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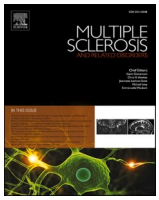

Original article

Late onset neuromyelitis optica spectrum disorders (LONMOSD) from a nationwide Portuguese study: Anti-AQP4 positive, anti-MOG positive and seronegative subgroups

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A B S T R A C T

Introduction: Several neuroimmunological disorders have distinct phenotypes according to the age of onset, as in multiple sclerosis or myasthenia gravis. It is also described that late onset NMOSD (LONMOSD) has a different phenotype.
Objective: To describe the clinical/demographic characteristics of the LONMOSD and distinguish them from those with early onset (EONMOSD).
Methods: From a nationwide Portuguese NMOSD study we analyzed the clinical/demographic characteristics of the LONMOSD.
Results: From the 180 Portuguese patients 45 had disease onset after 50 years old, 80% were female. 23 had anti-AQP4 antibodies (51.1%), 13 anti-MOG antibodies (28.9%) and 9 were double seronegative (20.0%). The most common presenting phenotypes in LONMOSD were transverse myelitis (53.3%) and optic neuritis (26.7%), without difference from EONMOSD ($p = 0.074$). The mean EDSS for LONMOSD was 6.0 (SD=2.8), after a mean follow-up time of 4.58 (SD=4.47) years, which was significantly greater than the mean EDSS of EONMOSD (3.25, SD=1.80)($p = 0.022$). Anti-AQP4 antibodies positive LONMOSD patients had increased

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disability compared to anti-MOG antibodies positive LONMOSD ($p = 0.022$). The survival analysis showed a reduced time to use a cane for LONMOSD, irrespective of serostatus ($p<0.001$).
Conclusions: LONMOSD has increased disability and faster progression, despite no differences in the presenting clinical phenotype were seen in our cohort.

1. Introduction

Neuromyelitis optica spectrum disorders (NMOSD) is a rare group of autoimmune diseases of the central nervous system. 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).

The typical onset of NMOSD is between the third and fourth decades of life, but initial symptoms and signs may occur later (Wingerchuk et al., 2015; Wingerchuk et al., 2007; Palace et al., 2019). These patients are classified as late onset NMOSD (LONMOSD), considering age at initial symptoms greater than 50 years (Palace et al., 2019; Kitley et al., 2012; Nagaishi et al., 2011; Collongues et al., 2014).

It is known that several neuroimmunological disorders have distinct phenotypes according to the age of onset, as in multiple sclerosis (Jakimovski et al., 2020; Mirmosayyeb et al., 2020) or myasthenia gravis (Barbaud et al., 2006; Gilhus and Verschuuren, 2015). It is also described that NMOSD has some different phenotypes according to the age of onset. (Cai et al., 2020; Nakahara et al., 2021) LONMOSD has been reported to be associated with a lower female-to-male ratio and worse prognosis due to higher frequency of spinal cord lesions, greater severity of symptoms and rapid disease progression, despite early aggressive immunosuppressive treatment (Collongues et al., 2014; Zhang et al., 2017).

More recently some studies also focused in a less frequent group in which the disease onset is after 70 years old, classified as very late onset NMOSD (Cai et al., 2020) with a more severe course and difficult therapeutic management (Cai et al., 2020; Lavandier et al., 2019).

2. Objective

The aim of this study is to describe the clinical and demographic characteristics of patients with late and very late onset NMOSD and distinguish them from those with early onset.

3. Methods

Recently the authors performed a nationwide Portuguese clinical and epidemiological NMOSD study (Santos et al., 2021). Using the data collected in that study we analyzed the clinical and demographic characteristics of the late onset NMOSD and compare them with those with early onset of the disease.

3.1. 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).

3.2. Clinical data collection

Clinical and demographic data were collected from patients' medical records. It 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, anti-AQP4 antibodies, anti-MOG antibodies, other auto-antibodies study, Expanded Disability Status Scale (EDSS) score and treatments used during the course of the 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.

3.3. 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.

3.4. Statistical analysis

For descriptive statistics, qualitative variables were studied using the absolute and relative frequencies. For the quantitative variables, the mean and standard deviation, or median and inter quartile range (p25–p75) (IQR) were calculated according to the normality of the distribution. Pearson's chi-squared test ($\chi 2$) was used to assess the association between some categorical variables and the NMOSD serological type. For quantitative variables, either independent sample t or Mann-Whitney test were used, according to the distribution. Kaplan Meyer survival curves were built with an EDSS$\geq$6 as the outcome. For these analyses, SPSS statistics, version 25 was used.

3.5. 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

From the 180 patients identified in the epidemiological Portuguese study (Santos et al., 2021) 45 were LONMOSD (25%). Six patients, 3% of the total cohort and 13% of the LONMOSD, were VLONMOSD. The distribution of cases by age of onset was 24 at 50–59, 15 at 60–69, 5 at 70–79 and 1 above 80 years old. The ratio F:M was 4:1 (36:9) in the LONMOSD subgroup and 2,6:1 (97:38) in EONMOSD. Mean disease duration was 7.16 (7.87) years for LONMOSD and 5.93 (6.05) years for EONMOSD ($p = 0.278$). We found a higher frequency of association to

Table 1
Early onset versus late onset NMOSD: clinical and demographic characteristics.

Variable	Total	Early onset135 (75%)	Late onset45 (25%)	P
Demographics				
Female, n(%)	133 (73.9)	97 (71.9)	36 (80.0)	0.331
Age at presentation (years), mean (SD)	38.3 (17.00)	31.7 (10.95)	59.8 (9.33)	<0.001
Diagnostic delay (years), mean (SD)	3.32 (6.66)	2.52 (3.17)	2.33 (4.08)	0.193
Follow-up time (years), mean (SD)	5.54 (5.43)	5.84 (5.68)	4.58 (4.47)	0.101
Other AI disorders, n (%)	30 (16.7)	18 (15.5)	12 (30.8)	0.035
Race, n (%)				
Caucasian	169 (93.9)	125 (96.6)	44 (97.8)	0.346
Afrodescendent	10 (5.6)	9 (6.7)	1 (2.2)	
Asian	1 (0.6)	1 (0.7)	0	
Clinical presentation, n (%)				
ON	64 (35.6)	52 (38.5)	12 (26.7)	0.074
TM	78 (43.4)	54 (40.0)	24 (53.3)	
ON+TM	12 (6.7)	9 (6.7)	3 (6.7)	
AP	5 (2.8)	5 (3.7)	0	
BSS	8 (4.4)	4 (3.0)	4 (8.9)	
Narcolepsy	1 (0.6)	0	1 (2.2)	
Supratentorial	5 (2.8)	5 (3.7)	0	
TM+BSS	2 (1.1)	1 (0.7)	1 (2.2)	
TM+Supratentorial	2 (1.1)	2 (1.5)	0	
ON+TM+AP	1 (0.6)	1 (0.7)	0	
TM+BSS+AP	2 (1.1)	2 (1.5)	0	
Number of relapses, mean (SD)	2.39 (1.98)	2.37 (2.2)	1.83 (0.75)	0.048
1st year, mean (SD)	1.31 (0.62)	1.26 (0.59)	1.50 (0.84)	0.965
2nd year, mean (SD)	0.33 (0.64)	0.37 (0.74)	0.17 (0.40)	0.919
Relapses in different topography, n(%)	57 (31.7)	45 (33.3)	12 (26.7)	0.463
Number of severe relapses, median (IQR)	1.0 (1.0–2.0)	1.0 (0.0–2.0)	1.0 (0.5–2.0)	0.855
Annualized relapse rate, mean (SD)	0.67 (0.62)	0.62 (0.68)	0.38 (0.22)	0.436

Results presented in the form of absolute frequency (percentage) – n (%) - for categorical variables and either mean (standard deviation) or median (interquartile range) – mean (SD) or median (IQR), respectively - for quantitative variables.
AI-autoimmune disorders; ON-optic neuritis; TM-transverse myelitis; BSS-brainstem syndrome; AP-area postrema syndrome.

other autoimmune disorders (AID) in the LONMOSD subgroup (15.5% EO vs 30.8% LO, $p = 0.035$). The mean interval between the age of symptoms onset and age of diagnosis was 3.32 (SD=6.66) years, which did not differ significantly between both groups ($p = 0.193$). Clinical and demographic details are described in the Table 1.

Regarding serostatus, 23 patients were anti-AQP4 Abs+ NMOSD, 13 anti-MOG Abs+ and 9 double seronegative. These results are described along with other laboratory findings in Table 2.

The most common clinical presentations were optic neuritis (ON) (38.5% of EONMOSD and 26.7% of LONMOSD) and transverse myelitis (TM) (40.0% of EONMOSD and 53.3% of LONMOSD), irrespective of the age of disease onset. A combination of ON and TM was present at onset of 6.7% for both groups. One LONMOSD case presented with narcolepsy, and 5 (3.7%) cases with supratentorial involvement. A subanalysis showed no significant difference in the presenting clinical phenotype between groups.

LONMOSD patients had an inferior mean number of relapses compared to EONMOSD ($p = 0.048$). Relapses were more common in the first year of the disease course, and the majority of them occurred in the same location as the initial attack in both LONMOSD and EONMOSD.

Table 2
Early onset versus late onset NMOSD: Laboratory findings.

	Total	Early onset135 (75%)	Late onset45 (25%)	P
Serostatus				
Anti-AQP4 Abs+, n (%)	77 (42.8)	54 (40.0)	23 (51.1)	0.347
Anti-MOG Abs+, n (%)	67 (37.2)	54 (40.0)	13 (28.9)	
Negative, n (%)	36 (20.0)	27 (20.0)	9 (20.0)	
CSF				
Pleocytosis, n (%)	72 (40.0)	53 (49.1)	19 (51.4)	0.851
Mean mononuclear count, median (IQR)	14.0 (8.0–55.0)	14.0 (7.0–36.0)	14.5 (9.5–82.0)	0.023
OCB, n (%)	25 (13.9)	20 (21.1)	5 (17.9)	0.796

Results presented in the form of absolute frequency (percentage) – n (%) - for categorical variables and either mean (standard deviation) or median (interquartile range) – mean (SD) or median (IQR), respectively - for quantitative variables.
OCB - oligoclonal bands.

The median number of severe relapses (relapses that motivated hospital admissions) was 1.0 (IQR=0.5–2.0) and did not differ significantly based on age of onset ($p = 0.855$). Overall, the annualized relapse rate was 0.38 (SD=0.22) for LONMOSD and 0.62 (SD=0.68) for EONMOSD ($p = 0.436$).

While the frequency of CSF pleocytosis was not different between groups, the median mononuclear count was significantly greater in LONMOSD ($p = 0.023$). Furthermore, double seronegative LONMOSD had a significantly greater median mononuclear count (88.0) when compared to EONMOSD (9.5) ($p = 0.038$). This difference was not observed in seropositive patients.

Oligoclonal bands were present in 20 EONMOSD (21.1%) and 5 LONMOSD (17.9%) patients, without a significant difference between groups ($p = 0.796$).

Treatment regimens were similar in early and late onset NMOSD, with steroids (95.6% of EONMOSD and 100% of LONMOSD) and azathioprine (48.4% of EONMOSD and 60.5% of LONMOSD) as the most commonly used agents (Table 3.). Mean final EDSS step was 6.0 (2.82) for LONMOSD, which was significantly greater than the 3.25 (1.80) in EONMOSD ($p = 0.022$). Moreover, anti-AQP4 Abs+ LONMOSD had a significantly higher EDSS step (7.6) when compared to anti-MOG Abs+ LONMOSD (4.5) ($p<0.001$). Time to EDSS score $\geq$ 6.0 for LONMOSD compared to EONMOSD and for LONMOSD serostatus groups is shown in Fig. 1.

A subanalysis of LONMOSD by serostatus showed that female sex

Table 3
Early onset versus late onset NMOSD: Treatment regimens and outcome.

	Total	Early onset135 (75%)	Late onset45 (25%)	P
Treatment, n (%)				
Steroids	174 (96.7)	129 (95.6)	45 (100)	0.339
Plasma exchange	46 (25.6)	35 (28.2)	11 (26.2)	0.845
IVIG	36 (20.0	26 (20.8)	10 (23.3)	0.830
AZA	85 (47.2)	59 (48.4)	26 (60.5)	0.117
RTX	48 (26.7)	38 (31.1)	10 (23.3)	0.435
MTX	5 (2.8)	3 (2.4)	2 (4.7)	0.603
MMF	21 (11.7)	19 (14.8)	2 (4.5)	0.107
Final EDSS, mean (SD)	3.34 (2.30)	3.25 (1.80)	6.0 (2.82)	0.022

Results presented in the form of absolute frequency (percentage) – n (%) - for categorical variables and either mean (standard deviation) or median (interquartile range) – mean (SD) or median (IQR), respectively - for quantitative variables.
IVIG-intravenous immunoglobulins; AZA-azathioprine; RTX-rituximab; MTX-methotrexate; MMF-mycophenolate mofetil.

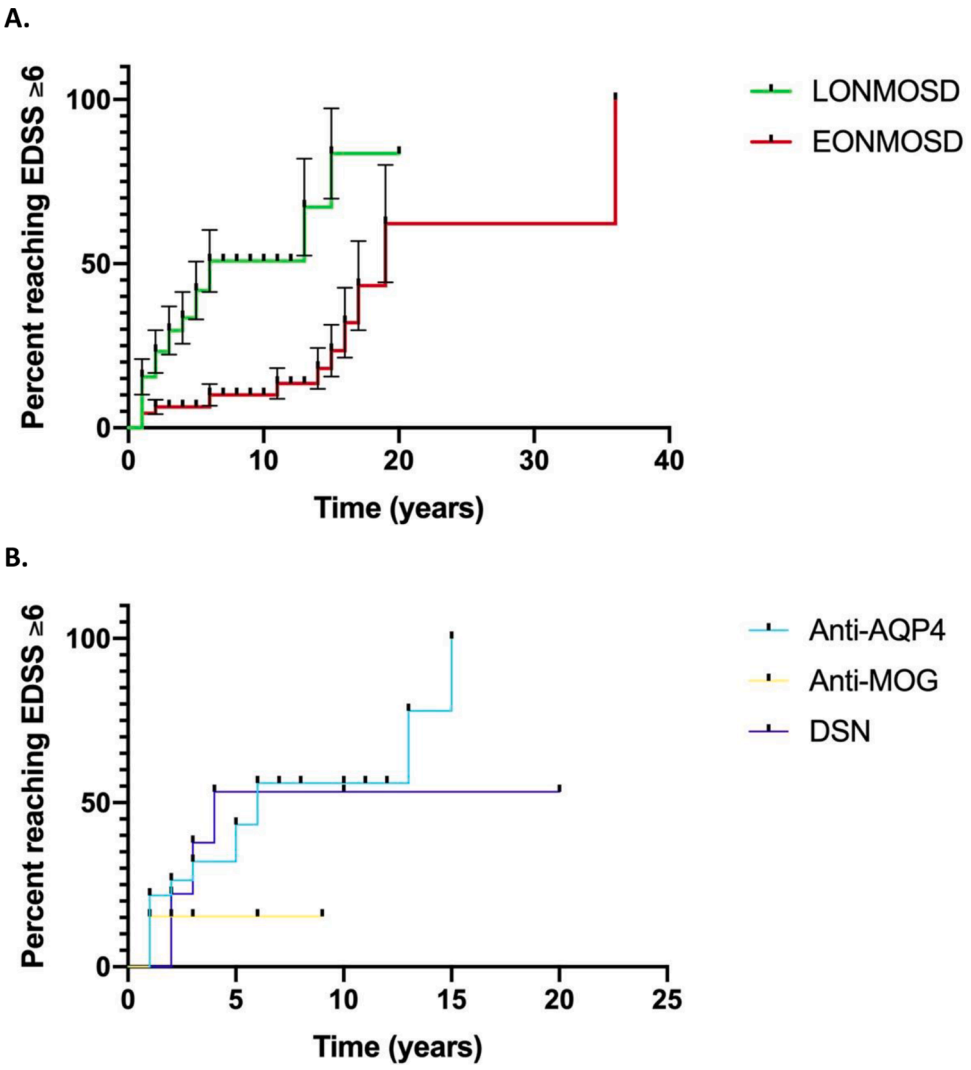

Fig. 1. (A) Years from onset to use a cane (EDSS score of 6.0) in patients with LONMOSD (green) compared to EONMOSD (red). Patients with LONMOSD tend to reach an EDSS score of 6 sooner than EONMOSD ($p<0.001$). (B) Comparison of years of onset to EDSS ≥6 in LONMOSD according to serostatus: no trends towards increased risk of disability was appreciable between groups ($p = 0.493$).

was more significantly associated with anti-AQP4 Abs+ compared to anti-MOG Abs+ and seronegative cases ($p = 0.015$). The association with other AI disorders was also more common in anti-AQP4 Abs+ cases compared to anti-MOG Abs+ ($p = 0.015$).

Tables 4–6 describe a subgroup analysis of anti-AQP Abs+, anti-MOG Abs+ and double seronegative NMOSD, respectively. Double seronegative LONMOSD have higher CSF mononuclear cell count ($p = 0.038$) and final EDSS ($p = 0.046$) when compared to double seronegative EONMOSD.

5. Discussion

Our study describes the clinicodemographic characteristics of the Portuguese cohort of LONMOSD. In our cohort, the proportion of LONMSD was 25%, which is higher than in the LATAM cohort (17%) (Carnero Contentti et al., 2020) but lower than other multicenter NMOSD cohorts from Europe and Asia, where the prevalence of LONMOSD was reported to be up to 29% and 41.5%, respectively (Palace et al., 2019; Collongues et al., 2014; Zhang et al., 2017; Mao et al., 2015). The distribution of our sample through ethnicity groups follows the same pattern as previous cohorts from near geographical areas (Collongues et al., 2014).

The higher frequency of association with other AI disorders in the late onset group may be unexpected, given that several immune-mediated diseases start during early adulthood. A previous study in 142 anti-AQP4 Abs+ NMOSD found that 10 EONMOSD had concomitant AI disorders compared to 3 LONMOSD, although it was not statistically significant (Zhang et al., 2017a). Our results may reflect the fact that diseases like thyroiditis and rheumatoid arthritis may have a late adult onset (Cooper and Stroehla, 2003), and the increased prevalence of AI disorders in LONMOSD may also reflect the cumulative incidence of these conditions.

We found no difference between NMOSD groups considering the presenting phenotype and treatment regimens. These results are in line with previously reported cohorts. The LATAM study also found no significant differences in NMOSD clinical course, clinical presentation, or type of acute and preventive NMOSD treatment between LONMOSD and EONMOSD patients (Carnero Contentti et al., 2020). However, previous studies suggested that EONMOSD was more frequently associated with ON than LONMOSD (Mirmosayyeb et al., 2020; Zhang et al., 2017a). Furthermore, other studies pointed to a correlation between older age of onset and spinal cord involvement in NMOSD, besides showing an association with the longitudinal lesion extension (Collongues et al., 2014; Nakahara et al., 2021). A recent cohort also showed that the initial attack was more likely to affect the spinal cord in LONMOSD and optic nerves in EONMOSD, irrespective of age at disease onset (Wang et al.,

Table 4
Subanalysis of Anti-AQP4 Abs+ NMOSD cases.

Variable	Early onset54 (70.1%)	Late onset23 (29.9%)	P
Demographics			
Female, n(%)	46 (59.7)	22 (28.6)	0.265
Age at presentation (years), mean (SD)	28.2 (10.85)	63.4 (8.99)	<0.001
Diagnostic delay (years), mean (SD)	2.7 (3.91)	2.6 (6.08)	0.759
Follow-up time (years), mean (SD)	5.5 (4.70)	5.4 (4.54)	0.933
Race, n(%)			
Caucasian	47 (61.0)	22 (28.6)	0.504
Afrodescendent	6 (7.8)	1 (1.3)	
Asian	1 (1.3)	0	
Clinical presentation, n (%)			
ON	19 (24.7)	6 (7.8)	0.344
TM	22 (28.6)	13 (16.9)	
ON+TM	2 (2.6)	2 (2.6)	
AP	5 (6.5)	0	
BSS	3 (3.9)	1 (1.3)	
Narcolepsy	0	1 (1.3)	
ON+TM+AP	1 (1.3)	0	
TM+BSS+AP	2 (2.6)	0	
Number of relapses, mean (SD)	2.4 (0.55)	2.0 (0.707)	0.036
1st year, mean (SD)	1.0 (0.0)	1.6 (0.89)	0.926
2nd year, mean (SD)	0.2 (0.45)	0.4 (0.55)	0.980
Relapses in different topography, n(%)	25 (32.5)	6 (7.8)	0.098
Number of severe relapses, median (IQR)	1.0 (1.0–3.0)	1.0 (1.0–2.0)	0.436
Annualized relapse rate, mean (SD)	0.7 (0.53)	0.5 (0.34)	0.229
CSF			
Pleocytosis, n(%)	15 (26.8)	10 (17.9)	0.389
Mononuclear count, median (IQR)	9.0 (7.0–67.0)	14.5 (9.5–47.5)	0.533
OCB, n(%)	7 (16.3)	4 (9.3)	0.469
Treatment, n(%)			
Steroids	53 (68.8)	23 (29.9)	0.339
Plasma exchange	20 (29.4)	8 (11.8)	0.730
IVIG	11 (15.9)	5 (7.2)	0.950
AZA	29 (42.0)	16 (23.2)	0.370
RTX	22 (31.9)	6 (8.7)	0.124
MTX	2 (2.9)	2 (2.9)	0.410
MMF	12 (17.4)	2 (2.9)	0.114
Final EDSS, mean (SD)	3.4 (1.29)	7.5 (1.12)	0.247

Results presented in the form of absolute frequency (percentage) – n (%) - for categorical variables and either mean (standard deviation) or median (interquartile range) – mean (SD) or median (IQR), respectively - for quantitative variables.
AI-autoimmune disorders; ON-optic neuritis; TM-transverse myelitis; BSS-brainstem syndrome; AP-area postrema syndrome; OCB-oligoclonal bands; IVIG-intravenous immunoglobulins; AZA-azathioprine; RTX-rituximab; MTX-methotrexate; MMF-mycophenolate mofetil.

Table 5
Subanalysis of Anti-MOG Abs+ positive NMOSD cases.

Variable	Early onset54 (80.6%)	Late onset13 (19.4%)	p
Demographics			
Female, n(%)	34 (50.7)	9 (13.4)	0.672
Age at presentation (years), mean (SD)	33.4 (10.66)	56.7 (0.58)	<0.001
Diagnostic delay (years), mean (SD)	4.8 (10.19)	2.2 (3.72)	0.370
Follow-up time (years), mean (SD)	5.1 (5.39)	2.7 (2.39)	0.126
Race, n(%)			
Caucasian	52 (77.6)	13 (19.4)	0.481
Afrodescendent	2 (3.0)	0	
Clinical presentation, n(%)			
ON	24 (35.8)	6 (9.0)	0.197
TM	17 (25.4)	5 (7.5)	
ON+TM	7 (10.4)	0	
BSS	1 (1.5)	2 (3.0)	
Supratentorial	4 (6.0)	0	
TM+Supratentorial	1 (1.5)	0	
Number of relapses, mean (SD)	2.0 (2.57)	1.7 (0.58)	0.408
1st year, mean (SD)	1.22 (0.55)	1.0 (0.0)	0.294
2nd year, mean (SD)	0.39 (0.78)	0.33 (0.58)	0.823
Relapses in different topography, n(%)	13 (19.4)	4 (6.0)	0.618
Number of severe relapses, median (IQR)	1.0 (0.0–1.0)	1.0 (0.5–1.0)	0.955
Annualized relapse rate, mean (SD)	0.7 (0.74)	0.5 (0.42)	0.526
CSF			
Pleocytosis, n (%)	25 (42.4)	5 (8.5)	0.953
Mononuclear count, median (IQR)	18.5 (11.5–48–0)	12.0 (6.0–261.0)	0.062
OCB, n(%)	9 (18.0)	0	0.148
Treatment, n (%)			
Steroids	49 (73.1)	13 (19.4)	0.254
Plasma exchange	6 (9.7)	1 (1.6)	0.719
IVIG	7 (11.1)	2 (3.2)	0.793
AZA	17 (27.4)	5 (8.1)	0.618
RTX	7 (11.3)	1 (1.6)	0.599
MMF	5 (7.5)	0	0.254
Final EDSS, mean (SD)	2.64 (1.61)	4.5 (1.80)	0.234

Results presented in the form of absolute frequency (percentage) – n (%) - for categorical variables and either mean (standard deviation) or median (interquartile range) – mean (SD) or median (IQR), respectively - for quantitative variables.
AI-autoimmune disorders; ON-optic neuritis; TM-transverse myelitis; BSS-brainstem syndrome; AP-area postrema syndrome; OCB-oligoclonal bands; IVIG-intravenous immunoglobulins; AZA-azathioprine; RTX-rituximab; MTX-methotrexate; MMF-mycophenolate mofetil.

2022). This study include a relatively small number of anti-MOG abs + cases ($n = 130$) compared to 360 anti-AQP4+ patients, and the authors were unable to replicate the same findings for the first. As thus, the phenotypic differences between LONMOSD and EONMOS might reflect this enrichement in anti-AQP4 abs+ NMOSD cases in the published cohorts. Still, we did not find any association with initial attack type even when restricting the analysis to anti-AQP4 abs+ cases. A robust analysis of 238 NMOSD patients (193 anti-AQP4 abs+ and 15 anti-MOG abs+ cases) has also found no association between serostatus and clinical phenotype, in agreement with our findings (Sepulveda et al., 2019). Larger cohorts are needed to clarify this issue in the future.

Our finding of increased CSF cell count in LONMOSD patients with pleocytosis is interesting. Previous studies either did not explore (Wang et al., 2022) or did not found a significant difference in the presence of pleocytosis between groups, with no reference to the mean cell count in those patients with pleocytosis (Collongues et al., 2014).

Our results suggest that this difference may be driven by the seronegative group, which is more represented in this cohort than in previous works (Sepulveda et al., 2019). Despite other disorders having been excluded by the practicing neurologist prior to diagnosing NMOSD, the possibility of this pleocytosis to reflect other immune mediated disorders whose clinical and imaging characteristics resemble NMOSD must be considered. Another possibility is that this represents a pattern associated with the seronegative LONMOSD variant. Further studies are needed to clarify if this association is related to features of the immune response that distinguishes LONMOSD from EONMOSD.

In our cohort, LONMOSD was associated with fewer relapses compared to EONMOSD, recapitulating previous findings (Zhang et al., 2017a). Despite this, there was no significant difference in the median number of severe relapses and annualized relapse rate.

Our survival analysis showed that LONMOSD significantly correlated to less time to reach increased disability, corroborating the findings from previous studies (Sepulveda et al., 2019). Residual disability after previous attacks, an important predictor of the final EDSS step, was unaccounted for, which would have been relevant in considering predictors

Table 6
Subanalysis of double seronegative NMOSD cases.

Variable	Early-onset27 (75.0)	Late-onset9 (25.0)	P
Demographics			
Female, n(%)	17 (47.2)	5 (13.9)	0.693
Age at presentation (years), mean (SD)	32.2 (10.74)	50.5 (0.71)	<0.001
Diagnostic delay (years), mean (SD)	3.5 (4.06)	1.8 (3.49)	0.268
Follow-up time (years), mean (SD)	8.1 (7.44)	5.4 (6.02)	0.338
Race, n (%)			
Caucasian	26 (72.2)	9 (25.0))	0.558
Afrodescendent	1 (2.8)	0	
Clinical presentation, n (%)			
ON	9 (25.0)	0	0.074
TM	15 (41.7)	6 (16.7)	
ON+TM	0	1 (2.8)	
BSS	0	1 (2.8)	
Supratentorial	1 (2.8)	0	
TM+BSS	1 (2.8)	1 (2.8)	
TM+Supratentorial	1 (2.8)	0	
Number of relapses, mean (SD)	2.6 (1.37)	1.5 (0.71)	0.452
1st year, mean (SD)	1.4 (0.67)	1.0 (0.0)	0.301
2nd year, mean (SD)	0.27 (0.65)	0	0.690
Relapses in different topography, n(%)	7 (19.4)	2 (5.6)	0.824
Number of severe relapses, median (IQR)	1.0 (1.0–2.0)	0.5 (1.0–3.0)	0.955
Annualized relapse rate, mean (SD)	0.6 (0.59)	0.7 (0.75)	0.653
CSF			
Pleocytosis, n(%)	13 (43.3)	4 (13.3)	0.657
Mononuclear count, median (IQR)	9.5 (4.0–16.8)	88.0 (50.0–436.0)	0.038
OCB, n(%)	4 (13.3)	1 (3.3)	0.712
Treatment, n (%)			
Steroids	27 (75.0)	9 (25.0)	n.a.
Plasma exchange	9 (25.0)	2 (5.6)	0.531
IVIG	8 (22.2)	3 (8.3)	0.835
AZA	13 (38.2)	5 (14.7)	0.855
RTX	9 (26.5)	3 (8.8)	0.886
MTX	1 (2.8)	0	0.558
MMF	2 (5.6)	2 (4.5)	0.401
Final EDSS, mean (SD)	3.8 (1.89)	4.5 (2.83)	0.046

Results presented in the form of absolute frequency (percentage) – n (%) - for categorical variables and either mean (standard deviation) or median (interquartile range) – mean (SD) or median (IQR), respectively - for quantitative variables.
AI-autoimmune disorders; ON-optic neuritis; TM-transverse myelitis; BSS-brainstem syndrome; AP-area postrema syndrome; OCB-oligoclonal bands; IVIG-intravenous immunoglobulins; AZA-azathioprine; RTX-rituximab;.

of progression. Despite this, our results are in line with previous studies that show a correlation between older onset and time to reach disability milestones, with the correlation with other factors having been established previously (Carnero Contentti et al., 2020; Sepulveda et al., 2019). Interestingly, there was no significant difference between LONMOSD and EONMOSD in terms of severe relapses, which reduces the contribution of this factor to disability accumulation. A decrease in repair mechanisms, immune tolerance and worse response to immunosuppressive therapy are plausible culprits for the greater EDSS step in LONMOSD (Collongues et al., 2014).

Anti-AQP4 Abs+ among LONMOSD was associated with increased disability, as measured by EDSS, compared to anti-MOG Abs+, confirming previous findings (Sepulveda et al., 2019). A recently published study by Wang et al. (2022) included the largest cohort of LONMOSD patients ($n = 122$) and found that anti-AQP4 Abs+ LONMOSD had worse prognostic outcomes, namely transverse myelitis, blindness, and motor dysfunction, as well as higher EDSS scores

This underscores the importance of serostatus in NMOSD management. However, we were unable to replicate the association between Anti-AQP4 Abs+ and time to disability. Furthermore, this same study reported an even worse outcome for patients with double seronegative LONMOSD, which was not observed in our cohort (Sepulveda et al., 2019).

This study has several limitations that should be accounted for. First, its retrospective design and heterogeneous composition of serostatus groups. In this regard, it reflects the composition of the Portuguese NMOSD population since it concerns all cases collected by the national registry. Secondly, anti-AQP4 Abs+ and anti-MOG Abs+ were tested with different assays and according to local protocols. Lastly, we did not control for possible confounders when determining the time to reach ambulation disability. Likewise, we are unable to rule out that age related comorbidities or vascular risk factors contribute to the outcome.

6. Conclusions

This cohort, which includes the three subtypes of the disease, Anti-AQP4 Abs+, Anti-MOG Abs+ and seronegative, shows that one in every four patients with NMOSD has a late onset form of the disease. Recognition of this subgroup of patients is relevant since the disease course appears to be characterized by less relapses, but with a more aggressive course and less time to reach increased disability. No difference in the presenting phenotype and acute or chronic treatment regimens was found in this cohort. LONMOSD correlated to less time to reach increased disability as shown by survival analysis, in agreement with the literature. Anti-AQP4 Abs+ were associated with increased disability, underscoring the importance of studying the serostatus in NMOSD management.

Declaration of Competing Interest

Authors declare no conflict of interest regarding this work.

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