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Neuromyelitis optica spectrum disorders: A nationwide Portuguese clinical epidemiological study

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ABSTRACT

Introduction: Neuromyelitis optica spectrum disorder (NMOSD) is a rare disorder in which astrocyte damage and/or demyelination often cause severe neurological deficits.
Objective: To identify Portuguese patients with NMOSD and assess their epidemiological/clinical characteristics.
Methods: This was a nationwide multicenter study. Twenty-four Portuguese adult and 3 neuropediatric centers following NMOSD patients were included.
Results: A total of 180 patients met the 2015 Wingerchuk NMOSD criteria, 77 were AQP4-antibody positive (Abs+), 67 MOG-Abs+, and 36 seronegative. Point prevalence on December 31, 2018 was 1.71/100,000 for NMOSD, 0.71/100,000 for AQP4-Abs+, 0.65/100,000 for MOG-Abs+, and 0.35/100,000 for seronegative NMOSD. A total of 44 new NMOSD cases were identified during the two-year study period (11 AQP4-Abs+, 27 MOG-Abs+, and 6 seronegative). The annual incidence rate in that period was 0.21/100,000 person-years for NMOSD, 0.05/100,000 for AQP4-Abs+, 0.13/100,000 for MOG-Abs+, and 0.03/100,000 for seronegative NMOSD.
AQP4-Abs+ predominated in females and was associated with autoimmune disorders. Frequently presented with myelitis. Area postrema syndrome was exclusive of this subtype, and associated with higher morbidity/mortality than other forms of NMOSD. MOG-Ab+ more often presented with optic neuritis, required less immunosuppression, and had better outcome.
Conclusion: Epidemiological/clinical NMOSD profiles in the Portuguese population are similar to other European countries

1. Introduction

Neuromyelitis optica spectrum disorder (NMOSD) is a rare relapsing inflammatory disease of the central nervous system (CNS), (Weinshenker and Wingerchuk, 2017; Flanagan and Weinshenker, 2014) but probably the most common of non-multiple sclerosis (MS) inflammatory demyelinating diseases (IDDs) of the CNS (Flanagan and Weinshenker, 2014; Leite et al., 2012).

The discovery in 2005 of circulating pathogenic immunoglobulin G1 (IgG1) antibodies against the astrocyte water channel protein aquaporin 4 (AQP4) associated with neuromyelitis optica revolutionized the understanding of the disease (Papadopoulos, 2009).

The diagnostic criteria developed by the International Panel for NMOSD Diagnosis in 2015 (Wingerchuk et al., 2015) enabled to diagnose (i) NMOSD with AQP4 antibodies (AQP4-Abs+) with at least one of six core clinical symptoms and (ii) NMOSD seronegative for AQP4 antibodies with at least two of six core clinical features (one of which being one of the three most common: optic neuritis, transverse myelitis, or area postrema syndrome) and evidence from magnetic resonance imaging (MRI). (Wingerchuk et al., 2015; Papp et al., 2018) Anti-myelin oligodendrocyte glycoprotein (MOG) antibodies (MOG-Abs+) are detected in a proportion of NMOSD patients who are seronegative for AQP4 antibodies (Jarius et al., 2016; Sato et al., 2014).

NMOSD is now a well-established heterogeneous CNS IDD, distinct and with a different treatment approach from MS (Trebst et al., 2014). Early diagnosis with prompt acute treatment and adequate prophylactic chronic immunotherapy are crucial to prevent disability. Data about NMOSD epidemiology and disease characteristics are crucial for adequate healthcare resource allocation, namely related to the use of specific therapies, and have been missing in Portugal until now (Weinshenker and Wingerchuk, 2017). This led us to conduct a national study on these patients, although according to the Portuguese population characteristics and healthcare services we could suspect to find analogous results to other European countries, especially those with similar latitude.

2. Objective

A clinical-based NMOSD survey was undertaken in the Portuguese population using the revised diagnostic criteria (2015 IPND criteria) (Wingerchuk et al., 2015), with the main objective of characterizing its epidemiology (point prevalence and incidence). The analysis of demographics and clinical features was the secondary aim.

3. Materials and methods

3.1. Study setting

According to the 2018 National Statistics Institute (INE) report, the resident Portuguese population on December 31, 2018 was 10,276,617 persons (INE and de Portugal, 2020).

In Portugal, NMOSD patients are mostly treated in public hospitals (part of the National Health Service). Even when diagnosis is suspected in private institutions, patients are referred to public hospitals for further workup and management. They are usually followed and treated by neurologists who also manage MS patients. This study was planned and discussed during meetings of the Portuguese Multiple Sclerosis Study Group (*Grupo de Estudos de Esclerose Múltipla*) and Portuguese Society of Neurology, to ensure that all neurologists treating NMOSD patients would be included, from patient identification to respective data collection.

This was a nationwide multicenter study. Public hospitals with at least one neurologist or Neuropediatrics Unit treating MS patients were invited to attend meetings and participate in the study.

Data was collected between January 1, 2018 and December 31, 2019. Both public and private hospitals managing NMOSD patients were invited to participate. Their names are listed in the author affiliations' section. All patients with NMOSD identified during that time period were included, namely those with disease onset before January 1, 2018 – prevalent population – and those with disease onset and NMOSD diagnosis during the study period – incident population.

A total of 43 Portuguese public hospitals and one private hospital were invited to participate. Seventeen did not treat NMOSD patients, only referring them to larger institutions. The remaining 24 adult centers and three Neuropediatric Units were included in the study.

All patients signed informed consent to participate, and anonymized data was inserted in an electronic database.

3.2. Identification of NMOSD patients

Patient information was retrieved from hospital clinical databases and/or Neurology clinical records. NMOSD diagnosis was confirmed by reviewing patients' medical records. Retrieved information included sociodemographic and clinical data. Patients who had not been tested for anti-AQP4 and/or anti-MOG antibodies or who were antibody-negative by local standard assays were asked to provide a blood sample for antibody testing.

During the study period, all neurologists from participating centers

were regularly contacted to register new patients, complete missing clinical data, and collect patients' blood samples as appropriate.

3.3. NMOSD subgroup classification

Patients with NMOSD diagnosis were identified based on the 2015 IPND criteria (Wingerchuk et al., 2015) and divided in three groups: AQP4-Abs+ subgroup, MOG-Abs+ subgroup, and seronegative subgroup. All cases were validated by the two neurologists who were principal investigators in this study (ES, MJS).

3.4. Clinical data collection

Clinical and demographic data were collected from patients' medical records and included date of birth, current age, gender, ethnicity, age at NMOSD onset, age at NMOSD diagnosis, presence of other autoimmune disorders, clinical presentation, number of relapses, MRI results, cerebrospinal fluid (CSF) results, AQP4-Abs, MOG-Abs, and other autoantibodies study, Expanded Disability Status Scale (EDSS) score, and treatments used during the course of disease. Brain and spinal cord MRI imaging was performed in all patients and data was collected based on the reports, and not on protocoled analysis of the images.

All data was recorded in a single anonymous database specifically designed for this study.

3.5. AQP4 and MOG antibody assays

All patients positive for anti-AQP4 or anti-MOG antibodies according to local tests were not tested again. Those tests included three different assays: a commercial fixed cell-based assay (Euroimmun®, Germany), a live, *in house,* cell-based assay (Oxford, UK), and a live cell Fluorescence-Activated Cell Sorting (FACS) assay (Mayo Clinic, EUA).

Negative results for anti-AQP4 were reanalysed and if again negative the sample was tested for anti-MOG antibodies. These tests were conducted in Centro Hospitalar Universitario do Porto (CHUP) using a fixed cell-based assay (Euroimmun®, Germany) according to the manufacturer's instructions. Results were considered positive if a typical fluorescence pattern was observed on transfected cells.

3.6. Statistical analysis

Point prevalence date was set on December 31, 2018 for all patients alive and resident in the study area. The 10,276,671 residents in Portugal on December 31, 2018 comprised prevalence denominator. Annual incidence rate refers to all incident cases in the country throughout the 2018-2019 study period. To estimate the number of person-years for the incidence denominator, the mid period number of 10,276,671 estimated residents was multiplied by two. Prevalence and annual incidence rate were calculated per 100,000 inhabitants for all NMOSD patients and respective subgroups.

Poisson distribution was used to calculate confidence intervals for incidence and prevalence (Papp et al., 2018). Pearson's chi-squared test ($\chi 2$) was used to assess the association between some categorical variables and the NMOSD serological type. In one case, the independent variable (age) was continuous and comparison of means was performed. For these analyses, SPSS statistics, version 25 was used.

3.7. Ethical approval

Ethical approval for patient data collection and blood collection when necessary was obtained from the Ethics Committees of participating hospitals and from the Portuguese Data Protection Authority (*Comissão Nacional de Proteção de Dados*). Informed consent was additionally retrieved from participating patients.

4. Results

4.1. Epidemiological characteristics

A total of 191 patients with NMOSD diagnosis according to Wingerchuk 2015 criteria were identified. Of these, 180 had known serological information for AQP4 and MOG antibodies and were included and eleven patients who only had information on AQP4 antibodies were excluded.

Of the 180 patients included, 144 were seropositive (77 had AQP4 and 67 MOG antibodies) and 36 seronegative. Four patients with anti-AQP4 antibodies died during the study period, three due to the disease and one from a respiratory infection.

The point prevalence on December 31, 2018 was 1.71/100,000 for NMOSD (95% confidence interval [CI] 1.47-1.98), 0.71/100,000 for AQP4-Abs+ subgroup (95% CI 0.56-0.90), 0.65/100,000 for MOG-Abs+ subgroup (95% CI 0.50-0.83), and 0.35/100,000 for seronegative subgroup (95% CI 0.24-0.48).

Forty-four new NMOSD cases were identified during the study period (27 in 2018 and 17 in 2019), 38 of which seropositive (11 AQP4-Abs+, 27 MOG-Abs+) and six seronegative.

The annual incidence rate between 2018-2019 was 0.21 per 100,000 person-years for NMOSD (44/[10276617 × 2]; 95% CI 0.16-0.29), 0.05 per 100,000 person-years for AQP4-Abs+ subgroup (95% CI 0.03-0.10), 0.13 per 100,000 for MOG-Abs+ subgroup (95% CI 0.09-0.20), and 0.03 per 100,000 person-years for seronegative subgroup (95% CI 0.01-0.06).

4.2. Demographic and clinical features

Tables 1 and 2 show the main demographic and clinical features of the three patient subgroups and Fig. 1 depicts the number of NMOSD cases by serological type, age at first symptoms, and sex.

The female:male ratio was significantly higher in the AQP4-Abs+ subgroup compared with the other two patient subgroups (7.5:1 vs 1.8:1 in MOG-Abs+ subgroup vs 1.7:1 in seronegative subgroup, p=0.001) (Fig. 1). Non-Caucasians accounted for 10.4% of patients in AQP4-Abs+ subgroup, 3.0% in MOG-Abs+ subgroup, and 2.8% in seronegative subgroup (p=0.327).

Table 1
Demographic characteristics of NMOSD patients with AQP4 antibodies, MOG antibodies, and seronegativity.

CharacteristicValues	AQP4 Abs+ (n=77)	MOG Abs+ (n=67)	Seronegative (n=36)	*P value*
Gender				0.001
Female	68 (88%)	43 (64%)	22 (61%)	
Male	9 (12%)	24 (36%)	14 (39%)	
Age (years)				0.087
Mean	48.4	41.7	44.7	
Min-Max	10-87	4-84	19-75	
SD	2.1	2.3	2.3	
Age group				0.08
<=18	1	8	0	
19-39	24	24	14	
40-64	34	25	20	
>65	18	10	2	
Ethnicity				0.327
African	7 (9%)	2 (3%)	1 (3%)	
Asiatic	1 (1%)	0 (0%)	0 (0%)	
Caucasian	69 (90%)	65 (97%)	35 (97%)	
Follow-up duration				0.097
(years)	30	35	10	
<=2	21	16	11	
>2 and <6	24	16	15	
6 or +				

AQP4 Abs+, anti-astrocyte water channel protein aquaporin 4 antibodies-positive; MOG Abs+, anti-myelin oligodendrocyte glycoprotein antibodies-positive.
Bold entries indicate statistical significance ($p < 0.05$).

Table 2
Clinical features of NMOSD patients with AQP4 antibodies, MOG antibodies, and seronegativity.

Characteristics Values	AQP4 Abs+ (n=77)	MOG Abs+ (n=67)	Seronegative (n=36)	*P value*
Age of onset Mean	40.7	35.8	38.0	0.218
Min-Max	4-87	2-78	9-67	
SD	17.9	17.6	12.9	
Age group at disease	8	13	1	0.094
onset < =18	29	26	21	
19-39	32	24	13	
40-65	8	4	1	
>65				
Interval between disease				0.023
onset and diagnosis	49 (64%)	47 (70	21 (58%)	
< 2 years	16 (21%)	%6 (9%)	6 (17%)	
2-5	7 (9%)	4 (6%)	8 (22%)	
6-10	5 (6%)	10 (15%)	1 (3%)	
> 11 years				
First symptom				0.016
1.optic neuritis	25 (32%)	30 (45%)	9 (25%)	
2.transverse myelitis	35 (46%)	22 (33%)	21 (58%)	
3.1 and 2	4 (5%)	7 (10%)	1 (3%)	
4.area postrema	5 (7%)	0 (0%)	0 (0%)	
syndrome	4 (5%)	3 (4%)	1 (3%)	
5.brainstem syndrome	1 (1%)	0 (0%)	0 (0%)	
6.narcolepsia	0 (0%)	4 (6%)	1 (3%)	
7.supratentorial	0	0	2	
symptoms	0	1	1	
2. and 5.	1	0	0	
2. and 7.	2	0	0	
3. and 4.				
2. and 4. and 5.				
Nr of relapses in the first				0.317
two years of disease 1	42 (58%)	44 (69%)	23 (64%)	
(ukn n=10) 2	22 (31%)	11 (18%)	7 (19%)	
3	5 (7%)	6 (10%)	2 (6%)	
>=4	3 (4%)	2 (3%)	4 (11%)	
Disability stage/EDSS	17 (28%)	38 (58%)	13 (42%)	0.002
Score 0.0 to 2.5	22 (37%)	18 (27%)	9 (29%)	
3.0 to 4.5	3 (5%)	5 (8%)	0 (0%)	
5.0 to 5.5	18 (30%)	5 (8%)	9 (29%)	
>=6				

AQP4 Abs+, anti-astrocyte water channel protein aquaporin 4 antibodies-positive; EDSS, Expanded Disability Status Scale; Max, maximum; Min, minimum; MOG Abs+, anti-myelin oligodendrocyte glycoprotein antibodies-positive; Nr, number; SD, standard deviation; Ukn, unknown.
Bold entries indicate statistical significance ($p < 0.05$).

Age at disease onset was variable in all three subgroups, ranging from childhood to elderly, although AQP4-Abs+ disease seemed to affect, on average, older people compared to the other subgroups.

The first disease clinical manifestation was myelitis in 45.4% and optic neuritis in 32.5% of patients in the AQP4-Abs+ subgroup, whereas in MOG-Abs+ subgroup optic neuritis was the most common clinical presentation (44.8%), followed by myelitis (32.8%). In the seronegative subgroup, 58.3% of patients presented with myelitis and 25.0% with optic neuritis (p=0.016). Clinical area postrema syndrome was only identified in seven patients in the AQP4-Abs+ subgroup, being an isolated presentation in five.

Simultaneous optic neuritis and myelitis at presentation was more common in the MOG-Abs+ subgroup (10.4% vs 5.2% in AQP4-Abs+ subgroup vs 2.8 in seronegative subgroup; p=0.016). Nine percent (6/67) of MOG-Abs+ patients had bilateral and simultaneous optic neuritis (Table 2).

Most patients reported one or two relapses in the first two years of disease (Table 2). Differences in relapse number between the three subgroups were not statistically significant.

Although no significant differences were observed in disease severity as measured by EDSS at nadir between the three subgroups, most severe EDSS cases (7−10 score) belonged to the AQP4-Abs+ subgroup (Table 2).

Association with other autoimmune diseases was more common in AQP4-Abs+ subgroup (26.7% vs 4.5% in anti-MOG Abs+ subgroup vs 12.9% in seronegative subgroup; p<0.0001).

4.3. MRI findings

MRI findings in the study cohort are depicted in Table 3. We got MRI data from 139 patients of the 180 NMOSD patients identified.

Brainstem involvement was significantly higher in MOG-Abs+ patients. Lesions in the area postrema, which contrast with the clinical syndrome, were identified in all patient subgroups (Table 3).

Involvement of both optic nerves extending to the chiasma was recorded in 20% of all NMOSD patients. Isolated chiasma involvement was not found in the seronegative subgroup.

Supratentorial lesions were more frequent in the MOG-Abs+ subgroup, although the difference was not statistically significant. MRI with typical acute demyelinating encephalomyelitis features was not identified in this cohort.

Conus medullaris lesions were more frequently found in MOG-Abs+ patients, whereas lesions in other spinal cord segments were equally reported in the three NMOSD subgroups.

4.4. Laboratory findings

Table 4 depicts laboratory findings in the three considered patient subgroups. Other autoantibodies (ANA, anti-SSA, anti-SSB, or anti-thyroid) were more common in the AQP4-Abs+ subgroup (39.4% vs 3.3% in anti-MOG Abs+ subgroup vs 6.4% in seronegative subgroup, p<0.0001).

CSF data was available in 80.6% of patients (145/180). Pleocytosis was present in 44.6% of the AQP4-Abs+ subgroup, 50.8% of the MOG-Abs+ subgroup, and 56.6% of the seronegative subgroup, which represented non-statistically significant differences (p=0.553). Average cell number was 34.1 cells/uL in AQP4-Abs+ subgroup, 45.8 cells/uL in MOG-Abs+ subgroup, and 90.4 cells/uL in seronegative subgroup (p=0.349). Oligoclonal bands were present in 25% of the AQP4-Abs+ subgroup, 18% of the MOG-Abs+ subgroup, and 16.7% of the seronegative group (p=0.563; Table 4).

4.5. Treatment according to NMOSD subgroup

Treatment received according to NMOSD patient subgroup are shown in Table 5. All patients with AQP4-Abs+ and seronegative disease and 92.5% of patients with MOG-Abs+ disease were treated with steroids in relapse. Plasma exchange was used in 46.7% (28/60) of AQP4-Abs+, 11.3% (7/62) of MOG-Abs+, and 30.6% (11/36) of seronegative subgroup (p=0.001).

Regarding chronic immunosuppressant treatment, rituximab was preferentially used in the AQP4-Abs+ subgroup (p=0.002). The number of immunosuppressants used was higher in the AQP4-Abs+ subgroup (p<0.0001).

5. Discussion

The reported prevalence of NMOSD is approximately 1/100,000 among Caucasians, with an annual incidence of <1/million population (Hor et al., 2020; Dale et al., 2018 Jun). However, prevalence may be up to 10/100,000 in certain racial groups. Still, this is a low prevalence compared with MS, which ranges from 1–2/100,000 in the equatorial region and 150– 200/100,000 in Canada and northern Europe (Hor et al., 2020).

Few NMOSD epidemiological studies have used the 2015 IPND criteria (Wingerchuk et al., 2015) or included AQP4-Abs+, MOG-Abs+, and double seronegative subgroups (Papp et al., 2018; Papp et al., 2020; Houzen et al., 2017 Nov 7).

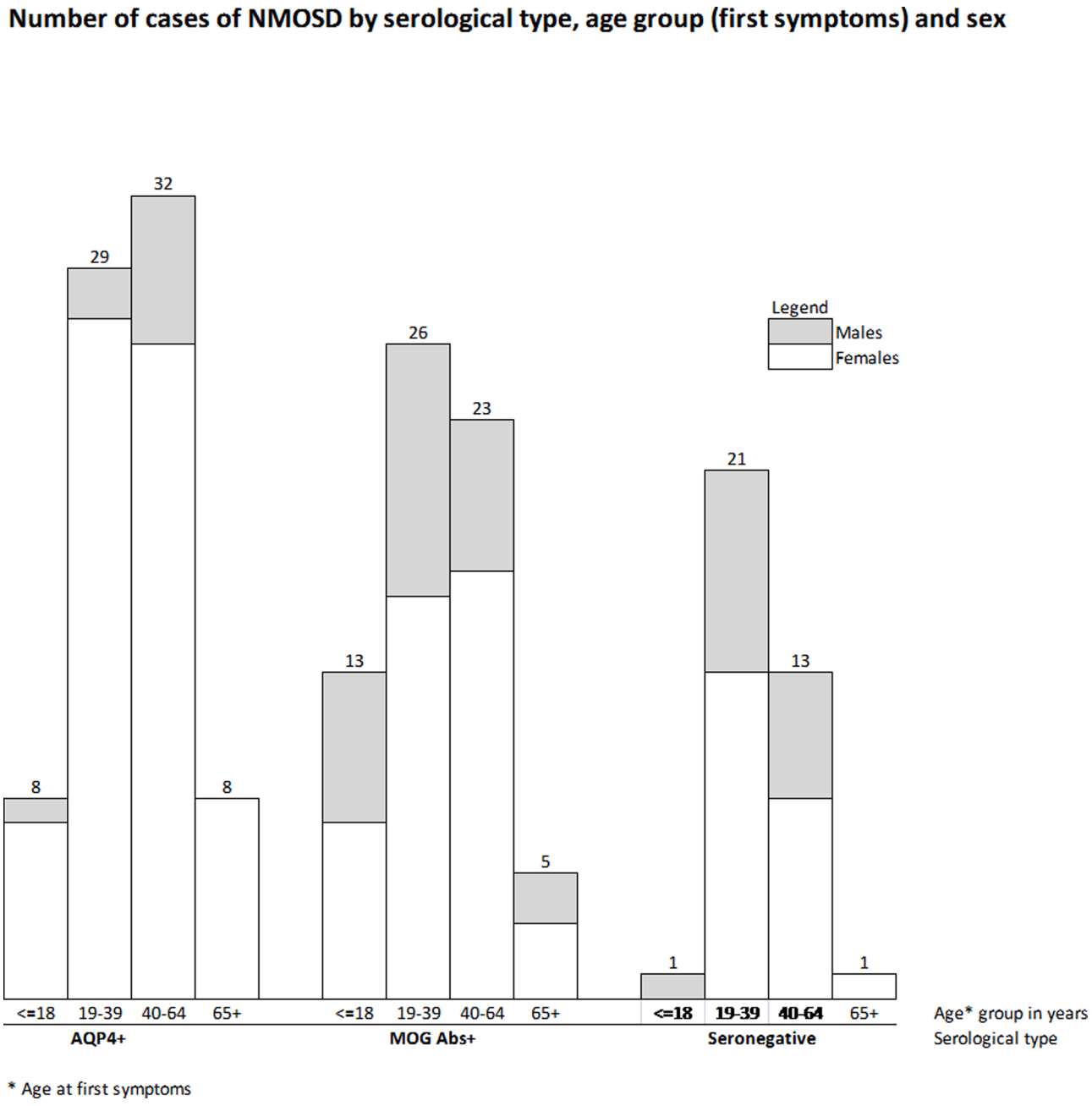

Fig. 1. Number of NMOSD cases by serological type, age at first symptoms, and sex.

Prevalence values identified in this study are similar to those found in Caucasian populations in Europe (Jarius et al., 2016; Hor et al., 2020), Middle East (Eskandarieh et al., 2017 Nov), and Australia (Sepúlveda et al., 2018) and lower than those reported in non-Caucasian populations using the same diagnostic criteria (Papp et al., 2020; Bukhari et al., 2017).

NMOSD prevalence in Australia and New Zealand has been estimated in 0.55/100,000 (Bukhari et al., 2017), in Catalonia in 0.89/100,000 (Sepúlveda et al., 2018), in Denmark in 1.09/100,000 (Papp et al., 2018), and in Sweden in 1.04/100,000 inhabitants (Jonsson et al., 2019).

A study from South Denmark reported an AQP4-Abs+ disease prevalence of 1.68/100,000 and of MOG-Abs+ disease of 4.4/100,000 (Asgari et al., 2019). In the present study, prevalence of AQP4-Abs+ disease was 0.71/100,000 (95% CI 0.56-0.90) and of MOG-Abs+ disease was 0.65/100,000 (95% CI 0.50-0.83).

Incidence rates found in this study in the Portuguese population were also similar to those reported in Europe (Catalonia and Denmark) and Australia (Hor et al., 2020; Sepúlveda et al., 2018; Hor et al., 2018).

Regarding the possible effect of latitude on prevalence, data from Catalonia is the one closest to Portugal (Sepúlveda et al., 2018).

The present multicenter nationwide study is the first investigating epidemiological and clinical features of the Portuguese NMOSD population, including adults and pediatric patients. In absence of a centralized national disease or laboratory results registry, the medical records of patients included in databases of the 27 Portuguese centers where NMOSD patients are followed was the only source of identification of patients for inclusion in this study. Using a laboratory source was not an option in this study, since patients from different hospitals are tested in different laboratories, including private ones.

With these limitations in mind and considering that NMOSD patients are almost always diagnosed and treated by or referred to neurologists,

Table 3
Imaging characteristics of NMOSD patients with AQP4 antibodies, MOG antibodies, and seronegativity.

Topography	Total (n=139)	AQP4 Abs+ (n=60)	MOG Abs+ (n=50)	Seronegative (n=29)	P value
Supratentorial (%)	87 (63)	18 (30)	24 (48)	9 (31)	0.106
Transverse myelitis (%)	96 (69.1)	42 (70)	31 (62)	23 (79.3)	0.34
Spinal cord segments, median (IQR)	4 (2-8)	5 (2-9)	5 (2-8)	4 (2-7)	0.69
LETM n(%)	66 (47.5)	28 (46.7)	22 (44)	16 (55.2)	0.79
VLETM, n (%)	13 (9.4)	6 (10)	4 (8)	3 (10.3)	0.87
Cervical, n (%)	65 (46.8)	28 (46.7)	24 (48)	13 (44.8)	0.98
Dorsal, n (%)	63 (45.3)	24 (40)	22 (44)	17 (58.6)	0.197
Conus medullaris, n (%)	12 (8.6)	2 (3.3)	8 (16)	2 (6.9)	0.06
Diencephalon, n (%)	15 (10.8)	3 (5.0)	10 (20)	2 (6.9)	0.04
Brainstem,n (%)	40 (28.8)	10 (16.7)	20 (40)	10 (34.5)	0.02
Cerebellum, n(%)	23 (16.6)	8 (13.3)	12 (24)	3 (10.3)	0.245
Postrema Area, n (%)	5 (3.6)	2 (3.3)	2 (4)	1 (3.45)	1
Optica nerve+chiasm, n(%)	28 (20.1)	12 (20)	14 (28)	2 (6.9)	0.08
Chiasm, n(%)	7 (5.0)	3 (5.0)	4 (8.0)	0 (0)	0.326

AQP4 Abs+, anti-astrocyte water channel protein aquaporin 4 antibodies-positive; AQP-4-/MOG-, anti-astrocyte water channel protein aquaporin 4 antibodies and Anti-myelin oligodendrocyte glycoprotein antibodies negative; EDSS, Expanded Disability Status Scale; LETM, longitudinally extensive transverse myelitis; Max, maximum; Min, minimum; MOG Abs+, anti-myelin oligodendrocyte glycoprotein antibodies-positive; Nr, number; SD, standard deviation; Ukn, unknown; VLETM, very long longitudinally extensive transverse myelitis >12 segments.
Bold entries indicate statistical significance ($p < 0.05$).

Table 4
Laboratory findings of NMOSD patients with AQP4 antibodies, MOG antibodies, and seronegativity.

Characteristics Values	AQP4 Abs+ (n=77)	MOG Abs+ n=67)	Seronegative (n=36)	*P value*
CSF pleocytosis				0.553
Yes	25 (45%)	30 (51%)	17 (57%)	
No	31 (55%) (Not performed/ UKN =21)	29 (49%) (Not performed/ UKN =8)	13 (43%) (Not performed/ UKN =6)	
CSF cells				0.349
Mean	34.1	45.7	90.4	
SD	10.9	15.6	55.5	
Median	12.0	18.0	11.5	
Min - Max	4 — 216	3 – 442	4 – 785	
CSF oligoclonal bands				0.563
Yes	11 (26%)	9 (18%)	5 (17%)	
No	32 (74%) (Not performed/ UKN=31)	41 (82%) (Not performed/ UKN=17)	25 (83%) (Not performed/ UKN=6)	
Other positive autoantibodies in the serum Yes				<0.0001
No	26 (39%) 40 (61%) (Not performed/ UKN=11)	2 (3%) 60 897%) (Not performed/ UKN =8)	2 (6%) 29 (94%) (Not performed/ UKN=5)	

AQP4 Abs+, anti-astrocyte water channel protein aquaporin 4 antibodies-positive; CSF, cerebrospinal fluid; Max, maximum; Min, minimum; MOG Abs+, anti-myelin oligodendrocyte glycoprotein antibodies-positive; Nr, number; SD, standard deviation; Ukn, unknown.
Pleocytosis was considered when >5 cells/ul.
Bold entries indicate statistical significance ($p < 0.05$).

this study was publicized several times in MS Portuguese meetings.

This was a prospective study conducted over a two-year period, during which centers were regularly contacted to identify patients and register data. Due to this, the authors believe that the number of NMOSD patients identified is likely to be close to the reality of NMOSD patients in Portugal.

Clinical and demographic data retrieved from this study are similar to those previously reported in other studies (Wingerchuk et al., 2015; Trebst et al., 2014; Jonsson et al., 2019; Quek et al., 2012). In the AQP4-Abs+ subgroup, there is stronger female predominance, other autoimmune disorders are more common, and non-Caucasians are more frequent. Myelitis is more frequently the initial manifestation in AQP4-Abs+ patients, whereas optic neuritis is the more common onset symptom in MOG-Abs+ counterparts. In this study, area postrema syndrome was highly predictive of AQP4-Abs+ status.

In MRI study, MOG-Abs+ patients more frequently presented lumbar and conus medullaris lesions. Optic neuritis with chiasm involvement, diencephalon, and brainstem were more frequent in AQP4-Abs+ patients.

Prognosis seems better in MOG-Abs+ compared to both AQP4-Abs+ and seronegative disease. As described in other studies, compared with other patient subgroups AQP4-Abs+ patients are more likely to have more relapses and more severe disease, present higher EDSS, (Sato et al., 2014; Wingerchuk et al., 1999) and require more aggressive treatment.

6. Conclusions

This was a nationwide study of AQP4-Abs+, MOG-Abs+, and double seronegative NMOSD classified according to the 2015 IPND criteria, which enabled to retrieve an epidemiological and clinical picture of the Portuguese NMOSD population.

NMOSD associated with AQP4 or MOG antibodies is a very rare autoimmune condition of the CNS, with both disease subgroups presenting distinguishing features. AQP4-Abs+ NMOSD is more often associated with female sex, non-Caucasian race, and other autoimmune diseases, has myelitis as main presenting syndrome, and is characterized by more severe attacks, with frequent use of plasma exchange or intravenous immunoglobulin and need for second-line immunosuppressants. MOG-Abs+ NMOSD is more often associated with (sometimes bilateral) optic neuritis as the commonest presenting feature, simultaneous optic neuritis and myelitis presentation, supratentorial disease, good intravenous methylprednisolone response, and lower requirement for chronic immunosuppression.

NMOSD epidemiological, demographic, and clinical characteristics in Portugal are similar to those from other published series, including in Europe, suggesting an effective clinical and laboratory NMOSD diagnosis throughout the country. Although it is a rare disorder, the significant number of patients we found justifies the existence of reference centers, favouring clinical assessment and treatment by teams with growing and accumulated experience.

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CRediT authorship contribution statement

Ernestina Santos: Investigation, Resources, Conceptualization, Methodology, Validation, Formal analysis, Data curation, Funding acquisition, Writing – original draft, Writing – review & editing. **Ana Luísa Rocha:** Investigation, Resources. **Vanessa Oliveira:** Investigation, Resources. **Daniela Ferro:** Investigation, Resources. **Raquel**

Table 5
Treatment received by NMOSD patients with AQP4 antibodies, MOG antibodies, and seronegativity.

Characteristics	AQP4 Abs+ (n=77)	MOG Abs+ (n=67)	Seronegative (n=36)	*P* value
Steroids				0.056
Yes	76	62	36	
No	1	5	0	
Plasma Exchange				0.001
(missing = 14)	28	7	11	
Yes	40	55	25	
No				
IV IG (missing = 12)				0.148
Yes	16	9	11	
No	53	54	25	
Azathioprine				0.003
(missing = 15)	45	22	18	
Yes	24	40	16	
No				
Rituximab (missing				0.002
= 15)	29	8	12	
Yes	41	54	22	
No				
Methotrexate				0.155
(missing = 12)	4	0	1	
Yes	66	62	35	
No				
Mycophenolate				0.029
Mofetil	14	5	2	
(missing = 8) Yes	55	62	34	
No				
Other				0.297
Yes	1	4	2	
No	76	63	34	
Nr of therapies				0.007
received	1	3	0	
0	11	26	5	
1	24	25	15	
2	21	9	9	
3	12	2	4	
4	5	1	3	
5	2	0	0	
6	1	0	0	
8				
Nr of therapies				<
received	2.79	1.76	2.58	0.0001
(missing = 1) Mean	0.16	0.12	0.19	
SD	3.00	2.00	2.00	
Median	0 - 8	0 - 5	1 - 5	
Min - Max				

AQP4 Abs+, anti-astrocyte water channel protein aquaporin 4 antibodies-positive; CSF, cerebrospinal fluid; IV IG, intravenous immunoglobulin; Max, maximum; Min, minimum; MOG Abs+, anti-myelin oligodendrocyte glycoprotein antibodies-positive; Nr, number; SD, standard deviation.
Bold entries indicate statistical significance ($p < 0.05$).

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