratio (OR) and 95% confidence interval (CI) were also demonstrated. Values of $p < 0.05$ were considered statistically significant. The statistical analysis was performed with SPSS for Windows, version 20.0 (SPSS Inc, Chicago, IL, USA) and significance was defined as $p < 0.05$.

Results

Characteristics of the study participants

The demographic and clinical characteristics of the NMO patients evaluated in the present study did not differ according to their anti-AQP4 serological status (Table 1). The majority were women (95.5%) and non-Caucasians (72.7%). The mean age of the patients was 43.0 (13.5) years, the mean age at disease onset was 36.2 (12.0) years, the disease duration was 6.7 (4.3) years, and the EDSS mean was 4.6 (1.8). The relapsing clinical course was more frequent (95.5%), three (13.7%) patients had infections associated with NMO, such as human immunodeficiency virus type 1 (HIV-1) infection, tuberculosis, and *Human herpesvirus type 3* (HHV-3) infection. Moreover, six (27.3%) patients presented other autoimmune disorders.

The seropositivity for anti-AQP4 was obtained in 12 (54.5%) patients. No association was observed between the seropositivity for anti-AQP4 and the disability EDSS (OR: 0.3636, 95% CI: 0.0279–4.742, $p = 0.852$). Moreover, no correlation was observed between the levels of anti-AQP4 and the EDSS (Pearson's correlation, $r = -0.1962$, $p = 0.5410$).

Frequency of autoimmune disorders and autoantibodies

As Table 2 shows, the frequency of autoantibodies did not differ according to the anti-AQP4 serological status of NMO patients. Among them, seven (33.3%) were seropositive for anti-nucleosome antibodies, six (27.3%) for ANA, and five (22.7%) for anti-TPO. The anti-dsDNA evaluated using IFA and ELISA and RF were not detected in all the 22 patients with NMO; ANCA was negative in 21 (95.5%) patients and inconclusive in one (4.5%) patient (Patient 4) that also presented seropositivity for ANA. Anti-CCP was detected in one (4.5%) patient (Patient 18), although in low levels (5.6 U/l). Table 3 shows the serum levels of other autoantibodies of NMO patients that presented other autoimmune conditions. With

https://doi.org/10.1017/neu.2016.49 Published online by Cambridge University Press

Table 1. Demographic and clinical characteristics of patients with neuromyelitis optica from Southern Brazilian population, according to the anti-aquaporin 4 (AQP4) status

	Anti-AQP4 + ($n = 12$)	Anti-AQP4 − ($n = 10$)	Total ($n = 22$)	p value
Sex				
Female	11 (91.7)	10 (100.0)	21 (95.5)	0.162
Male	1 (8.3)	0 (0.0)	1 (4.5)	
Age (years)	40.3 (±14.7)	46.2 (±11.7)	43.0 (±13.5)	0.322
Ethnicity				
Caucasian	3 (25.0)	3 (30.0)	6 (27.3)	0.583
Non-Caucasian	9 (75.0)	7 (70.0)	16 (72.7)	
BMI (kg/m^2)	25.7 (±3.7)	25.6 (±5.81)	25.7 (±4.6)	0.968
Age at onset (years)	33.2 (±12.5)	39.9 (±10.9)	36.2 (±12.0)	0.206
Disease duration (years)	7.1 (±4.1)	6.3 (±4.7)	6.7 (±4.3)	0.628
Clinical course				
Relapsing	12 (100.0)	9 (90.0)	21 (95.5)	0.455
Monophasic	0 (0.0)	1 (10.0)	1 (4.5)	
EDSS*	4.5 (±1.8)	4.7 (±1.9)	4.6 (±1.8)	0.883
Initial event				
Unilateral ON	7 (58.3)	1 (10.0)	8 (36.7)	
Bilateral ON	2 (16.7)	1 (10.0)	3 (13.6)	0.071
Myelitis	2 (16.7)	6 (60.0)	8 (36.7)	
Myelitis + ON	1 (8.3)	2 (20.0)	3 (13.6)	
Second event				
Unilateral ON	4 (33.4)	2 (20.0)	6 (27.3)	
Bilateral ON	0 (0.0)	1 (10.0)	1 (4.5)	0.662
Myelitis	6 (50.0)	5 (50.0)	11 (50.0)	
Unknown†	2 (16.7)	2 (20.0)	4 (18.2)	
Infection associated with NMO‡				
Yes	2 (16.7)	1 (10.0)	3 (13.6)	1.000
No	10 (83.3)	9 (90.0)	19 (86.4)	
Other autoimmune disorders§				
Yes	3 (25.0)	3 (30.0)	6 (27.3)	1.000
No	9 (75.0)	7 (70.0)	16 (72.7)	
Unilateral blindness‖				
Yes	4 (33.3)	1 (10.0)	5 (22.7)	0.323
No	8 (66.7)	9 (90.0)	17 (77.3)	
Corticosteroids (mg/day)*	17.5 (±13.5)	19.0 (±11.9)	18.1 (±12.5)	0.788
Azathioprine (mg/day)*	140.9 (±20.22)	140.0 (±21.1)	140.4 (±20.1)	0.921

Anti-AQP4 −, anti-AQP4 negative; anti-AQP4 +, anti-AQP4 positive; BMI, body mass index; EDSS, Expanding Disability Status Scale; NMO, neuromyelitis optica; ON, optical neuritis.

* Logarithmic transformation of continuous data was used in the analyses when the variables were not normally distributed or when there was heterogeneity of variance (as assessed with the Levene test).

† Data were missing in four patients.

‡ Human immunodeficiency virus, tuberculosis, and herpes zoster.

§ Hypothyroidism, hyperthyroidism, juvenile idiopathic arthritis, systemic lupus erythematosus, systemic sclerosis, Raynaud's phenomenon.

‖ None of the patients had bilateral blindness. Categorical variables were expressed as absolute number (n) and percentage (%) and assessed by χ^2 test or Fisher Exact test ($p < 0.05$). Continuous variables were expressed as mean and ± SD. The differences in continuous variables between groups using Student's t-test.

the frequency of seropositivity for thyroid autoantibodies and the thyroid hormone serum levels, two (9.0%) NMO patients were diagnosed with autoimmune hypothyroidism (Hashimoto thyroiditis) and one (4.5%) with autoimmune hyperthyroidism (Grave's disease). Other autoimmune disorders observed were JIA ($n = 1$), SLE, and systemic sclerosis ($n = 1$) and Raynaud's phenomenon ($n = 1$).

When the epidemiological, clinical, and laboratorial characteristics of the NMO patients were compared according to their presence or absence of autoimmune disorders, only the presence of antinuclear autoantibodies was more frequent among those with autoimmune disorders ($n = 4$) compared with those without autoimmune disorders ($n = 2$) (χ^2 test, $p < 0.05$). When the NMO patients with seropositivity for, at least, one autoimmune antibody ($n = 18$) were compared with those without autoantibodies ($n = 4$), difference was not observed in the clinical forms of NMO, EDSS, and age at onset

https://doi.org/10.1017/neu.2016.49 Published online by Cambridge University Press

Table 2. Seropositivity for autoantibodies in patients with neuromyelitis optica from Southern Brazilian population, according to their anti-aquaporin 4 (AQP4) status

Autoantibodies	Anti-AQP4 + ($n = 12$)	Anti-AQP4 − ($n = 10$)	Total ($n = 22$)	p value
Anti-nucleosome*	4 (33.3)	3 (33.3)	7 (33.3)	1.000
Antinuclear antibodies	5 (41.7)	1 (10.0)	6 (27.3)	0.162
Anti-thyroperoxidase	4 (33.3)	1 (10.0)	5 (22.7)	0.323
Anti-thyroglobulin	2 (16.7)	3 (30.0)	5 (22.7)	0.624
Anti-SSA/Ro	1 (8.3)	1 (10.0)	2 (9.1)	1.000
TRAb	0 (0.0)	1 (10.0)	1 (4.5)	0.455
Anti-CCP	1 (8.3)	0 (0.0)	1 (4.5)	1.000
Anti-dsDNA	0 (0.0)	0 (0.0)	0 (0.0)	–
Anti-SSB/La	0 (0.0)	0 (0.0)	0 (0.0)	–
Anti-Sm	0 (0.0)	0 (0.0)	0 (0.0)	–
Anti-RNP	0 (0.0)	0 (0.0)	0 (0.0)	–
Anti-Scl70	0 (0.0)	0 (0.0)	0 (0.0)	–
Rheumatoid factor	0 (0.0)	0 (0.0)	0 (0.0)	–
ANCA†	0 (0.0)	0 (0.0)	0 (0.0)	–

ANCA, anti-cytoplasmic neutrophil antibodies; anti-AQP4 −, anti-AQP4 negative; anti-AQP4 +, anti-AQP4 positive; anti-CCP, anti-cyclic citrullinated peptide; anti-dsDNA, antibodies to double-stranded DNA; anti-RNP, anti-ribonucleoprotein antibodies; anti-Sm, anti-Smith antibodies; TRAb, thyroid-stimulating hormone receptor antibodies.

Data were expressed as absolute number (n) and percentage (%) and were evaluated using Fisher Exact test.

* Anti-nucleosome were evaluated in 21 patients.

† One patient had inconclusive ANCA pattern because he was also seropositive for antinuclear antibodies.

of disease (χ^2 test, $p > 0.05$); however, NMO patients with autoimmune antibodies showed a trend towards higher frequency of unilateral optical neuritis (ON) when compared with those without seropositivity for the autoantibodies (χ^2 test, $p = 0.059$).

Discussion

The main finding of the present study was that autoimmune thyroid diseases were the most frequent autoimmune disorder among the NMO patients, followed by SLE with systemic sclerosis, JIA, and Raynaud phenomenon. This result underscores that autoimmune thyroid diseases are the most common autoimmune diseases associated with NMO (16), such as Graves' disease, benign thyroid tumours, and Hashimoto's thyroiditis (19). One patient of the present study was seropositive for anti-Tg and anti-nucleosome antibodies and exhibited Raynaud's phenomenon, which has been described in association with several autoimmune diseases or conditions, mostly autoimmune rheumatic diseases (29). In the present study, three patients were simultaneously seropositive for anti-nucleosome and ANA; and four were simultaneously seropositive for anti-nucleosome and anti-AQP4. The only male patient (Patient 22) showed seropositivity for anti-TPO, ANA, anti-SSA/Ro, and anti-AQP4. One patient (Patient 18) who had HIV/AIDS showed also seropositivity for anti-AQP4.

Among the six patients that showed seropositivity for ANA, five of them were simultaneously seropositive for ANA and anti-AQP4 only one had the diagnosis of SLE and systemic sclerosis, simultaneously. Even though neurologic complications of SLE may occur in up to 75% of patients, transverse myelitis is uncommon, occurring in only 2% of patients (30). In 2007, Birbaum et al. (31) reported the first example of positive anti-AQP4 to confirm the diagnosis of NMO in an African-American woman with SLE who had several relapses after NMO spectrum disorders onset. The relationship between SLE and CNS inflammatory demyelinating diseases, such as MS and NMO, has been poorly understood (16). In 2011, for the first time it was reported a clinical case of a 62-year-old woman who had relapsing anti-AQP4 positive longitudinally extensive transverse myelitis and developed systemic sclerosis (32). Regarding the JIA, it is a chronic inflammatory disease characterised by chronic synovitis, and sometimes associated with extra-articular manifestations, mainly fever, rash, pericarditis, and uveitis. JIA has been reported in patients with other autoimmune diseases, such as thyroid and coeliac diseases (33–35). In the present cohort, one NMO patient had JIA, a rare overlapping autoimmune condition, reported also in a cohort of NMO-IgG seropositive children (36).

We also found that the epidemiological, clinical, and laboratory features of the NMO patients did not differ significantly between the anti-AQP4 status, presence of autoimmune disorders, as well as the seropositivity of autoantibodies showed by the NMO patients. Divergent from our results, previous study showed that anti-AQP4 IgG seropositive NMO differs clinically

https://doi.org/10.1017/neu.2016.49 Published online by Cambridge University Press

Table 3. Serum levels of autoantibodies obtained from patients with neuromyelitis optica from Southern Brazilian population

Patients	Sex	Age	Anti-AQP 4	ANA	Anti-nucleosome	Anti-dsDNA	Anti-SSA	Anti-SSB	Anti-Scl70	Anti-RNP	Anti-Sm	Anti-TPO	Anti-Tg	Autoimmune Disorder
1	F	44	1/40	<1 : 160	5.27	5.26	1.79	1.47	0.63	0.63	2.03	<0.16	<1.0	–
2	F	39	Neg	<1 : 160	5.18	8.75	6.08	1.21	0.64	0.64	0.98	<0.16	<1.0	–
3	F	45	1/320	1 : 160*	50.20	13.59	2.83	1.54	1.02	1.02	2.66	0.16	<1.0	–
4	F	47	Neg	1 : 1280†	>270.0	18.77	5.69	2.01	0.51	0.51	2.33	35.76	369.49	Hypothyroidism
5	F	20	1/160	1 : 320‡	19.03	10.96	7.09	3.09	2.37	2.37	3.22	<0.16	<1.0	JIA
6	F	50	1/40	<1 : 160	11.97	11.65	3.24	4.44	0.59	0.59	2.61	<0.16	<1	–
7	F	39	1/320	1 : 5120§	37.54	16.03	4.05	1.34	0.76	0.78	1.96	<0.16	0.67	SLE + SSc
8	F	33	Neg	<1 : 160	33.56	10.06	2.17	0.87	0.55	3.67	0.35	<0.16	10.76	RP
9	F	47	Neg	<1 : 160	8.00	7.31	2.21	2.00	0.70	2.61	1.77	0.20	0.55	–
10	F	57	1/160	1 : 640‡	11.72	4.30	5.08	1.69	0.68	3.26	1.02	1514.52	13.41	Hypothyroidism
11	F	48	Neg	<1 : 160	8.62	8.34	1.82	10.27	0.51	11.12	2.42	<0.16	0.59	–
12	F	28	Neg	<1 : 160	31.53	6.97	155.11	4.46	0.32	3.40	1.48	<0.16	2.27	–
13	F	65	Neg	<1 : 160	5.96	4.64	2.83	2.00	0.65	6.06	1.48	7.60	10.26	–
14	F	39	Neg	<1 : 160	16.16	5.94	2.15	6.19	0.93	5.38	2.97	0.35	1.02	–
15	F	32	1/10	<1 : 160	48.10	7.59	2.07	1.39	0.27	1.94	0.19	0.47	2.99	–
16	F	68	1/40	<1 : 160	15.46	13.32	10.50	2.19	0.21	7.50	0.01	0.60	3.38	–
17	F	16	1/320	<1 : 160	9.67	7.17	1.91	1.33	0.70	2.27	1.55	307.49	56.64	–
18	F	33	1/320	<1 : 160	2.52	5.53	2.30	1.95	0.67	5.20	2.24	0.40	1.6	–
19	F	58	Neg	<1 : 160	1.54	1.99	2.66	4.04	0.52	12.87	0.75	0.50	<1.0	–
20	F	58	Neg	<1 : 160	1.50	–	2.30	1.33	0.93	0.63	0.01	0.31	1.82	Hyperthyroidism
21	F	47	1/10	<1 : 160	46.97	15.96	2.17	0.56	0.38	8.95	2.12	6.23	3.09	–
22	M	33	1/80	1 : 320‡	4.59	4.64	234.30	17.77	1.32	3.02	4.24	0.67	0.97	–

ANA, antinuclear antibodies, indirect immunofluorescence assay with HEp2 cells, positive when values ≥1 : 160; anti-AQP 4, anti-aquaporin 4, indirect immunofluorescence assay; anti-dsDNA, anti-double-stranded DNA; anti-RNP, anti-ribonucleoprotein antibodies; anti-Sm, anti-Smith antibodies; anti-Tg, anti-thyroglobulin antibodies; anti-TPO, anti-thyroid peroxidase antibodies; F, female; JIA, juvenile idiopathic arthritis; M, male; age expressed as years; Neg, negative; RP, Raynaud's phenomenon; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

* Nucleolar fluorescent pattern.

† Homogeneous nuclear fluorescent pattern.

‡ Speckled nuclear fluorescent pattern.

§ Centromere fluorescent pattern; anti-nucleosome and anti-dsDNA: enzyme immunoassay, positive when values >20 U/ml; anti-SSA/Ro, anti-SSB/La, anti-Smith antibodies (anti-Sm), anti-RNP, anti-Scl70: enzyme immunoassay, positive when values ≥25 U/ml; anti-TPO and anti-Tg were positive when values were ≥5.6 and ≥4.0 IU/ml, respectively.

and epidemiologically from seronegative disease: strong predominance in women, more severe clinical attacks, higher spinal cord lesion load, and frequent association with coexisting autoimmunity (37).

The epidemiological, clinical, and immunological characteristics of the patients with NMO of the present cohort are consistent with previous studies carried out in different population worldwide, such as to be more frequent among women, non-Caucasians, and higher age at onset of disease than the observed among the Brazilian MS cohorts (38,39), as well as other cohorts (37,40,41). The population of the present study showed that a female : male ratio was 21 : 1. This value is much higher than that reported previously (37,40). The sample of NMO included in the present study corresponded to the entire cohort of the NMO patients attended at the regional reference neurological outpatient clinic and this female preponderance may underscore that female sex hormones are able to affect B cell biology and thus enhance the possibility of autoantibody production (42). Moreover, gender may determine whether NMO follows a relapsing or monophasic course with an association between female and the relapsing course (43).

The relapsing clinical course was more frequent (95.5%) among the patients of the present study, in agreement with previous reports, which is more commonly in women and associated with older age at onset, longer time interval between index events, less severe motor impairment with the first myelitis attack, and with the presence of systemic autoimmunity (11,44).

The present study demonstrated that the seropositivity for anti-AQP4 was 54.5%, smaller than the frequency of 64.3% obtained in a Brazilian sample of 28 patients with NMO (45). NMO patients that do not have detectable levels of NMO-IgG may represent a group for which AQP4 is not the target antigen for autoantibody. Other CNS antigens such as the Kir4.1 present on astrocytes might be targets for autoantibodies in those NMO patients. It is possible that there is a unique and rare specificity of NMO-IgG that is particularly pathogenic but that cannot be detected by current diagnostic techniques (46).

The lower seropositivity of anti-AQP4, as well as for other autoantibodies obtained in the present study compared with those previously described could be explained by the prednisone treatment of the NMO patients. The patients of the present study had no relapses during the research and were treated with prednisone in association with azathioprine or mycophenolate mofetil. There is evidence that anti-AQP4 levels are reduced in patients under immunosuppressive treatment and without relapses (47,48). On the other hand, Jarius et al. (48) observed that anti-AQP4 antibodies were detected during remission as well as during relapses, both in untreated NMO patients and in patients under immunosuppressive treatment, suggesting that this antibody can be of diagnostic importance independently of treatment status or disease activity.

The lack of anti-AQP4 IgG seropositivity in a subset of NMO patients suggests that the myelitis and ON can be caused by other mechanisms, such as connective tissue disorders (7), paraneoplastic disorders (13,49), or infectious diseases, providing strong evidence in favour of the hypothesis of NMO being aetiopathogenetically heterogeneous (9).

In the present cohort, 13.6% NMO patients presented infections that preceded the NMO onset, such as tuberculosis, HIV-1, and HHV-3 infections. These results were in agreement with previous studies (50,51). NMO following pulmonary tuberculosis has also been reported (50) and these authors suggested that the close temporal relationship to pulmonary tuberculosis is not coincidental and the syndrome is most likely due to an immune reaction to tuberculosis rather than the use of anti-tuberculosis medication. *Mycobacterium tuberculosis* surface antigens may trigger the formation of cross-reactive antibodies against AQP4 (51).

NMO without an identified cause can be seen in the course of HIV infection, even at an early stage of the disease, before immunosuppression occurs. Optic neuritis can occur in patients infected with HIV and the role of HIV itself is now well established. Intramedullar involvement in the course of HIV infection may result from HIV itself (vacuolar myelopathy) (52). Moreover, several different types of herpes infections, such as HHV-3 and HHV-5 or cytomegalovirus have been reported in patients with apparent NMO (53).

Limited information exists about treatment of NMO in patients with infectious diseases, such as tuberculosis and HIV infection. A clinical trial conducted in Chinese NMO patients demonstrated a marked beneficial effect of anti-tuberculosis treatment on the course of patients with steroid refractory NMO (54). These authors showed that anti-tuberculosis treatment may lead to the recovery of important neurological functions and all our patients responded positively to therapy. EDSS score and visual acuity improved and abnormalities in the spinal cord, observed by magnetic resonance imaging (MRI), markedly decreased over time. Anti-tuberculosis treatment also significantly reduced the rate of relapse. With the advent of the highly active antiretroviral therapy, immunosuppressants once strictly contraindicated in HIV positive individuals are now attempted, especially in those less immunocompromised (54–57).

https://doi.org/10.1017/neu.2016.49 Published online by Cambridge University Press

Taken together, the results of the present study underscored that the NMO patients present high frequency of autoimmune disorders and autoantibodies against cellular antigens. Further studies with large number of NMO patients may contribute to improve our understanding of the NMO pathogenesis and the coexisting autoimmunity.

Acknowledgements

The authors thank the patients and the University Hospital of State University of Londrina for technical and administrative supports. Authors' Contributions: W.L.C.J.P.: conceived and designed the study, enrolled and attended clinically the patients, acquired clinical data, interpreted the results, drafted and revised the manuscript, and gave final approval for the version to be published; E.M.V.R.: conceived and designed the study, interpreted the results, drafted and revised the manuscript, and gave final approval for the version to be published; A.P.K., S.R.O.: acquired demographical, epidemiological, and clinical data, collected and processed the blood samples of the patients, and gave final approval for the version to be published; M.A.B.L., D.F.A., T.F.: acquired laboratory data, performed the laboratory tests, and gave final approval for the version to be published; A.N.C.S.: conceived and designed the study, performed the statistical analysis, interpreted the results; and gave final approval for the version to be published; L.J.V.S., P.R.V.P.R.: enrolled and attended clinically the patients, acquired clinical and imaging data, and gave final approval for the version to be published; D.R.K.-M.: attended the patients, interpreted the imaging data, revised critically the manuscript for important intellectual content, and gave final approval for the version to be published.

Financial Support

The study was supported by Coordination for the Improvement of Higher Level of Education Personnel (CAPES) of Brazilian Ministry of Education; Institutional Program for Scientific Initiation Scholarship (PIBIC) of the National Council for Scientific and Technological Development (CNPq), and State University of Londrina (PROPPG).

Conflicts of Interest

None.

References

1. Sellner J, Hemmer B, Mühlau M. The clinical spectrum and immunobiology of parainfectious neuromyelitis optica (Devic) syndromes. J Autoimmun 2010;**34**:371–379.
2. Jacob A, Matiello M, Wingerchuk DM, Lucchinetti CF, Pittock SJ, Weinshenker BG. Neuromyelitis optica: changing concepts. J Neuroimmunol 2007;**187**:126–138.
3. Matiello M, Jacob A, Wingerchuk D, Weinshenker BG. Neuromyelitis optica. Curr Opin Neurol 2007;**20**:255–260.
4. Kim W, Kim SH, Kim HJ. New insights into neuromyelitis optica. J Clin Neurol 2011;**7**:115–127.
5. Jarius S, Wildemann B. The history of neuromyelitis optica. J Neuroinflammation 2013;**10**:1–12.
6. Wingerchuk DM, Lennon VA, Lucchinetti CF, Pittock SJ, Weinshenker BG. The spectrum of neuromyelitis optica. Lancet Neurol 2007;**6**:805–815.
7. Jarius S, Jacobi C, De Seze J et al. Frequency and syndrome specificity of antibodies to aquaporin-4 in neurological patients with rheumatic disorders. Mult Scler 2011;**17**:1067–1073.
8. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. J Exp Med 2005;**202**:473–477.
9. Brusse E, Tijssen C. Neuromyelitis optica with endocrinopathy: further evidence of a new syndrome. J Neuroophthalmol 2001;**25**:151–155.
10. Sergio P, Mariana B, Alberto O et al. Association of neuromyelitis optica (NMO) with autoimmune disorders: report of two cases and review of the literature. Clin Rheumatol 2010;**29**:1335–1338.
11. Asgari N, Owens T, Frøkiaer J, Stenager E, Lillevang ST, Kyvik KO. Neuromyelitis optica (NMO) – an autoimmune disease of the central nervous system (CNS). Acta Neurol Scand 2011;**123**:369–384.
12. Fujihara K. Neuromyelitis optica and astrocytic damage in its pathogenesis. J Neurol Sci 2011;**306**:183–187.
13. Jarius S, Paul F, Franciotta D et al. Neuromyelitis optica spectrum disorders in patients with myasthenia gravis: ten new aquaporin-4 antibody positive cases and a review of the literature. Mult Scler 2012;**18**:1135–1144.
14. Leite MI, Coutinho E, Lana-Peixoto M et al. Myasthenia gravis and neuromyelitis optica spectrum disorder: a multicenter study of 16 patients. Neurology 2012;**78**:1601–1607.
15. Maruta K, Sonoda Y, Uchida Y, Takahashi T, Fukunaga H. A case of neuromyelitis optica associated with anti-aquaporin 4 antibody and other autoantibodies. Nihon Ronen Igakkai Zasshi 2012;**49**:491–495.
16. Wingerchuk DM, Weinshenker BG. The emerging relationship between neuromyelitis optica and systemic rheumatologic autoimmune disease. Mult Scler 2012;**18**:5–10.
17. Sato DK, Lana-Peixoto M, Fujihara K, De Seze J. Clinical spectrum and treatment of neuromyelitis optica spectrum disorders: evolution and current status. Brain Pathol 2013;**23**:647–660.
18. Freitas E, Guimarães J. Neuromyelitis optica spectrum disorders associated with other autoimmune diseases. Rheumatol Int 2014;**35**:243–253.
19. Zhang B, Zhong Y, Wang Y et al. Neuromyelitis optica spectrum disorders without and with autoimmune diseases. BMC Neurol 2014;**14**:162.
20. Pittock SJ, Lennon VA, De Seze J et al. Neuromyelitis optica and organ-specific autoimmunity. Arch Neurol 2008;**65**:78–83.
21. Uzawa A, Mori M, Sawai S et al. Cerebrospinal fluid interleukin-6 and glial fibrillary acidic protein levels are increased during initial neuromyelitis optica attacks. Clin Chim Acta 2013;**421**:181–183.

https://doi.org/10.1017/neu.2016.49 Published online by Cambridge University Press

22. Nagaishi A, Takagi M, Umemura A et al. Clinical features of neuromyelitis optica in a large Japanese cohort: comparison between phenotypes. J Neurol Neurosurg Psychiatry 2011;**82**:1360–1364.
23. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. Neurology 2006;**66**:1485–1489.
24. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997; **40**:1725.
25. Shiboski SC, Shiboski CH, Criswell L et al. American College Rheumatology classification criteria for Sjogren's syndrome: a data-driven, expert consensus approach in the Sjogren's International Collaborative Clinical Alliance cohort. Arthritis Care Res 2012;**64**:475–487.
26. Aletaha D, Neogi T, Silman A et al. Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism Collaborative Initiative. Arthritis Rheum 2010;**62**: 2569–2581.
27. Petty RE, Southwood TR, Manners P et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol 2004;**31**:390–392.
28. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;**33**:1444–1452.
29. Kayser C, Corrêa MJU, Andrade LEC. Raynaud's phenomenon. Rev Bras Reumatol 2009;**49**:48–63.
30. D'cruz DP, Mellor-Pita S, Joven B et al. Transverse myelitis as the first manifestations of systemic lupus erythematosus or lupus-like disease: good functional outcome and relevance of antiphospholipid antibodies. J Rheumatol 2004;**31**:280–285.
31. Birnbaum J, Kerr D. Devic's syndrome in a woman with systemic lupus erythematosus: diagnostic and therapeutic implications of testing for the neuromyelitis optica IgG autoantibody. Arthritis Rheum 2007;**57**:347–351.
32. Franciotta D, Zardini E, Caporali R et al. Systemic sclerosis in aquaporin-4 antibody-positive longitudinally extensive transverse myelitis. J Neurol Sci 2011;**303**: 139–141.
33. Mihailova D, Grigorova R, Vassileva B et al. Autoimmune thyroid disorders in juvenile chronic arthritis and systemic lupus erythematosus. Adv Exp Med Biol 1999;**455**:55–60.
34. Alpigiani MG, Cerboni M, Bertini I et al. Endocrine autoimmunity in young patients with juvenile chronic arthritis. Clin Exp Rheumatol 2002;**20**:565–568.
35. Stagi S, Giani T, Simonini G, Falcini F. Thyroid function, autoimmune thyroiditis and coeliac disease in juvenile idiopathic arthritis. Rheumatology 2005;**44**:517–520.
36. Mckeon A, Lennon VA, Lotze T et al. CNS aquaporin-4 autoimmunity in children. Neurology 2008;**71**:93–100.
37. Jarius S, Ruprecht K, Wildemann B et al. Contrasting disease patterns in seropositive and seronegative neuromyelitis optica: a multicentre study of 175 patients. J Neuroinflammation 2012;**9**:14.
38. Kallaur AP, Oliveira SR, Simão AN et al. Tumor necrosis factor beta (TNF-β) *Nco*I polymorphism is associated with multiple sclerosis in Caucasian patients from Southern Brazil independently from HLA-DRB1. J Mol Neurosci 2014; **53**:211–221.
39. Lana-Peixoto MA. Devic's neuromyelitis optica: a critical review. Arq Neuropsiquiatr 2008;**66**:120–138.
40. Flanagan EP, Cabre P, Weinshenker BG et al. Epidemiology of aquaporin-4 autoimmunity and neuromyelitis optica spectrum. Ann Neurol 2016. doi:10.1002/ana.24617.
41. Rivera JF, Kurtzke JF, Booth VJA, Corona VT 5th. Characteristics of Devic's disease (neuromyelitis optica) in Mexico. J Neurol 2008;**255**:710–715.
42. Grimaldi CM. Sex and systemic lupus erythematosus: the role of the sex hormones estrogen and prolactin on the regulation of autoreactive B cells. Curr Opin Rheumatol 2006;**18**:456–461.
43. Wingerchuk DM. Neuromyelitis optica: effect of gender. J Neurol Sci 2009;**286**:13–18.
44. Matà S, Lolli F. Neuromyelitis optica: an update. J Neurol Sci 2011;**303**:13–21.
45. Adoni T, Lino AM, Marchiori PE, Kok F, Callegaro D. Seroprevalence of NMO-IgG antibody in Brazilian patients with neuromyelitis optica. Arq Neuropsiquiatr 2008;**66**: 295–297.
46. Graber DJ, Levy M, Kerr D, Wade WF. Neuromyelitis optica pathogenesis and aquaporin 4. J Neuroinflammation 2008;**29**:5–22.
47. Takahashi T, Fujihara K, Nakashima I et al. Anti-aquaporin-4 antibody is involved in the pathogenesis of NMO: a study on antibody titre. Brain 2007;**130**:1235–1243.
48. Jarius S, Aboul-Enein F, Waters P et al. Antibody to aquaporin-4 in the long-term course of neuromyelitis optica. Brain 2008;**131**:3072–3080.
49. Ducray F, Roos-Weil R, Garcia PY et al. Devic's syndrome-like phenotype associated with thymoma and anti-CV2/CRMP5 antibodies. J Neurol Neurosurg Psychiatry 2007;**78**:325–327.
50. Silber MH, Willcox PA, Bowen RM, Unger A. Neuromyelitis optica (Devic's syndrome) and pulmonary tuberculosis. Neurology 1990;**40**:934–938.
51. Sridhar S, Chan JF, Yuen KY. Fatal anti-aquaporin-4 seropositive neuromyelitis optical spectrum disorder in tuberculosis. BMC Infect Dis 2014;**14**:470.
52. Blanche P, Diaz E, Goert B, Sicard D, Rivoal O, Brezin A. Devic's neuromyelitis optica and HIV-1 infection. J Neurol Neurosurg Psychiatry 2000;**68**:795–796.
53. Machado C, Amorim J, Rocha J, Pereira J, Lourenço E, Pinho J. Neuromyelitis optica spectrum disorder and varicella-zoster infection. J Neurol Sci 2015;**358**:520–521.
54. Feng YQ, Guo N, Huang F, Chen X, Sun QS, Liu JX. Anti-tuberculosis treatment for Devic's neuromyelitis optica. J Clin Neurosci 2010;**17**:1372–1377.
55. Ciufreda D, Pantaleo G, Pascual M. Effects of immunosuppressive drugs on HIV infection: implications for solid-organ transplantation. Transpl Int 2007;**20**: 649–658.
56. Feyissa AM, Singh P, Smith RG. Neuromyelitis optica in patients with coexisting human immunodeficiency virus infections. Mult Scler 2013;**19**:1363–1366.
57. Salazar R, Cerghet M, Shad A, Markowitz NP. NMO-IgG positive relapsing longitudinally extensive transverse myelitis (LETM) in a seropositive HIV patient. Clin Neurol Neurosur 2013;**115**:1873–1875.

https://doi.org/10.1017/neu.2016.49 Published online by Cambridge University Press