Multiple Sclerosis and Related Disorders 36 (2019) 101413

Contents lists available at ScienceDirect

Multiple Sclerosis and Related Disorders

journal homepage: www.elsevier.com/locate/msard

Long-term outcome and prognosis in patients with neuromyelitis optica spectrum disorder from Serbia

Jelena Drulovic[a,b,*], Vanja Martinovic[a], Irena Dujmovic Basuroski[a,b,c], Sarlota Mesaros[a,b], Simone Mader[d,e], Brian Weinshenker[f], Tatjana Pekmezovic[g]

[a] Clinic of Neurology, Clinical Center of Serbia, Dr Subotica 6, 11000 Belgrade, Serbia
[b] Faculty of Medicine, University of Belgrade, Dr Subotica 8, 11000 Belgrade, Serbia
[c] Department of Neurology, University of North Carolina School of Medicine, North Carolina, USA
[d] Feinstein Institute for Medical Research, Center for Autoimmune, Musculoskeletal and Hematopoietic Diseases, Northwell Health, Manhasset, New York, USA
[e] Institute of Clinical Neuroimmunology, Biomedical Center and University Hospital, Ludwig-Maximilians University Munich, D-82152 Martinsried, Germany
[f] Department of Neurology, Mayo Clinic, Rochester, Minnesota, USA
[g] Institute of Epidemiology, Faculty of Medicine, University of Belgrade, Visegradska 26A, 11000 Belgrade, Serbia

ARTICLE INFO

Keywords:
Neuromyelitis optica spectrum disorder (NMOSD)
Long-term outcome
Opticospinal Impairment Scale (OSIS)
NMOSD diagnostic criteria

ABSTRACT

Background: Neuromyelitis optica spectrum disorder (NMOSD) most commonly, although not exclusively, targets optic nerves and spinal cord. Untreated, early and severe disability is common. We evaluated the long-term outcome in NMOSD patients diagnosed according to the 2015 criteria.

Methods: We retrospectively analyzed 74 patients from the hospital-based NMOSD cohort at the Clinic of Neurology, Belgrade, Serbia, who fulfilled the 2015 NMOSD criteria. We identified patients based on 2015 criteria; 51.4% of whom would not have fulfilled 2006 criteria. Median follow-up was 6.9 years. Aquaporin-4 (AQP4) IgG was tested in all patients using a cell-based indirect immunofluorescence assay. The level of neurological disability was assessed by the Expanded Disability Status Scale (EDSS) score, and by Opticospinal Impairment Scale (OSIS), visual acuity (VA) and motor function subscores.

Results: The disease course was monophasic in 17.6% patients and relapsing in the remainder; none developed progressive disease. AQP4-IgG was detected in 89.2% of patients. 45 of 74 patients were treated with immunosuppressants, 40 with azathioprine, 3 with mycophenolate mofetil, 1 with cyclophosphamide, 1 with mitoxantrone, and 2 patients with rituximab. The median intervals from onset to EDSS 4.0, 6.0 and 7.0 were 6.5 years, 11.9, and 22.0 years, respectively. Higher baseline EDSS was associated with risk of attaining EDSS 4.0, 6.0 and 7.0; a shorter first inter-attack interval for reaching EDSS 4.0 and 6.0; longer time to the start of treatment for reaching EDSS 7.0. Worse visual acuity at the disease onset predicted faster assignment of OSIS VA = 6 and VA = 8. Severe visual deficit (OSIS V_A 6) was reached earlier after optic neuritis (median time, 10.0 years) or combined opticospinal onset (median time, 11.4 years) than after myelitis onset (median time, 18.0 years) ($p = 0.002$).

Conclusion: Our results support the benefits of early diagnosis and treatment of NMOSD, especially in persons with severe optic and spinal disability at onset.

1. Introduction

Untreated, neuromyelitis optica spectrum disorder (NMOSD) is a devastating disease with a poor prognosis. International consensus criteria published in 2015 liberalized the diagnosis, roughly doubling the number of recognized cases (Wingerchuk et al., 2015; Hamid et al., 2017). New diagnostic criteria may have changed the prognosis of the disease by identifying earlier and perhaps milder cases, although severity of disability is not a component of either 2015 or 2006 criteria.

The aim of our study was to evaluate the long-term outcome of a large cohort of Serbian NMOSD patients satisfying 2015 diagnostic criteria. Others have also addressed the long term outcome of NMOSD in the contemporary era (Wingerchuk et al., 1999; Ghezzi et al., 2004; Rivera et al., 2008; Bichuetti et al., 2009; Cabre et al., 2009; Collongues et al., 2010; Fragoso et al., 2019), but the mean follow-up has been usually short. Kitley et al. evaluated a large NMOSD cohort;

* Corresponding author
E-mail address: drulovicjelena@gmail.com (J. Drulovic).

https://doi.org/10.1016/j.msard.2019.101413
Received 16 August 2019; Received in revised form 22 September 2019; Accepted 25 September 2019

Table 1
Features tested for their predictive role in NMOSD.

Variables
Demographic
- Age
- Gender
Clinical
- Fulfilment of 2006 NMO diagnostic criteria
- Age at onset
- Disease course (monophasic / relapsing-remitting)
- Time from disease onset to diagnosis
- Time from disease onset to maintenance treatment
- Type of maintenance treatment : corticosteroids, immunosupressant, TPE
- Disease onset core clinical characteristics[a]
- EDSS score at the disease onset (nadir)
- OSIS V_A at the disease onset (nadir)
- OSIS motor function at the disease onset (nadir)
- Disease onset type: monofocal/multifocal
- First inter-attack interval duration
- Total number of attacks and annualized rate of attacks within first, second, and fifth year after the onset of disease
- Number of relapses to reach EDSS score 4.0, 6.0 and 7.0
- Time to reach EDSS score 4.0, 6.0 and 7.0
- Time to reach OSIS V_A 6 and 8
- Time to reach OSIS motor function 5 and 7
- Outcome (alive/deceased)
Paraclinical
- AQP4-IgG status and titer
- Brain MRI lesions: present/absent
- Brain MRI: lesion location[b]
- Cervical/dorsal spinal cord MRI lesions: present/absent
- Cervical/dorsal spinal cord MRI: lesion characteristics (T1-hypointensity, T2-hyperintensity, T1-gadolinium enhancement)
- Cervical/dorsal spinal cord MRI: lesion > 70% of the transverse spinal cord transections
- Cervical/dorsal spinal cord MRI: total lesion length
- CSF findings (proteins, number and cell phenotypes, IgG index, oligoclonal IgG bands)

NMOSD, neuromyelitis optica spectrum disorders; TPE, therapeutic plasma exchange; EDSS, Expanded Disability Status Scale; OSIS, Opticospinal Impairment Score; V_A, visual acuity; AQP4, aquaporin-4; MRI, magnetic resonance imaging; CSF, cerebrospinal fluid.

[a] Optic neuritis, acute myelitis, area postrema syndrome, acute diencephalic syndrome, acute brainstem syndrome, symptomatic cerebral syndrome;.

[b] Optic nerve, area postrema, periependymal brainstem, diencephalic, non-specific supratentorial/ infratentorial.

the mean follow-up was 24 months in patients with monophasic illness and 121.8 months in those with relapsing disease (Kitley et al., 2012). Additionally, we analyzed potential prognostic factors for survival and other long-term outcomes in our NMOSD patients using observational retrospective cohort design.

2. Material and method

We evaluated 74 Caucasian NMOSD patients from the Clinic of Neurology, Clinical Centre of Serbia, Belgrade, who fulfilled the 2015 diagnostic criteria (Wingerchuk et al., 2015) and were followed from onset to March 31, 2017. Median follow-up was 6.9 years (range, 6 months–34.5 years). Neurological examination was performed in 3–6 month intervals in all patients (depending on the individual disease activity and treatment safety profile) additional evaluations were performed at the time of suspected relapses. All deaths were identified and main cause of death was obtained from death certificates.

Demographic, clinical, and paraclinical data were evaluated as potential predictors of long-term outcome (Table 1). We determined the proportion of patients satisfying 2015 (Wingerchuk et al., 2015) who did not satisfy 2006 criteria (Wingerchuk et al., 2007) to address the impact of change in outcome attributable to changes in diagnostic criteria. Clinical manifestations of NMOSD were classified according to the 2015 NMOSD criteria (Wingerchuk et al., 2015). We assessed neurological impairment using the Expanded Disability Status Scale (EDSS) score (Kurtzke, 1983), and additionally the Opticospinal Impairment Scale (OSIS), Visual acuity and Motor function subscores (Pittock et al., 2013). NMOSD relapses were defined by new neurological symptoms associated with a change on neurologic examination or objective clinical worsening of the existing symptoms that would last for ≥24 h (except for area postrema symptoms when the symptom duration needed to last for more than 48 h to qualify for an NMOSD relapse) (Pittock et al., 2013; Shosha et al., 2018).

Aquaporin-4 (AQP4)-IgG was tested in the Neuroimmunology Laboratory, Clinical Center of Serbia, Belgrade, Serbia in all patients using a commercial cell-based indirect immunofluorescence assay (Euroimmun AG). Titers of 1:10 or higher were considered to be positive. In addition, antibodies against myelin-oligodendrocyte (MOG-IgG) in serum were tested in a subset of 47 randomly selected NMOSD patients for whom enough sample volume was available at the Feinstein Institute for Medical Research, Manhasset, New York using a live cell-based assay (Mader et al., 2018).

Cerebrospinal fluid (CSF) analysis (protein level, cell count and phenotype, IgG index, oligoclonal IgG bands) was performed by the standard methods in 66 patients. Brain, cervical and thoracic spinal cord magnetic resonance imaging (MRI) was performed in all patients in whom there was no contraindication ($n = 71$). In 3 patients in whom MRI could not be performed (both brain and spinal cord MRI, $n = 1$, spinal cord MRI, $n = 2$), the diagnosis of NMOSD was established based on the clinical presentation and serum AQP4-IgG positivity (Wingerchuk et al., 2015). Longitudinally-extensive transverse myelitis (LETM) was defined as a continuous spinal cord MRI lesion extending ≥3 vertebral segments on T2-weighted sagittal MRI sequences (Wingerchuk et al., 2015; Wingerchuk et al., 2007).

The study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade. All patients gave informed consent. For the tests performed at the Feinstein Institute for Medical Research, Manhasset, New York, the patients signed an additional informed consent.

2.1. Statistical analysis

For comparison between groups, chi square tests and ANOVA were applied, as appropriate. The life table method was used to estimate the time to the assignment of EDSS scores 4.0, 6.0, and 7.0. We analyzed whether there is a difference in the proportion of various symptoms at disease onset according to age at onset. Survival probability was analyzed using the Kaplan–Meier procedure. The log-rank test was used to assess differences in survival curves. The predictive value of all variables presented in Table 1 was assessed by univariate and multivariate Cox proportional hazard regression models. Variables significant at the 0.05 level were further analyzed in the multivariate Cox proportional hazard regression model and considered significant if $p < 0.05$.

3. Results

3.1. Serbian NMOSD cohort characteristics

Demographic and clinical characteristics of patients from our study are presented in Table 2. Of NMOSD study participants satisfying 2015 criteria, 38 (51.4%) patients did not satisfy 2006 criteria. In two patients in whom brain MRI was not performed, we could not determine whether 2006 criteria were satisfied. The ratio of women to men was 5.7:1. The median age at onset was 40 years (range 7–68). LETM was detected in 85% of patients at the time of an acute myelitis attack and 55% outside of an attack. Median time from the disease onset to diagnosis and the start of maintenance corticosteroids and/or immunosuppressant treatment was approximately 3 years.

89.2% of our patients were AQP4-IgG positive. Median NMOSD-IgG titer was 1:640. Additionally, two of seven of AQP4-IgG negative

Table 2
Demographic and clinical characteristics of patients from the Serbian NMOSD cohort.

Variable	NMOSD
Gender, No (%)	
Female Male	63 (85.1) 11 (14.9)
[a]Age, years	49.9 (10.0–76.0)
[a]Age at onset, years	40 (7–68)
[a]Time from onset to diagnosis, months	35 (0.4–256)
[a,b]Time from onset to treatment, months	40 (0.3–383)
NMOSD clinical symptoms at onset, N (%)	
Acute myelitis	41 (55.4)
Optic neuritis	33 (44.6)
Area postrema syndrome	12 (16.2)
Acute brainstem syndrome	19 (25.7)
Acute diencephalic syndrome	7 (9.5)
Symptomatic cerebral syndrome	3 (4.1)
NMOSD onset, N (%)	
Monofocal Multifocal	51 (68.9) 23 (31.1)
Disease course, N(%)	
[c]Monophasic Relapsing	13 (17.6) 61 (82.4)
[a]First interattack interval, months	21 (0.8–200)
[a]Number of attacks	
0–1 year 0–2 years 0–5 years	1 (1–5) 1 (1–5) 2 (1–14)
[a]Annualized attack rate	0.57 (0.06–7.45)
[a]EDSS score at disease onset (nadir)	3 (1.5–9.5)
[a]EDSS score at last follow-up visit	3 (1.0–10.0)
Median time to reach EDSS score 4.0, years	6.5 (2.1–11.1)
Median time to reach EDSS score 6.0, years	11.9 (7.9–15.9)
Median time to reach EDSS score 7.0, years	22.0 (14.5–29.3)
[d]OSIS V_A at disease onset (nadir)	2.2 ± 3.0
[d]OSIS V_A at last follow-up visit	2.6 ± 3.1
Median time to reach OSIS V_A 6, years	15.7 (13.1–18.3)
Median time to reach OSIS V_A 8, years	18.9 (16.6–21.1)
[d]OSIS Motor function at disease onset (nadir)	2.4 ± 3.1
[d]OSIS Motor function at last follow-up visit	3.2 ± 2.9
Median time to reach OSIS Motor function 5, years	11.0 (5,7–16.3)
Median time to reach OSIS Motor function 7, years	18.0 (14.3–21.5)
[a]Duration of follow-up, years	6.9 (0.5–34.5)
Deaths, N (%)	7 (9.5%)

[a] Median (range);.
[b] Maintanance corticosteroids and/or immunosuppressants;.
[c] A single NMOSD attack during follow-up;.
[d] mean (± SD).

patients in this subgroup were MOG-IgG positive.

During the follow-up interval (median 6.9 years, range 0.5–34.5), 13 patients did not relapse (17.6%), and 61 (82.4%) did. The mean follow-up/duration of disease in monophasic patients was 2.7 ± 1.4 years and in relapsing patients 9.3 ± 6.2 years ($p = 0.001$). None developed progressive disease. Age of onset (divided into 3 groups: < 30 years; 31–50 years; 51 + years) did not differ between those with optic neuritis or myelitis onset ($p = 0.110$). Furthermore, there was no difference in the mean age of disease onset between patients presenting with myelitis (40.1 ± 15.5 years) and those with ON (35.2 ± 14.3 years; $p = 0.728$). Optic neuritis was the presenting feature in 26 of 61 patients (43%) in the relapsing group and 7 of 13 patients (54%) in the monophasic group ($p = 0.460$). In relapsing patients who presented with optic neuritis, 69% relapsed with ON (which is more frequent than expected based on overall distribution of relapses, $p = 0.001$); this subgroup had a median of 2 relapses per patient (range, 1–6). In contrast, myelitis was the presenting feature in 35 of 61 patients (58%) in the relapsing group and 6 of 13 (46%) patients in the monophasic group ($p = 0.460$). In relapsing patients who presented with myelitis, 22 of 35 (63%) relapsed with myelitis (which is more frequent than expected based on overall distribution of relapses, $p = 0.001$); this subgroup had a median of 2 relapses per patient (range, 1–9). In our relapsing patients who presented with ON, 69% relapsed with ON and had a median of 2 relapses per patient (range, 1–6); out of 41 patients who presented with myelitis, 18 (43.9%) had ON relapses (median 1 relapse per patient, range 1–5). Seven out of 33 (21.2%) patients who presented with ON became blind, and 2 out of 41 (4.9%) who presented with myelitis. Mean number of ON attacks in ON onset group was 1.9 ± 1.2 and in myelitis onset group, each patient had one ON relapse which led immediately to blindness. There is no statistically significant difference in number in relapses between ON onset and myelitis onset groups ($p = 0.374$), possibly due to small number of units of observation.

Among those with monophasic disease course, 5 had isolated LETM (affecting 5–19 vertebral segments); 2 had isolated unilateral optic neuritis (ION); 1 each experienced bilateral ON (BON), BON + acute diencephalic clinical syndrome, BON + LETM, BON + LETM + area postrema syndrome, LETM + acute brainstem syndrome + area postrema syndrome + encephalopathy, and BON + LETM + acute brainstem syndrome + area postrema syndrome + encephalopathy. Twelve of these patients had disease duration less than 5 years, and the remaining patient less than 10 years. All received maintenance treatment after the first relapse. Twelve of 13 patients with a monophasic disease (92.3%) tested positive for AQP4-IgG. One woman with monophasic NMOSD, seronegative for AQP4-IgG but seropositive for MOG-IgG (titer, 1:1280), experienced bilateral optic neuritis and LETM satisfying the 2015 diagnostic criteria for AQP4-IgG seronegative NMOSD (Wingerchuk et al., 2015).

MRI lesions affecting only optic nerve and spinal cord on the first MRI ever performed were present in 44 out of 73 patients (60.3%) whereas other non-opticospinal brain lesions were observed in 29 (39.7%) patients (Table 3). Spinal cord lesions on the first spinal cord MRI ever done, all performed at the time of acute myelitis, were present in 90.1%. Myelitis most frequently involved the cervical spinal cord ($n = 28$; 40%); thoracic spinal cord in 14 patients (20%), and both

Table 3
Brain and spinal cord MRI findings on the first MRI ever performed in the Serbian NMOSD patient cohort.

Brain MRI lesions, No (%)	
Present	29/73 (39.7)
Absent	44/73 (60.3)
Brain MRI lesion location, No (%)	
Optic nerve Diencephalon Area postrema Periependymal brainstem	12/44 (27.3) 2/44 (4.5) 7/44 (15.9) 7/44 (15.9)
Supratentorial Infratentorial	37/44 (84.1) 16/44 (36.4)
Spinal cord MRI lesions[a], No (%)	
Present Absent	64/71 (90.1) 7/71 (9.9)
Spinal cord MRI lesion localisation, No (%)	
Cervical T2[b] Cervical T2 ≥3 vertebral segments[b] Cervical T1 gado + [c] Thoracic T2[b] Thoracic T2 ≥3 vertebral segments[b] Thoracic l T1 gado + [c] Cervical + thoracic T2 ≥3 vertebral segments[b]	27/64 (42.2) 22/64 (34.4) 11/27 (40.7) 14/64 (21.9) 13/64 (20.3) 3/14 (21.4) 23/64 (35.9)
Spinal cord lesion length[d]	6.0 (1–19)
Spinal cross-sectional area affected > 70%, No (%)	12/64 (18.8)

[a] Cervical and/or thoracic;.
[b] T2-Weighted hyperintensity;.
[c] T1-Weighted hypointensity with post-contrast gadolinium enhancement;.
[d] Median (range).

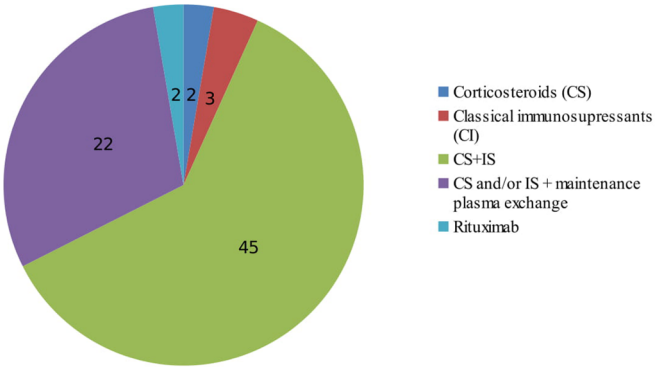


Fig. 1. Maintenance treatment.

cervical and thoracic in 23 (33%). In 58 of 64 patients (90.6%), spinal cord MRI lesions extended over ≥3 vertebral segments while the remainder (6 of 64; 9.4%) had short-segment lesions affecting 1–2 vertebral segments. Optic nerve lesions were detected on MRI in 27.3% of patients.

Initial CSF findings, in all cases during an acute attack, showed a median CSF protein level of 0.45 g/L (range, 0.28–2.23 g/L), median CSF cell count of 4/μL (range, 0–378 mononuclear cells/μL; > 5 granulocytes/μL in 25% of patients), and oligoclonal IgG bands in 16 of 66 patients (24.2%).

Fig. 1 summarizes the maintenance treatment in our patients. Maintenance treatment was started after a median of 40 months from disease onset (Table 2). Most (45, 60.8%) were treated with a combined CS + IS treatment (azathioprine or mycophenolate mofetil or cyclophosphamide or mitoxantrone) either simultaneously or consecutively. 45 of 74 (60.8%) patients were treated with IS; 40 patients were treated with azathioprine, three with mycophenolate mofetil, one with cyclophosphamide, one with mitoxantrone, and two patients with rituximab. Two patients (2.7%) who refused immunosuppressants received only corticosteroids (CS) as maintenance therapy. Plasma exchange (PLEX) was used as an add-on maintenance treatment to CS and/or IS in 22 patients (29.7%) in whom the clinical disease activity could not have been controlled by CS and/or IS.

3.2. Disability in Serbian NMOSD cohort

Forty-four patients (58.5%) reached EDSS 4.0, 34 (48.6%) EDSS 6.0, and 32 (43.2%) reached EDSS 7.0 over 6.9-year follow-up at a median interval of 6.5 years, 11.9, and 22.0 years, for these respective levels of impairment (Table 2). There was no difference in median time to reach EDSS scores of 4.0, 6.0 and 7.0 in patients satisfying 2015, but not 2006 criteria (data not presented; $p = 0.680$, $p = 0.949$, and $p = 0.503$, respectively).

In patients experiencing acute myelitis as the first attack, the median time to reach EDSS scores of 4.0, 6.0, and 7.0 was 3.9 (95%CI 1.7–8.3) years, 11.5 (95%CI 2.6–21.2) years, and 21.0 (95%CI 13.1–28.5) years, respectively. Time to reach EDSS scores of 4.0, 6.0 and 7.0 in patients having an acute myelitis as onset attack (isolated or combined with other symptoms) was not different from that of the remaining patients. Thus the topography of the first attack had no detectable impact on the subsequent disability, as measured by EDSS.

Since EDSS is not equally sensitive to outcomes related to motor and visual deficit, we also used OSIS scores to estimate motor and visual deficit (Table 2). Twenty seven percent of patients reached OSIS VA 6 (counts fingers only in one or more eye), and 13.5% VA 8 (no light perception in one or more eye). Fewer than 50% reached those levels of disability in the entire cohort; the intervals at which these levels of visual impairment were reached by the first quartile were 2 and 3 years, respectively. 41.9% reached motor function subscore 5 (MRC grade 2 or worse weakness in one or more muscle groups in at least one limb), and 29.7% motor function subscore 7 (MRC grade 0–1 strength in all muscles in one or more limb), over the follow-up interval. The time to these levels of impairment was 3 years for both (median not observed in this data).

Visual acuity assessments were performed in all patients. According to the topography of the first attack, there was statistically significant difference in curves related to severe visual loss, assessed as OSIS VA 6 and OSIS VA 8. Severe visual disability (OSIS VA 6) was reached earlier when the first attack was ON (median time, 10.0 years) and opticospinal onset (median time, 11.4 years) than after myelitis onset (median time, 18.0 years) ($p = 0.002$). There was no difference in median time to reach severe visual loss in patients satisfying 2015, but not 2006 criteria (data not presented; $p = 0.371$).

The predictive value of all variables presented in Table 1, for reaching EDSS score and OSIS VA and Motor function subscores milestones, was assessed by univariate and multivariate Cox proportional hazard regression models. Only significant findings are presented.

In the univariate Cox proportional hazard regression analyses, older age at onset, longer time from onset to diagnosis and to the start of maintenance treatment, higher EDSS score at the nadir of the onset attack and longer spinal MRI lesions were predictive of reaching EDSS score milestones of 4.0, 6.0, or 7.0 (Table 4), while, among other variables, the effect of total number of relapses was also analyzed and was not significant (data not shown). In multivariate analyses, higher EDSS score at onset attack was an independent predictor of reaching all three endpoints (EDSS scores 4.0, 6.0 and 7.0); a shorter first inter-attack interval independently predicted faster assignment of EDSS scores 4.0 and 6.0. Longer time from onset to the start of maintenance treatment was an independent prognostic factor for the faster development of EDSS score 7.0.

Additionally, we analyzed predictive factors for reaching disability measured by OSIS VA and Motor function subscores as dependent variables. In the multivariate Cox regression analyses (dependent variable: OSIS VA = 6 and VA = 8), OSIS VA at the disease onset (nadir) was a consistent independent predictor of reaching both endpoints (OSIS VA = 6 and VA = 8) (HR = 1.463; 95%CI 1.251–1.711; $p = 0.001$, and HR = 1.491; 95%CI 1.192–1.866; $p = 0.001$, respectively) worse visual acuity at onset predicted faster assignment of VA = 6 and VA = 8. Furthermore, longer time from onset to diagnosis predicted faster assignment of VA = 8 (HR = 0.988; 95%CI 0.978–0.997; $p = 0.010$).

Regarding predictive factors for reaching motor disability, univariate Cox regression analyses (dependent variable: OSIS motor function = 5 and 7) demonstrated that age, age at onset, time from disease onset to maintenance and IS treatment, and OSIS motor function at disease onset (nadir) were predictive for reaching OSIS motor function = 5 and 7 ($p < 0.05$). Multivariate analysis showed that worse weakness at onset (HR = 1.301; 95%CI 1.139–1.486; $p = 0.001$) and older age (HR = 1.063; 95%CI 1.025–1.102; $p = 0.001$) predicted faster achievement of motor function = 5. Patients with higher age at onset (HR = 1.056; 95%CI 1.025–1.089; $p = 0.001$) had a higher probability to reach motor function = 7 earlier.

3.3. Survival in Serbian NMOSD cohort

Seven patients (9.5%) died during the follow-up, and the causes of death were: NMOSD-related respiratory failure ($n = 4$), sepsis ($n = 1$), myocardial infarction ($n = 1$), and accident ($n = 1$). Survival analysis is shown in Fig. 2. At 5, 10, 15 and 20 years, survival probabilities were: 98.6%, 94.6%, 82.7% and 63.0%, respectively. There was no difference in survival probability in patients satisfying 2015 but not 2006 criteria (data not presented; $p = 0.729$).

Early prognostic factors for longer survival in the univariate model were: younger age, younger age at onset, a shorter time from onset to the start of maintenance treatment, area postrema symptoms at onset, and a shorter time from onset to EDSS score 7.0 (Table 5). A

Table 4
Predictive factors of disability in 74 patients with NMOSD: Cox proportional hazard regression models.

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*	HR	95% CI	*p*
Outcome: EDSS score 4.0						
Age at onset	1.029	1.008–1.051	0.008			0.375
Time from onset to diagnosis	0.990	0.984–0.986	0.002			0.113
Time from onset to maintenance treatment	0.993	0.988–0.998	0.009			0.393
Spinal onset	0.447	0.235–0.850	0.014			0.398
Area postrema onset	0.481	0.234–0.988	0.046			0.773
First interattack interval duration	0.980	0.967–0.993	0.002	0.973	0.959–0.987	0.001
First attack EDSS score (at nadir)	1.415	1.252–1.599	0.001	1.535	1.260–1.870	0.001
Length of spinal lesion	1.076	1.015–1.141	0.014			0.656
Outcome: EDSS score 6.0						
Age at onset	1.040	1.014–1.065	0.002			0.373
Time from onset to diagnosis	0.990	0.984–0.987	0.005			0.229
Time from onset to maintenance treatment	0.989	0.981–0.996	0.002			0.195
Optic neuritis onset	2.075	1.013–4.250	0.046			0.093
Area postrema onset	0.376	0.172–0.823	0.014			0.786
Course of the disease	0.417	0.180–0.966	0.041			0.585
First interattack interval	0.984	0.970–0.988	0.027	0.967	0.940–0.995	0.021
First attack EDSS score (at nadir)	1.766	1.467–2.125	0.001	1.562	1.087–2.243	0.016
Length of spinal lesion	1.104	1.036–1.175	0.002			0.976
Motor index OSIS	1.479	1.197–1.827	0.001			0.068
Outcome: EDSS score 7.0						
Age at onset	1.044	1.017–1.073	0.001			0.327
Time from onset to diagnosis	0.991	0.984–0.998	0.016			0.065
Time from onset to maintenance treatment	0.989	0.981–0.997	0.006	0.960	0.930–0.991	0.011
Area postrema onset	0.350	0.157–0.779	0.010			0.609
First attack EDSS score (at nadir)	1.681	1.407–2.008	0.001	1.761	1.395–2.223	0.001
Length of spinal lesion	1.097	1.026–1.173	0.007			0.753

HR - Hazard ratio; 95%CI - 95% confidence interval; EDSS- Expanded Disability Status Scale; OSIS – Opticospinal Impairment Score; n.s.- non-significant.

multivariate Cox proportional hazard regression model did not reveal any independent predictor of survival in our NMOSD patients.

4. Discussion

We analyzed long-term clinical outcomes and predictive factors in large group of European Caucasian NMOSD patients diagnosed according to the revised 2015 criteria (Wingerchuk et al., 2015). The discovery of the specific biomarker for NMO/NMOSD (Lennon et al., 2004) that was included in the 2006 revision of the NMO diagnostic criteria (Wingerchuk et al., 2007) enabled earlier and more accurate establishment of NMO diagnosis. The 2015 criteria enable a diagnosis of NMOSD after a single relapse in AQP4-IgG seropositive individuals. Application of these criteria led to an increase in the rate of diagnosis of NMOSD by 63–76% (Hamid et al., 2017; Carnero Contentti et al., 2018). 38 (51.4%) patients satisfied 2015 but not 2006 criteria. Sixty-six of 74 patients (89.2%) were AQP4-IgG positive, using cell-based immunofluorescence assays on fixed transfected cells.

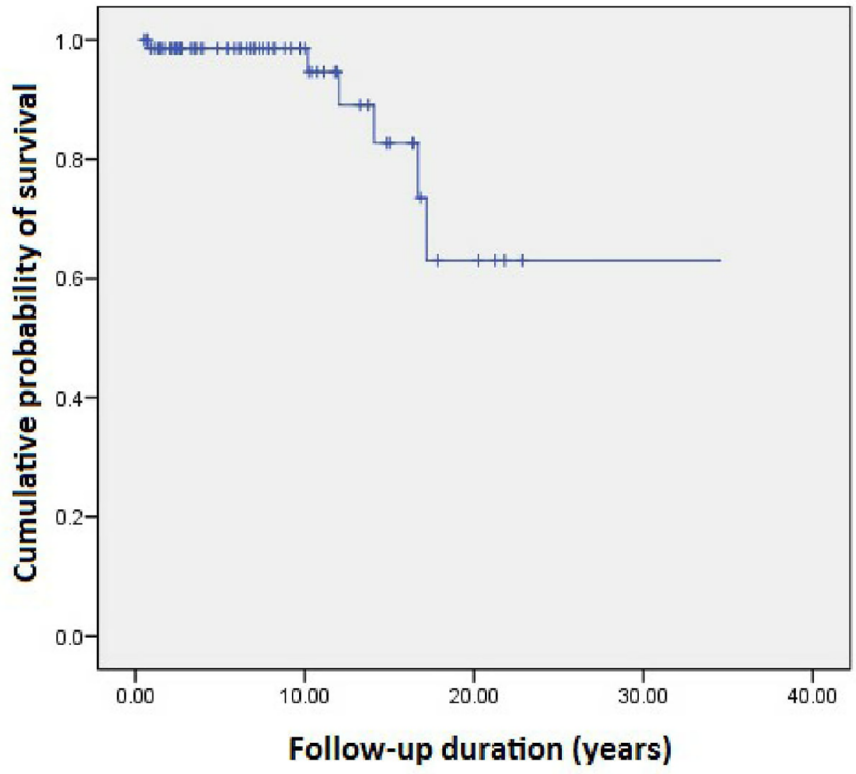
Demographic characteristics of our patients are consistent with previous studies. The ratio of women to men was 5.7; the median age at onset was 40 years (Ghezzi et al., 2004; Collongues et al., 2010; Fragoso et al., 2019). The disease course was exclusively relapsing-remitting and not progressive, consistent with other studies (Cabre et al., 2009; Collongues et al., 2010; Kitley et al., 2012; Wingerchuk et al., 2007). Over a median follow-up interval of 7 years, the disease course remained monophasic in 13 patients (17.6%). However, the mean follow-up was shorter (2.7 ± 1.4 years) in comparison to relapsing patients (9.3 ± 6.2 years) and we presumed that many patients classified as having monophasic course eventually will relapse. Twenty five patients did not relapse after 5 years and eleven after 10 years of follow-up.

Few longitudinal studies have been reported which characterize the long-term NMOSD course and factors predictive for development of disability (Ghezzi et al., 2004; Rivera et al., 2008; Cabre et al., 2009; Collongues et al., 2010; Kitley et al., 2012; Wingerchuk and Weinshenker, 2003). These studies were small (range, 34–125 subjects) and applied pre-2015 criteria. We identified patients based on 2015 criteria, 51.4% of whom would not have fulfilled 2006 criteria. Notably, attainment of EDSS 4.0, 6.0, and 7.0 as well as survival did not differ between those who did and did not satisfy 2006 diagnostic criteria. However, given the relatively small number of subjects and short follow-up in our study, further studies are required to address the impact of 2015 criteria on NMOSD prognosis.

Although a number of NMO/NMOSD epidemiological studies provide useful data, only a few evaluated disability outcomes (Table 6). Similar to our findings, French and Italian studies (Ghezzi et al., 2004; Collongues et al., 2010) reported that proportion of patients who reached EDSS 6.0 was 47–56%, after comparable interval from disease onset (10–12 years). The time to reach EDSS 7.0 was almost identical in our study as in a French study (22 vs 21 years) (Collongues et al., 2010). The mean time from onset to first effective treatment was 4.6 ± 5.9 years in the French study, compared to 3.3 (range, 0.1–31.9) years in ours.

The topography of the first attack had no impact on the subsequent disability, as measured by EDSS. However, as assessed by OSIS V_A 6 and OSIS V_A 8, achievement of severe visual loss occurred earlier in those with ON at onset. Severe visual deficit (OSIS V_A 6) was reached earlier after ON (median time, 10.0 years) or combined opticospinal onset (median time, 11.4 years) than after myelitis onset (median time, 18.0 years) ($p = 0.002$). There is no statistically significant difference in the mean number of ON attacks between ON onset group and myelitis onset group ($p = 0.374$), but we believe that severity of relapses might have had a crucial and more important role in the development of significant deficit than the number of relapses. Nonetheless, it is important to mention that patients presenting with ON become blind faster than those presenting with myelitis.

Higher EDSS score at onset attack was a consistent independent predictor of reaching all three EDSS endpoints; a shorter first inter-attack interval independently predicted faster assignment of EDSS scores 4.0 and 6.0. In the Italian study, reaching EDSS 6.0 was associated with residual EDSS at onset and mean annualized relapse rate (Ghezzi et al., 2004). Using the Cox multivariate regression analysis, we have not



	Follow-up duration (years)								
	0	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**
No of patients	74	44	26	12	3	1	1	1	-

Fig. 2. Survival probability: Belgrade NMOSD cohort.

Table 5
Significant predictors of survival in 74 patients from the Serbian NMOSD cohort: univariate Cox proportional hazard regression model.

Variable	Hazard Ratio	95% Confidence Interval	*p*
Age	1.101	1.006–1.205	0.036
Age at onset	1.133	1.033–1.242	0.008
Time to maintenance treatment	0.973	0.951–0.995	0.018
Area postrema syndrome at onset	0.107	0.014–0.803	0.030
Time to EDSS score 7.0	0.867	0.761–0.989	0.033

demonstrated that the effect of total number of relapses was significantly associated with reaching high EDSS scores. It might be explained by the notion that each patient's chance of reaching a high EDSS score was due either to residual EDSS after a single severe relapse or an accumulation after non-severe relapses. Although Collongues et al. have not identified any risk factors that could predict the EDSS, a high number of MRI brain lesions at diagnosis were predictive of a severe residual visual deficit (Collongues et al., 2010).

Worse visual acuity at disease onset and longer time from onset to diagnosis predicted faster assignment of severe visual loss, as assessed by OSIS V_A 6 and 8 subscores. Thus, it is worthwhile to consider the separate contributors to visual and spinal cord impairments; EDSS may lose considerable information as a summary measure in NMOSD.

Similarly to the notion that higher EDSS score at onset attack was a consistent independent predictor of reaching EDSS 4.0, 6.0, and 7.0, worse weakness at onset predicted faster achievement of severe motor deficit as measured by OSIS motor function = 5.

Factors associated with longer survival in the univariate model were: younger age, younger age at onset, a shorter time from onset to the start of maintenance treatment, area postrema symptoms at onset, and a longer time from onset to EDSS 7.0, although no independent predictors of survival were identified with multivariate analysis. Kitley et al. also reported that older age at disease onset was predictor of mortality and speculated that severe motor disability predisposes to life-threatening complications, such as pneumonia, disproportionately in older age patients (Kitley et al., 2012). Wingerchuk et al. previously demonstrated that the degree of motor recovery after myelitis event and the number of relapses in the first two years of disease predicted shorter survival (Wingerchuk and Weinshenker, 2003). Our results, in accordance with above mentioned, support the notion that survival and disability may be influenced by early and aggressive therapeutic interventions aimed at reduction in early attack frequency.

Our study has limitations. It is a single center observational study with relatively small sample size, which limits power. Therefore, larger sample size as well as longer follow-up is warranted.

High EDSS score at nadir, short first interattack interval, and long

Table 6
Studies analyzing clinical features of patients with NMO/NMOSD.

Author (Year)	Ghezzi et al. (2004)	Fragoso et al. (2019)	Collongues et al. (2010)	Kitley et al. (2012)	Drulovic et al. (2019)
Country	Italy	Brazil	France	UK, Japan	Serbia
Number of patients	46	153	125	106	74
Inclusion criteria	Two attacks of only ON and spinal cord dysfunction; brain MRI negative or non-specific of MS; oligoclonal bands negative	NMOSD 2015	NMO 2006	AQP4-IgG positive patients	NMOSD 2015
Median age at onset (years)	40[a]	28	34.5[a]	40.5[a]	40
Mean follow-up period (years)	8.8 ± 3.5	NA	10.0 ± 7.8	1.5[b] (monophasic) 8.8[b] (relapsing)	2.7 ± 1.4 (monophasic) 9.3 ± 6.8 (relapsing)
Median time from disease onset to first relapse (range) (months)	17 (1–120)	NA	30.8 ± 43.1[a]	14 (1–179)	21 (0.8–200)
Mean time from onset to first treatment (months)	NA	NA	55.2 ± 70.8	2.6 ± 5.8 (monophasic) 54.3 ± 64.6 (relapsing)	4.0 ± 5.8 (monophasic) 71.5 ± 79.8 (relapsing)
Patients (%)reached:					
-EDSS 4.0	NA	NA	70.8	NA	58.5
-EDSS 6.0	47	NA	55.8	NA	48.6
-EDSS 7.0	NA	12	29.2	23	43.2
Median time (years) from onset to reach:					
-EDSS 4.0	NA	NA	7	NA	6.5
-EDSS 6.0	10	NA	10	NA	12
-EDSS 7.0	NA	7	21	6	22
Proportion (%) of patients receiving IS treatments	NA	NA	81	95	68
Median time to effective treatment (years)	NA	NA	5	2.5 (UK) 5.5 (Japan)	3

*a Mean time;
**b Median time; IS – immunosuppressive; NA – not available.

time from onset to maintenance treatment independently predicted severe disability attainment in our study. Since EDSS was not as sensitive as OSIS to outcomes, OSIS should be considered as disability measure, especially its V_A subscore, for future studies. Finally, our results support the assertion that early effective treatment, especially in persons with severe visual and motor disability at onset of NMOSD, reduces the rate of attainment of severe disability.

Disclosures

Jelena Drulovic has been an advisor or speaker for Bayer HealthCare, Sanofi-Genzyme, Medis, Merck, Teva, Novartis, and Roche, and Principal investigator in clinical trial sponsored by MedImmune. She has received research support from the Ministry of Education and Science in Serbia (project no 175031).

Vanja Martinovic has been an investigator in clinical trial sponsored by MedImmune, and has received research support from the Ministry of Education and Science in Serbia (project no 175087).

Irena Dujmovic Basuroski: was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No 175031). Her current institution (UNC at Chapel Hill) received a grant support from The Bodford Family Transverse Myelitis Center Fund. She received lecture fees from Roche and travel compensation from The Guthy-Jackson Charitable Foundation. She has been sub-investigator in clinical trial sponsored by MedImmune.

Sarlota Mesaros has been an advisor or speaker for Medis, Merck, Teva, and Roche and has been an investigator in clinical trial sponsored by MedImmune. She has received research support from the Ministry of Education and Science in Serbia (project no 175031).

Simone Mader declares no conflict of interest.

Brian Weinshenker receives royalties from RSR Ltd, Oxford University, Hospices Civil de Lyon, and MVZ Labor PD Dr. Volkmann und Kollegen GbR for a patent of NMO-IgG as a diagnostic test for NMO and related disorders, served on adjudication committee for clinical trials in NMO being conducted by MedImmune and Alexion, and consulted for Chugai regarding a clinical trial for NMO.

Tatjana Pekmezovic has been an advisor or speaker for Sanofi-Genzyme, Medis, Merck, Teva, and Roche and has been an investigator in clinical trial sponsored by MedImmune. She has received research support from the Ministry of Education and Science in Serbia (project no 175087).

Declaration of Competing Interest

None

Acknowledgments

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grants no. 175031 and 175087).

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