



# Neuromyelitis optica in Portugal (NEMIPORT) – A multicentre study



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## ABSTRACT

**Background:** Neuromyelitis Optica (NMO) is an inflammatory demyelinating disease of the CNS. There have been few epidemiologic studies on NMO, none in Portugal.

**Objective:** To analyze the clinical, biological and MRI characteristics from a cohort of Portuguese patients who fulfilled the Wingerchuk 2006 NMO/NMOSD criteria. To identify and characterize those who had concomitant autoimmune disease or circulating autoantibodies.

**Methods:** We performed an observational, retrospective, multicenter study in 5 Hospital Centers in Portugal.

**Results:** Sixty-seven patients fulfilled the inclusion criteria. They were mainly Caucasian, 55 female. Median age at onset was 32.0 years and mean follow-up  $7.4 \pm 6.0$  years. Twenty-one patients were definite NMO and optic neuritis (ON) the most frequent initial presentation. Forty-six were classified as NMO spectrum disorders. The main subtypes were recurrent ON and single longitudinally extensive transverse myelitis. Twenty-four patients had positive AQP4-IgG. Twenty-three had other circulating autoantibodies. Fifteen out of 67 patients had concomitant autoimmune disease. There was a significant correlation between the presence of autoimmune disease and the positivity for AQP4-IgG. Five patients died, all definite NMO.

**Conclusion:** This is the first study about this rare disease in Portugal. Demographic features were similar to other studies. The existence of concomitant autoimmune disease was significantly associated with seropositivity for AQP4-IgG.

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## 1. Introduction

NMO is a rare inflammatory demyelinating disease of the central nervous system (CNS) [1], with an estimated prevalence of 0.3–4.4/100,000 [2]. It is more prevalent in females (9:1) [1]. In the past 10 years, different diagnostic criteria for NMO have been proposed, including those of Wingerchuk [1,3] and United States National Multiple Sclerosis Society – NMSS [4].

The main clinical features of NMO are optic neuritis (ON) and acute transverse myelitis (TM). Longitudinally extensive TM – LETM and TM with cervical extension to the brainstem are considered typical of NMO.

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Brain MRI findings at the onset of NMO are typically normal or show non-specific white-matter lesions that do not satisfy neuroimaging criteria for MS [5], even though they might raise doubts about differential diagnosis [6].

Laboratory studies in NMO typically comprise a prominent cerebrospinal fluid (CSF) pleocytosis (>50 leucocytes/mL) with a high proportion of neutrophils, although this is only present in a minority of patients. The presence of oligoclonal bands (OCB) of immunoglobulin G (IgG) restricted to the CSF is detected in only 15–30% of the patients [1]. The detection of AQP4-IgG is considered a serum biomarker [7,8] contributing to the diagnosis of NMO and to broaden its clinical spectrum. However, the AQP4-IgG seropositive rate depends on the assay type used [8–11]. The cell-based assay, currently the gold standard, has 100% specificity and between 68% (commercial CBA) and 73% (Oxford CBA) sensitivity in contrast with the commercial ELISA assay that has 97.7% specificity and 70% sensitivity [11].

A common denominator of autoimmunity in NMO is suggested by its association with other autoimmune conditions (10–40% of patients) such as thyroiditis, myasthenia gravis, systemic lupus erythematosus (SLE) and Sjögren's syndrome or more often (50%) by the presence of circulating autoantibodies in the absence of clinical symptoms or autoimmune disease [1,12,13].

Recovery in NMO is often incomplete and most patients follow a relapsing–remitting course with progressive increase of disability. Predictive factors for poor outcome include the presence of other autoimmune diseases, high frequency of relapses during the first two years and poor motor recovery following the index event [1].

There have been few epidemiologic studies on NMO and none in the Portuguese population. This work aims to characterize a group of Portuguese patients with definite and NMO spectrum disorders (NMOSD), according to the Wingerchuk 2006 criteria. Additionally, we intend to determine if there are demographic, clinical, analytical and imaging differences between patients with definite and NMOSD. Finally, we aim to explore the characteristics of patients with concomitant autoimmune disease.

## 2. Material and methods

We performed an observational and retrospective study of NMO patients in 5 Hospital Centers in Portugal. Four are tertiary hospitals and one is a district hospital, following approximately two-thirds of the approximately 5000 patients with demyelinating diseases in the country [14]. Data was collected from March to June 2012. We investigated patients with definite NMO or NMOSD, according to the Wingerchuk 2006 criteria [1,3]. Definite NMO criteria: optic neuritis and acute myelitis and at least two of three supportive criteria: 1. Contiguous spinal cord MRI lesion extending over 3 vertebral segments; 2. Brain MRI not meeting diagnostic criteria for multiple sclerosis; 3. NMO-IgG seropositive status. NMOSD – Limited forms of neuromyelitis optica: Idiopathic single or recurrent events of longitudinally extensive myelitis ( $\geq 3$  vertebral segment spinal cord lesion seen on MRI); recurrent or simultaneous bilateral optic neuritis; optic neuritis or longitudinally extensive myelitis associated with systemic autoimmune disease; optic neuritis or myelitis associated with brain lesions typical of neuromyelitis optica (hypothalamic, corpus callosal, periventricular, or brainstem).

The majority were hospitalized patients that were posteriorly followed-up in general neurology or demyelinating diseases outpatient clinic. We included all patients observed since 1990.

The inclusion criteria were as follows: patients who fulfill criteria for definite and NMOSD (Wingerchuk 2006); patients with brain/spinal MRI (report and/or image); determination of the AQP4-IgG made in the laboratories of the Mayo Clinic or Oxford,

according to the *visual observation-fluorescence cell-based assay* technique. The exclusion criteria were findings consistent with other pathologies including inflammatory demyelinating (namely MS), infectious, vascular, metabolic diseases or CNS malformation and patients without MRI image or report.

Data was obtained from hospital databases of the different participating centers, through consultation of medical records. Epidemiologic data was assessed for each patient including demographic information (age, gender, ethnicity, place of birth); clinical (age of onset, initial clinical disability according to initial EDSS score, pattern of evolution – relapsing–remitting, monophasic or progressive – according to the number of attacks during the follow-up period, optico-spinal interval, time between first and second attack, annualized relapse rate – if applicable, progression time to EDSS 3, 6, 8, 10 – based on stable disability measurement after attack recovery and stabilization, concomitant autoimmune diseases); laboratory (CSF during the first attack, seropositivity for AQP4-IgG, presence of OCB restricted to the CSF and other immunological studies); imaging studies (brain and spinal MRI) and treatment (type of treatment in relapses and global treatment received). MRI at disease onset and additional/subsequent ones were reviewed and evaluated by a neuroradiologist (number, topography, morphology and patterns of contrast enhancement of lesions). LETM lesions were considered when extending for 3 or more vertebral segments.

All patients were asked to provide written informed consent after the study approval by ethics committees, boards of the participating centers and National Commission for Data Protection (NCDP). In the case of deceased patients, data was collected with NCDP permission.

All data analysis was performed using SPSS version 17.0. Demographic and clinical data is presented by descriptive statistics. Means, medians, and ranges were calculated, 95% confidence intervals (CI) were estimated and  $p$  values <0.05 were considered significant.

## 3. Results

### 3.1. Demographic and clinical features (Table 1)

We found 67 patients fulfilling the inclusion criteria (NMO and NMOSD). From these, 55 (65.7%) were female and the majority (95.0%) were Caucasian. The mean age at disease onset was  $36.0 \pm 15.0$  years, with a mean follow-up time of  $7.4 \pm 6.0$  years. Spinal symptoms were the initial presentation in 31 patients (46.3%) and ON in 30 patients (44.8%). Only 3 patients had concomitant myelitis and ON (4.5%) at presentation. In the definite NMO group the majority of patients presented with ON (12 patients, 57.1%) and the median optico-spinal interval was 38 months.

The mean time to an EDSS of 3, 6 and 8 was respectively  $4.5 \pm 4.9$  months (in 25 patients),  $9.9 \pm 22.5$  months (in 17 patients) and  $9.3 \pm 8.4$  months (in 7 patients).

Twenty-one out of 67 (31.3%) patients were classified as definite NMO and 46 (68.7%) as NMOSD. LETM and a negative initial brain MRI (Barkhof criteria) [15] were the main supportive criteria (10 patients) for definite NMO. Six patients had the three supportive criteria for definite NMO. In the NMOSD group, recurrent ON (13) and isolated LETM (12) were the most frequent presentations.

Five patients died (three from pneumonia, one from pancreatic cancer and one was NMO related – cervical lesion causing respiratory insufficiency).

Autoimmune diseases were observed in 15 patients (22.4%), the most frequent was SLE (5 patients), followed by autoimmune

**Table 1**

Demographic and clinical characteristics of the NMO cohort.

Characteristics	N <sup>a</sup>	% (n)	
Female	67	65.7 (55)	
Caucasian	67	95 (64)	
	N <sup>a</sup>	Mean (SD)	Median (min; max)
Age at disease onset	67	36.0 (15.0)	32 (3; 76)
Age at diagnosis	65	40.7 (15.0)	39 (13; 79)
Follow-up (years)	67	7.4 (6.0)	4 (1; 24)
Optic-spinal interval (months)	23	45.1 (48.7)	19 (0; 168)
S1–S2 Interval (months)	46	44.0 (63.2)	19 (2; 276)
Annualized relapse rate (at 24 months)	59	0.9 (0.7)	0.5 (0.5; 3.5)
Time to EDSS 3 (months)	25	4.5 (4.9)	2 (0; 18)
Time to EDSS 6 (months)	17	9.9 (22.5)	3 (0; 96)
Time to EDSS 8 (months)	7	9.3 (8.4)	11 (0; 24)
	N <sup>a</sup>	% (n)	
Deaths	67	7.5 (5)	
Relapsing–remitting course	67	72 (48)	
Monophasic course	67	28 (19)	

<sup>a</sup> Number of patients with available and or applicable information.**Table 2**

Biological characteristics of the NMO cohort.

	N <sup>a</sup>	% (n)
CSF		
Cell count/mm <sup>3</sup>	53	
≤5		64 (34)
]5; 50[		26 (14)
≥50		9 (5)
Oligoclonal bands	46	17 (8)
Proteins ≥ 45 mg/dL	50	42 (21)
IgG index > 0.73	34	26 (9)
Blood		
AQP4-IgG +	67	36 (24)
Detectable antibodies	50	34.3 (23)
Antinuclear antibodies		20 (10)
Anticardiolipin antibodies		18 (9)
Anti-SSA antibodies		16 (8)
Anti-β2 glycoprotein		16 (8)
Other antibodies		50 (25)

<sup>a</sup> Number of patients with available and or applicable information.

thyroiditis, primary antiphospholipid syndrome (3 patients each), myasthenia gravis, Sjogren's syndrome, primary biliary cirrhosis (2 patients each) and scleroderma (1 patient). Three patients had 2 autoimmune pathologies simultaneously.

### 3.2. Laboratorial features (Table 2)

The CSF analysis revealed that 34 patients had less than 5 leucocytes/mm<sup>3</sup>. Analysing the CSF pleocytosis according to the form of presentation we found that from the 30 patients that presented with ON 19 (63%) had normal CSF cell count (<5 cells/mm<sup>3</sup>), 2 (6.6%) had CSF pleocytosis between 5 and 50 cells/mm<sup>3</sup>, 1 (3%) had CSF pleocytosis >50 cells/mm<sup>3</sup> and in 8 patients these data was not available. From the 31 patients that presented with LETM 13 (42%) had normal CSF cell count, 11 (35%) had CSF pleocytosis between 5 and 50 cells/mm<sup>3</sup>, 3 patients (9.7%) had >50 cells/mm<sup>3</sup> and 4 patients had no available data. All the patients that presented with ON and had CSF pleocytosis had mononuclear predominance. From the patients that presented with LETM and had CSF pleocytosis 10 had mononuclear predominance.

Eight out of 46 patients had positive OCB.

AQP4-IgG was found in 24 patients (35.8%). Twenty-three out of 50 patients had positive autoantibodies.

**Table 3**

Imaging characteristics of the NMO cohort.

MRI features	N <sup>a</sup>	% (n)
Brain MRI pattern	66	
Normal		42 (28)
Non-specific		39 (26)
MS-like		12 (8)
NMO-like		6 (4)
Brain MRI lesions topography	66	
Supratentorial		47 (31)
Infratentorial		21 (14)
Periventricular		43 (28)
Juxtacortical		12 (8)
Brain MRI number lesions	66	
0		42.4 (28)
1–24		31.8 (21)
>25		25.8 (17)
Spinal MRI number of lesions	59	
0		23.7 (14)
1		57.6 (34)
2		13.6 (8)
>3		5.1 (3)
LETM		52.5 (31)
Cervical LETM		20.3 (12)
Dorsal LETM		32.2 (19)
Cervico-dorsal LETM		20.3 (12)

<sup>a</sup> Number of patients with available and or applicable information; 1 patient with LETM had only spinal MRI performed; 8 patients without spinal involvement had only brain MRI performed.

### 3.3. MRI features (Table 3)

In most patients, brain MRI showed no abnormalities (42.4%) or nonspecific findings (39.4%). Only 4 (6.1%) cases showed NMO-like lesions. LETM was detected in 31 patients, with dorsal segment being the most often involved (19 patients).

### 3.4. Treatment

Methylprednisolone (MP) was the most frequently used treatment in the acute phase (83.3%) followed by the combination of MP with intravenous immunoglobulin or plasma exchange (7.4% each). For preventive treatment, prednisolone and azathioprine were the mainly used treatments (25.6% and 18.6%, respectively); in 7 patients (16.3%) they were given simultaneously.

### 3.5. Group comparisons

#### 3.5.1. Definite NMO vs NMOSD (Table 4)

Comparing the group of definite NMO with the NMOSD we found a relapsing–remitting course in 90.5% and 63.0% respectively and a monophasic evolution in 9.5% and 37.0%, respectively ( $p=0.02$ ). If we selected only patients with more than 5 years of follow-up we reach a total of 33 patients, 15 definite NMO and 18 NMOSD. None of the definite NMO patients was monophasic. From the 18 NMOSD, 4 (22.2%) were monophasic and 14 had more than one attack. Although the percentage of monophasic patients in the NMOSD group is lower than when all the patients were considered (37.0%) it is still significantly higher than in the definite NMO group.

Median annualized relapse rate was 1.0 in the definite NMO group and 0.5 in the NMOSD ( $p=0.01$ ). Concerning the death rate, all patients that had died ( $n=5$ ; 7.5%) belonged to the NMO definite group ( $p=0.002$ ).

OCB were present in 38.5% of the definite NMO patients and in 9.1% of the NMOSD patients ( $p=0.03$ ).

The spinal MRI showed lesions in all patients with definite NMO and in 63.2% of the NMOSD group ( $p=0.001$ ).

No differences between the two groups were found in the remaining demographic, clinical, laboratorial and therapeutic data.

Regarding the presence of autoimmune diseases there were no statistically significant differences.

#### 3.6. Positive AQP4-IgG vs negative AQP4-IgG

Twenty-three out of 24 (95.8%) patients with AQP4-IgG and 19 out of 41 (46.3%) of those without antibody were female ( $p=0.0012$ ). From the AQP4-IgG positive group, eleven patients belong to the definite NMO group (52.4%) and 13 (28.3%) to the NMOSD ( $p=0.10$ ).

Positive AQP4-IgG patients had a mean age at diagnosis of 37.2 years; 10 patients (41.6%) presented with myelitis and 9 (37.5%) with ON; 18 patients (75%) followed a relapsing–remitting course and 6 (25%) were monophasic; 2 patients in this group died (8.3%).

The seropositive AQP4-IgG patients had significantly more concomitant autoimmune diseases than did patients with seronegative AQP4-IgG (41.7% vs 9.8%,  $p<0.004$ ).

Twenty-five percent of the AQP4-IgG seropositive patients were monophasic and 26.8% from the seronegative were monophasic and this difference was not statistically significant ( $p>0.05$ ).

No further significant differences were found between those two groups.

#### 3.7. Patients with autoimmune diseases

Concomitant autoimmune disease was present in 15 out of 67 patients, 5 belonging to the definite NMO group and 10 to the NMOSD.

Fourteen out of 15 were female, Caucasian, with a median age at disease onset of 32 years (19; 71), and a median follow-up of 4 years (1; 13). Eleven had a relapsing–remitting course. Seven patients (46.7%) had myelitis as the initial topography, 5 ON and 1 both. One of these 15 patients died. As previously reported, 10 out of the 15 patients with autoimmune diseases (66.7%) had positive AQP4-IgG antibody.

## 4. Discussion

This study, the first done in the Portuguese population regarding NMO, comprises a significant number of patients from the most representative national centers.

Although there are many epidemiologic studies from several countries, the methodologies namely the population sampling, the

inclusion criteria, the definitions of NMO and NMOSD and the variables studied vary greatly among them. For these reasons it is difficult to make direct comparisons.

The demographic characteristics of our cohort are similar to those reported in other studies [16,17] namely regarding the female:male ratio (4.6:1), the median age at onset (36.0 years) and the disease course (relapsing–remitting in 71.6%). Interestingly, in our study no patient had a progressive course. Although higher female:male ratios, higher age at disease onset and higher percentage of relapsing remitting course have been reported [18–20] namely in cohorts with populations sizes similar to Portugal [18,20]. The CSF results are different from what we can find in the literature. In fact, in the majority of the patients there was no CSF pleocytosis and 8 out of 46 (17.4%) had OCB (around 12% in other studies) [19–21]. We found that a higher percentage of patients presenting with LETM had CSF pleocytosis when compared to the patients that presented with ON. Like in previous reports, brain MRI at disease onset was mostly normal or showed non-specific brain lesions [18–20]. With respect to spinal cord lesions, the results were also consistent with those of prior studies showing LETM in 52.5% of patients. The percentage of AQP4-IgG positive patients is lower in our study (35.8%; CBA technique) than in the French [16] (54.0%; indirect immunofluorescence assay), the Danish [17] (62.0%; CBA technique), the Hong Kong [18] (88.9%, CBA) and the American multicentre one [19] (68.3%; indirect immunofluorescence assay). However, the total number of patients (namely of the NMOSD group) and the definition of NMOSD (some studies included in this group only patients that were AQP4-IgG seropositive) [18–20] must be taken into account. In fact, if we compare only with the definite NMO groups the results are in consonance between our study (52.4%) and the Danish [17] one (55.6%). AQP4-IgG positivity was not associated with a worse outcome, considering the number of deaths, annualized relapse rate or time to reach EDSS of 3, 6 or 8.

In spite of having found a higher percentage of patients with concomitant autoimmune disorders (15 patients, 22.4%) than it was reported in the French [16] (10.4%) and Hong Kong [18] (10.6%) studies, most of the encountered disorders were similar (mainly SLE and Sjogren's syndrome) except for the two cases of myasthenia gravis described in our cohort [13] and also in the Austrian one [20]. Since 66.7% of patients with autoimmune diseases had positive AQP4-IgG antibody, we hypothesize that its presence may be faced as an “autoimmune marker”.

With respect to the clinical data of the definite NMO group, we observed that the predominant initial topography in the optic nerves (57.1%) is in agreement with the Italian [21] and the American multicentre group [19], but in contrast with others [16,17,22]. As well, the opticospinal interval (24 months) was longer than in the Mexican [22] (3.5 months) and French [16] (15 months) studies. However, the annualized relapse rate (1.0) in our cohort was similar to the previous studies [16,17,21]. The mortality rate in this group (23.8%) was similar in the American Mayo Clinic cohort [23] (22.5%), but higher than in the Italian [21] (13.0%), Mexican [22] (2.9%), French [16] (3.2%) and Danish [17] (8.3%) cohorts. It is also worth of note that in the present study the presence of AQP4-IgG antibody was essential for definite NMO diagnosis in 5 cases (23.8%) whereas in the French study [16] it was needed in only 10.0% (12 cases) of the cases and in the Danish study [17] in 36.0% (15 cases).

Concerning the comparison between definite NMO with NMOSD, the first one seems to have a worse prognosis with a higher annualized relapse rate (1.0 vs 0.5) and mortality rate (all of the 5 deaths occurring in the former group). Also, the majority of patients in the definite NMO group had a relapsing–remitting course, whereas the monophasic course was significantly higher in the NMOSD patients. Positive oligoclonal bands were more frequent in the NMO definite group. Yet, this may due to a record bias, since this information was available only in a small number of



**Table 4**

Comparison between definite NMO and NMOSD groups.

	N <sup>a</sup>	Definite NMO (N=21)	N <sup>a</sup>	NMOSD (N=46)	p
Female, n (%)	16	76%	28	61%	0.22
Caucasian, n (%)	18	86%	45	98%	0.09
Age at onset (years), median (min; max)	21	32 (3; 60)	46	33.5 (13; 76)	0.42
Age at diagnosis (years), median (min; max)	19	40 (17; 65)	46	38 (13; 76)	0.85
Follow-up (years), median (min; max)	21	9 (1; 21)	46	4 (1; 24)	0.18
S1–S2 interval (months), median (min; max)	20	17.5 (20; 168)	26	19 (2; 276)	0.75
Annualized relapse rate, median (min; max)	20	1.0 (0.5; 3.5)	39	0.5 (0.5; 3.0)	0.01
Deaths (n)	21	5	46	0	0.002
Relapsing–remitting course, n (%)	21	19 (91)	46	29 (63)	0.02
Monophasic course, n (%)	21	2 (9)	46	17 (37)	
CSF pleocytosis	15		38		
≤5	10	67	24	63	0.72
]5; 50[	4	27	10	26	
≥50	1	7	4	11	
Oligoclonal bands, n (%)	13	5 (39)	33	3(9)	0.03
Proteins ≥ 45, n (%)	16	8(50)	34	13(38)	0.43
Brain MRI, n (%)	21		45		
Normal		6 (29)		20 (44)	0.19
Non-specific		10 (48)		18 (40)	
MS-like		2 (9)		6 (13)	
NMO-like		3 (14)		1 (2)	
Lesions topography, n (%)	21		45		
Supratentorial		12 (57)		20 (44)	0.34
Infratentorial		6 (29)		9 (20)	0.44
Periventricular		8 (40)		17 (38)	0.87
Juxtacortical		2 (10)		6 (13)	1.00
Spinal MRI, n (%)	21		38		
Without lesions		0		14 (37)	0.001
With lesions		21 (100)		24 (63)	

<sup>a</sup> Number of patients with available and or applicable information.

patients. There was no significant difference between definite NMO and NMOSD, regarding the presence of AQP4-IgG or other circulating autoantibodies. Compared with the Danish study [17], where the spectrum forms were also evaluated, we found a higher proportion of those disorders (68.7% vs 14.0%). We believe that this fact may be due to a selection bias, overestimating those cases where CNS inflammatory disease was suspected but did not match MS criteria, such as recurrent optic neuritis or idiopathic LETM.

Taking into account the comparison between AQP4-IgG seropositive and seronegative patients, we found a higher proportion of females among the positive ones, similarly to what have been previously reported [24]. Analysing the group of AQP4-IgG seropositive patients, we found similar clinical characteristics to what have been reported in the UK and Japan cohorts [25], including mean age at onset, percentage of female patients, disease course and form of presentation. The mortality rate was 8.3% in our cohort, higher than the Japanese but lower than the UK cohorts [25]. In fact, recent studies comparing seropositive and seronegative patients brought new concepts, including switching the NMO definition from a clinical phenotype to a biological one [24].

Being retrospective, this study has inherent limitations, namely the record consulting bias. The determination of AQP4-IgG was made according to the same technique. However, since it was performed at different times of the disease evolution, in some cases the titers might have been influenced by the ongoing treatment. Likewise, MRI available data was also performed at different times and might have been influenced by the ongoing treatment or even the disease progression.

In conclusion, this study corroborates the clinical heterogeneity of NMO in its broad sense. Finally, the association found between AQP4-IgG positivity and concomitant autoimmune disease, could be taken into account for future research studies.

### Conflict of interest

The authors declare that there is no conflict of interest.

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