Differential brainstem atrophy patterns in multiple sclerosis and neuromyelitis optica spectrum disorders

Abstract

Background: Multiple sclerosis (MS) and neuromyelitis optica spectrum disorders (NMOSD) are CNS inflammatory demyelinating disorders. It is clinically important to distinguish MS from NMOSD as treatment and prognosis differ. Brainstem involvement is common in both disorders.

Purpose: To investigate whether the patterns of brainstem atrophy on volumetric analysis in MS and NMOSD were different and correlated with clinical disability.

Study Type: Case control cross-sectional study

Subjects: 17 MS, 13 NMOSD and 18 healthy control (HC) subjects were studied.

Field Strength/Sequence: T1w and T2w spin-echo images were acquired by 3T scanner (Achieva, Philips Healthcare, Best, The Netherlands).

Assessment: Semi-automated segmentation and volumetric measurement of brainstem regions were performed. Anatomical information was obtained from whole brain T1w images using three-dimensional magnetization-prepared rapid gradient-echo (MPRAGE) imaging sequence (TR/TE/T: 7.0/3.2/800ms, voxel size: 1x1x1mm^3, scan time: 10min41s).

Statistical Tests: independent samples T-test, Mann-Whitney U test, partial correlation and multiple regression analysis

Results: Baseline characteristics were similar across the 3 groups, without significant difference in disease duration (p=0.354) and EDSS score (p=0.159) between MS and NMOSD subjects. Compared to HC, MS subjects had significantly smaller normalized whole brainstem (-5.2%, *p*=0.027), midbrain (-8.3%, *p*=0.0001) and pons volumes (-5.9%, *p*=0.048). while only the normalized medulla volume was significantly smaller in NMOSD subjects compared to HC (-8.5% vs HC, *p*=0.024). Normalized midbrain volume was significantly smaller in MS compared to NMOSD subjects (-5.0%, *p*=0.014) whereas normalized medulla volume was significantly smaller in NMOSD compared to MS subjects (-8.1%, *p*=0.032). Partial correlations and multiple regression analysis revealed that smaller normalized whole brainstem, pons and medulla oblongata volumes were associated with greater disability on Expanded Disability Status Scale (EDSS), Functional System Score (FSS)-brainstem and FSS-cerebellar in NMOSD subjects.

Data Conclusion: Differential patterns of brainstem atrophy were observed, with the midbrain being most severely affected followed by pons in MS, whereas only the medulla oblongata was affected in NMOSD.

Introduction

Multiple sclerosis (MS) is an important central nervous system inflammatory demyelinating disorder (CNS IDD) and the most common non-traumatic cause of neurological disabilities among young patients (1). It is an immune-mediated disorder with uncertain etiology and immunopathogenesis. The majority of MS patients have a course at onset typified by recurrent attacks of unilateral optic neuritis, short-segment myelitis, and involvement of cerebral hemispheres, cerebellum and brainstem separated by variable and unpredictable intervals, classified as relapsing-remitting multiple sclerosis (RRMS) (1). Histopathologically, MS is characterized by CNS demyelination, axonal injury and loss, lymphocytic inflammatory infiltrate and reactive astrocytes during acute neuroinflammation and chronic demyelination, partial remyelination, axonal and neuronal loss, astrogliosis, and brain and spinal cord atrophy in the chronic phase (2, 3).

Neuromyelitis optica spectrum disorders (NMOSD) belong to another group of CNS IDD. The majority of NMOSD patients have relapsing attacks of neuroinflammation, classically severe unilateral or bilateral optic neuritis, longitudinally extensive myelitis, and less commonly cerebral involvement affecting the dorsal medulla (area postrema), other brainstem regions, peri-ventricular and peri-ependymal regions of the diencephalon, as well as cerebral hemispheres which can mimic MS (4, 5). However, NMOSD is distinct from MS as the underlying pathophysiology, autoimmunity against the aquaporin-4 (AQP4) water channel, is present in the majority of NMOSD patients but absent in MS patients (6). This is evidenced by the detection of autoantibodies against

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aquaporin-4 (AQP4-IgG) in the serum of the majority of NMOSD (~75%), but not in MS patients (7). Inflammatory infiltrates composed of macrophages, neutrophils, eosinophils and variable amount of lymphocytes, demyelination, white and gray matter necrosis, vasculocentric deposition of immunoglobulins and complement activation products (C9neo) with hyalinized blood vessel walls, loss of AQP4 and astrocytes, cavitation, and cord atrophy are the typical pathologies observed in affected spinal cord of AQP4-IgG positive NMOSD patients (8, 9).

The prevalence of MS is much higher among Caucasians than Asians whereas NMOSDs are more prevalent among Asians including Hong Kong Chinese than Caucasians. Our recent single-center study revealed that MS and NMOSD account for 41.9% and 22.4% of CNS IDD in our patients respectively suggesting that MS and NMOSD are the two most common CNS IDD in Hong Kong Chinese (10). The distinction of MS from NMOSD is important as long-term disease-modifying therapies (DMTs) for MS such as interferon-β, fingolimod and natalizumab may be ineffective and even harmful for NMOSD (11-13). Early diagnosis of NMOSD confirmed with detection of AQP4-IgG is critically important as prompt initiation of immunosuppressive therapy is indicated to prevent relapses which are typically severe, disabling and even life-threatening (9, 14).

The brainstem is frequently affected, both clinically and radiologically, in both MS and NMOSD patients (15, 16). We hypothesized that the brainstem volume is significantly reduced in our MS and NMOSD patients, and the pattern of volume loss is different between MS and NMOSD as the medulla seems more commonly

and severely affected in NMOSD. , In this study, we aimed to measure the brainstem volume at midbrain, pons, and medulla level in a cohort of Chinese MS and NMOSD patients, and analyze whether the regional brainstem volumes correlate with neurological disability scores of the patients, which may help to understand the differences in patterns of neurological disability.

Methods

<u>Recruitment of subjects</u>

MR images of 17 RRMS patients, 13 NMOSD patients and 18 healthy control (HC) subjects were investigated. Diagnoses of MS and NMOSD were made according to the 2010 revisions to the McDonald Criteria of the International Panel on Diagnosis of MS (17) and 2006 revised diagnostic criteria for neuromyelitis optica (18) respectively. All the NMOSD subjects included also fulfill the recently revised diagnostic criteria (6). None of the HC subjects had a neurological or psychological condition, physical disability, or abnormality on brain MR images. Neurologic disabilities of the MS and NMOSD subjects were assessed by a neurologist (K.H.C.) at the time of MR scan, and graded according to Kurtzke Functional System Scores (FSS) and the Expanded Disability Status Scale (EDSS) . This study was approved by the local institutional review board. Informed consents were obtained from all participants.

<u>MRI acquisition</u>

Imaging was performed on a 3T scanner (Achieva, Philips Healthcare, Best, The Netherlands) with a body coil for excitation and an 8-channel head coil for reception. Anatomical information was obtained from whole brain T1-weighted (T1w) images acquired using three-dimensional magnetization-prepared rapid gradient-echo (MPRAGE) imaging sequence (TR/TE/T: 7.0/3.2/800ms, voxel size: 1x1x1mm^3, scan time: 10min41s). Additionally, T2-weighted (T2w) spin-echo images were acquired (44 axial slices with no inter-slice gap, slice thickness: 3mm, in-plane resolution: 1mmx1mm). Both T1w and T2w images were

examined to exclude pathology other than MS or NMOSD by a neuroradiologist (H.K.M.) with 24 years of experience in clinical work and research in neuroradiology.

Image processing and volumetric analysis

The T1w-MPRAGE images were first processed with the automatic structural imaging processing stream (19) in Freesurfer v5.3.0 (20). Cerebellar volumes were generated by the standard Freesurfer processing pipeline. Brainstem segmentation was then refined with the brainstem substructures tools (21). Visual checking and manual corrective voxel-editing of the brainstem segmentations were then performed by a neurologist (C.Y.L.) with experience in neuroimaging processing. Volumes of the brainstem structures were computed based on these corrected segmentations.

To account for differences in brain sizes attributable to variation in head size (22-24), all volumes were adjust for head size variation. Total intracranial volume (TICV) was estimated using SPM12 (http://www.fil.ion.ucl.ac.uk/spm /software/spm12) with its "Segment" and "Tissue Volumes" utilities (25). Hyperintense lesions on T2w images were manually outlined by a neurologist (C.Y.L.) with experience in neuroimaging processing and T2 lesion volume (T2LV) was then calculated using MIPAV (26); this was repeated by a neuroradiologist (H.C.C.) with experience in neuroimaging process which yielded an inter-observer Pearson correlation coefficient of 0.911 (p=0.000) with measurement by the neurologist, indicating high inter-observer consistency

Data and statistical analysis

All statistical analyses were performed with SPSS version 23. The volume estimates computed were normalized to the TICV using the residual method (23). The residual method predicts a normalized volume according to the correlation between the computed volume estimates and the TICV using the following equation:

$$\text{Volume}_{[\text{normalized}]} = \text{Volume}_{[\text{computed}]} + \alpha(\text{TICV}_{[\text{mean}]} - \text{TICV})$$

Both $\text{TICV}_{[\text{mean}]}$ and the coefficient α are calculated from the HC subjects; α is the gradient of the ordinary least-squares regression line between the computed volumes and TICV. A distinct α value was estimated for midbrain, pons, medulla oblongata, whole brainstem, cerebellum and whole brain correspondingly. Lesion volumes were also normalized according to individual head and brain size.

Different volumes of interest (VOIs) and other normally distributed continuous variables between groups were compared using independent samples T-test, while non-normally distributed or ordinal variables were compared using Mann-Whitney U test. Partial correlation was employed to assess associations between VOIs, different clinical scores and other continuous variables with correction for

age and sex. To find the predictive factors and models for EDSS and FSS, variables (VOIs or other clinical parameters) with p-value <0.1 in univariate analysis were further analyzed using multiple regression with backward selection method and adjustment of age and sex. VOIs with high collinearity e.g. brainstem volume and pons volume were tested in separate regression models. A p-value (2 tailed) less than 0.05 was considered statistically significant. The significant predictive models were compared based on the adjusted squared multiple correlation coefficients (R^2) and those with highest R^2 were presented as the final models.

Results

Baseline characteristics

The demographic, clinical and MR imaging characteristics of the subjects are summarized in Table 1. All the VOIs showed a significant linear relationship with TICV in the control group and were normalized accordingly. Comparing MS and NMOSD subjects, there was no significant difference in disease duration, history of use of immunotherapies, EDSS, FSS-cerebellar or FSS-brainstem.

Brain and brainstem atrophy

The comparative patterns of brainstem atrophy among the 3 groups were summarized in Table 2.

1. MS versus controls

The normalized volumes of whole brainstem, midbrain and pons were significantly reduced in MS subjects compared to HC. The differences were 1.27mL (5.2%, *p*=0.027), 0.50ml (8.3%, *p*=0.000) and 0.83mL (5.9%, *p*=0.048) respectively. The normalized brain volume (NBV), normalized supratentorial brain volume (NStBV) and WM volume were also significantly smaller in MS subjects compared to HC, with a mean difference of 85.35mL (7.6%, *p*=0.005), 85.97mL (8.8%, *p*=0.004) and 56.93mL (13.4%, *p*<0.0001) respectively. There was no significant difference in age, gender, normalized volume of medulla oblongata, cerebellum or TICV.

2. NMOSD versus controls

The normalized volume of medulla oblongata was reduced in NMOSD group compared to HC with a mean difference of 0.37mL (8.5%, *p*=0.024). Otherwise there was no statistically significant difference in whole brain or other VOIs. There was also no significant difference in age, gender or TICV.

3. MS versus NMOSD

MS subjects had smaller midbrain volumes with a mean difference of 0.29mL (5.0%, *p*=0.014) and NMO subjects had smaller medulla oblongata volumes with a mean difference of 0.35mL (8.1%, *p*=0.032). Figure 2 shows a comparison of MR images of an RRMS subject and an NMOSD subject. The NBV, NStBV and WM volume were reduced in MS subjects with a mean difference compared to

NMOSD of 80.06mL (7.2%, *p*=0.009), 78.39mL (8.1%, *p*=0.009) and 45.56mL (11.0%, *p*=0.004). Total, cerebral and infratentorial T2LV were significantly larger in MS. The volumes of pons, whole brainstem, cerebellum and GM showed no significant difference. There was also no significant difference in age, gender or TICV. Figure 1 showed T1w images with brainstem segmentation of an MS subject and an NMO subject.

MS and NMOSD subgroup analysis

In MS subjects (Table 3), the normalized brainstem volume (NBsV) showed a moderate to strong positive correlation with NStBV (*r*=0.628, *p*=0.027).

No statistical significant correlation was found of EDSS with whole brain volume or T2LV (total, cerebral or infratentorial). On the other hand, longer disease duration was associated with higher EDSS (*r*=0.567, *p*=0.028), while smaller NBsV (*r*=-0.461, *p*=0.084), smaller normalized midbrain volume (*r*=-0.444, *p*=0.097), and the use of DMTs (median EDSS 2.75 vs 1.0, *p*=0.082) showed a trend towards higher EDSS. Multiple regression analysis using age, sex, disease duration, use of DMTs and NBsV or midbrain volume was performed attempting to predict the EDSS, but a statistically significant model could not be found. For FSS-cerebellar in MS subjects, the only variable with significant correlation was normalized whole brain volume (*r*=-0.603, *p*=0.017) and multiple regression analysis revealed a significant predictive model (adjusted R^2=0.480, *p*=0.032) (Table 4). No significant association was found between FSS-brainstem and other tested variables.

Similar partial correlation analyses were performed in NMOSD subjects (Table 5). Larger infratentorial T2LV was associated with smaller NBsV and normalized pons volume. Disease duration or AQP4 IgG positivity has no significant association with EDSS or FSS. Multiple regression analysis was performed and the significant predictive models for the clinical disability scores were summarized in Table 6. NBsV significantly predicted all three clinical scores, while normalized medulla oblongata volume and normalized pons volume predicted FSS-brainstem and FSS-cerebellar.

Discussion

Brainstem involvement is a major cause of significant disability in MS and NMOSD. Our previous study of Chinese RRMS and NMOSD patients revealed that the brainstem is commonly affected in both RRMS (two-thirds) and NMOSD (44%) patients (15, 16). It is noteworthy that the frequency of brainstem T2W hyperintense lesions detected in the current study is much lower than our previous study, because all of these patients have been aggressively treated with immunotherapies and some lesions had resolved. Although more than half of our patients had cerebral lesions detected on MRI, the lesion volumes were small. It is increasingly recognized that brainstem lesions on MRI are common in NMOSD patients (5, 27). One study from China suggested medulla oblongata lesions as a predictor of more severe neurologic deficits and worse prognosis in NMOSD (28). Other studies reported whole brain, white matter, focal cortical and deep gray matter atrophy in NMOSD patients compared to HC (29-31), but no consistent pattern was found.

Our study showed that medulla oblongata volume was significantly reduced in NMOSD subjects compared to both HC and RRMS subjects , even though our NMOSD patients had relatively short disease duration. Importantly, smaller medulla oblongata volume was associated with more severe brainstem and cerebellar symptoms. There was no significant atrophy compared to HC in gray or white matter, supratentorial or other brainstem regions. Therefore, our findings suggested the importance of medulla volume as an independent marker of disease severity and disability in NMOSD patients, even when brainstem lesions are absent on conventional MRI (after treatment).

Studies on brainstem atrophy in MS are limited. Previous studies mainly examined the whole brainstem volume, and consistently reported reduced brainstem volume in MS patients compared to HC (32, 33). One study that included both RRMS and SPMS patients with a median disease duration of 7 years reported a reduction of brainstem volume up to 20.6% and brainstem atrophy was associated with higher EDSS score but not supratentorial volume loss (32). A more recent study showed a 5.2% NBsV reduction in RRMS compared to HC, which was similar to our finding, despite a longer mean disease duration of about 19 years (33). The authors reported no significant association between NBsV and different clinical measures of motor dysfunction including EDSS in the regression models (33).

Our study extends those findings by identifying that midbrain volume is reduced most (8.3%) followed by pons volume (5.9%) in RRMS patients relative to HC.

This is consistent with a recent study of RRMS patients with median disease duration of 9 years using cross-sectional area (CSA) as a measurement reflective of regional brainstem size, which reported a similar descending trend of atrophy from midbrain to medulla (34). Statistically significant medulla atrophy was not detected in MS patients compared to HC in our study. This may be due to our small sample size and relatively short disease duration of studied subjects. Liptak et al. reported that medulla oblongata volume is associated with disability and spinal cord damage in a cohort of MS patients (71% RRMS) with a mean disease duration of 7.1 years (35), supporting medulla atrophy is an important pathology in MS. Brain atrophy and lesion load on MRI are important biomarkers in MS (36, 37) and are shown to associate with long-term disabilities . Our findings showed that whole brain and regional (supratentorial, whole brainstem, midbrain and pons) atrophy were evident in MS subjects, and normalized brainstem volume was positively correlated with supratentorial brain volume. This, together with the descending trend of atrophy from midbrain to medulla, may suggest that brainstem atrophy in MS results from both axonal loss due to Wallerian degeneration of long fiber tracts secondary to cerebral hemispheric lesions and direct axonal injury from brainstem inflammation. No statistical significant association was noted between EDSS and brain volume or T2LV in our study, which could be again related to the small sample size.

The pathogenetic basis of the observed medulla atrophy in NMOSD patients are uncertain. The area postrema in the dorsal medulla is of particular interest in

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NMOSD as the lack of intact blood brain barrier in the area postrema allows access of AQP4 IgG in peripheral blood to the CNS in this region. This is consistent with the clinical observation of area postrema syndrome characterized by refractory nausea, hiccups and/or vomiting in NMOSD patients. Among 15 NMO patients studied, Mayo investigators found unilateral or bilateral lesions in the medullary floor of the fourth ventricle and area postrema of 6 patients (40%) (27). AQP4 loss was prominent in lesions of all 6 patients and the AQP4 loss areas showed tissue rarefaction or vacuolation, blood vessel wall thickening, marked parenchymal and perivascular infiltration by T cells (CD3+ and CD8+), B cells and plasma cells, prominent activation of parenchymal microglia and perivascular macrophages, and prominent astroglial reaction. Complement deposition was observed in 3 patients but there was no astrocyte loss or acute neuronal pathology in all 6 patients (27). We believe that axonal loss complicating repeated attacks of neuroinflammation due to reactive astrocytosis triggered upon AQP4-IgG binding to astrocytic membrane AQP4 may be the pathophysiological basis of medulla atrophy in NMOSD. The common involvement of the medulla oblongata in Asian NMOSD subjects may possibly be due to a high permeability of the blood-brain barrier in the medulla of Asians and hence increased accessibility to AQP4-IgG, or a high level of AQP4 expression in the medulla of Asians.

While the symptoms and clinical presentation of relapses can be similar in MS and NMOSD, their disease course, treatment strategies and prognosis are

markedly different. Along with detection of AQP4-IgG, other clinical and paraclinical features including MRI can be used to differentiate the two (38). Apart from conventional MRI, other advanced MRI techniques have been employed to study the differences of the two diseases (39). However, these modalities remain largely for research purpose. Our study has shown a differential pattern of atrophy in MS and NMOSD subjects with similar EDSS levels, specifically in the brainstem. Furthermore, our findings were consistent with other studies reporting that MS subjects have more prominent whole brain, supratentorial and white matter atrophy, and higher T2LV (both supratentorial and infratentorial) compared to NMOSD subjects. These regional volume changes reflect different underlying pathophysiological mechanisms and pathologies.

Our study has several limitations. First, our sample size is small. Some of the findings and regression models cannot be ascertained with statistical significance. Second, our imaging protocol did not include fluid attenuated inversion recovery (FLAIR) or proton density (PD) weighted sequence, and therefore the T2LV which was manually delineated could subject to intra- and inter-observer differences. We suggest in future volumetric studies FLAIR sequence should be performed for reproducible measurement of T2LV. Third, our study design and imaging protocol did not include measurement of mean upper cervical cord area (MUCCA), which has been reported to be an important predictor of motor dysfunction and disability in MS subjects (33, 40). We also suggest the addition of other MS-specific functional score such as the Multiple

Sclerosis Functional Composite which may measure the degree of disability better.

In conclusion, a different pattern of brainstem atrophy was found between RRMS and NMOSD subjects with similar disease duration, functional and disability status. We postulate that these patterns reflect different pathophysiological mechanisms and pathologies underlying the two diseases. Although volumetric measurement of brainstem structures is mainly used in research at present, its value as a biomarker for diagnosis and disease progression in MS and NMOSD deserve clarification by longitudinal studies.

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Table 1. Demographic, clinical and MRI characteristics of the subjects

	MS	NMOSD	HC
Age at MRI (mean ± SD), years	41.0 ± 9.4	44.2 ± 13.4	43.4 ± 12.3
Gender (% female)	12/17 (70.6%)	11/13 (84.6%)	9/18 (50%)
Disease duration (median), years	6 (IQR 2.5-11)	4 (IQR 2.5-6.5)	–
EDSS (median)	2 (IQR 1.5-4)	3.5 (IQR 2-4)	–
FSS-brainstem			–
Median (IQR)	0 (0-1.5)	0 (0-2)	
Mean ± SD	0.71 ± 0.85	0.54 ± 0.88	
FSS-cerebellar (median)			–
Median (IQR)	1 (0-2)	0 (0-2)	
Mean ± SD	1.29 ± 1.16	0.85 ± 1.21	
CSF oligoclonal bands	11/13 [a] (84.6%) ***	3/13 (23.1%) ***	–
AQP4-IgG positivity	0/17 ***	10/13 (76.9%)***	–
History of use of immunotherapies	12/17 (70.6%)	13/13 (100%)	–
TICV (mean), L	1.30 ± 0.14	1.27 ± 0.14	1.37 ± 0.15
NBV (mean), L	1.03 ± 0.10 **, ††	1.11 ± 0.04 **	1.12 ± 0.04 ††
- GM	0.664 ± 0.065	0.699 ± 0.049	0.693 ± 0.032
- WM	0.368 ± 0.048 **, †††	0.414 ± 0.023 **	0.425 ± 0.030 †††
NStBV (mean), L	0.89 ± 0.10 **, ††	0.97 ± 0.04 **	0.98 ± 0.04 ††
NCbV (mean), L	0.119 ± 0.013	0.120 ± 0.015	0.117 ± 0.006
NBsV (mean), mL	23.37 ± 1.38 †	24.00 ± 2.23	24.64 ± 1.82 †
- Midbrain	5.50 ± 0.27 *, †††	5.79 ± 0.35 *	6.00 ± 0.29 †††
- Pons	13.22 ± 1.01 †	13.79 ± 1.56	14.05 ± 1.35 †
- Medulla oblongata	4.33 ± 0.27 *	3.98 ± 0.56 *, ɸ	4.35 ± 0.30 ɸ
Normalized total T2LV (mean), mL	14.15 ± 11.63 ***	0.38 ± 0.53 [b] ***	–
- Cerebral	13.76 ± 11.41 ***	0.34 ± 0.54 [b] ***	–
- Infratentorial	0.40 ± 0.61 **	– [b] **	–

Abbreviations: MS= multiple sclerosis; NMOSD= neuromyelitis optica spectrum disorders; HC= healthy controls; SD= standard deviation; IQR= interquartile range; EDSS= expanded disability status scale; FSS= functional system score; CSF= cerebrospinal fluid; AQP4-IgG= aquaporin-4 autoantibody; TICV= total intracranial volume; NBV= normalized brain volume; GM= gray matter; WM= white matter; NStBV= normalized supratentorial brain volume; NCbV= normalized cerebellar volume; NBsV= normalized brainstem volume; T2LV= T2 lesion volume

[a.] 4 of the patients have no CSF oligoclonal bands results available.

[b.] 7 out of 13 of the NMO subjects had supratentorial lesions (range 0.07-1.4ml) and only 1 out of 13 had infratentorial lesions of 0.39ml.

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ for comparison between MS and NMOSD subjects.

† $p<0.05$, †† $p<0.01$, ††† $p<0.001$ for comparison between MS and control subjects.

ɸ $p<0.05$ for comparison between NMOSD and control subjects.

Table 2 Difference of mean brain and brainstem volumes among groups

	MS – HC		NMOSD – HC		MS – NMOSD	
NBV, mL	-85.35 (7.6%)	**	-5.29 (0.5%)		-80.06 (7.2%)	**
GM	-28.37 (4.1%)		6.15 (0.9%)		- 34.52 (4.9%)	
WM	-56.93 (13.4%)	***	-11.37 (2.7%)		-45.56 (11%)	**
NStBV, mL	-85.97 (8.8%)	**	-7.58 (0.8%)		-78.39 (8.1%)	**
NBsV, mL	-1.27 (5.2%)	*	-0.63 (2.7%)		-0.64 (2.5%)	
Midbrain	-0.50 (8.3%)	***	-0.21 (3.5%)		-0.29 (5.0%)	*
Pons	-0.83 (5.9%)	*	-0.25 (1.8%)		-0.58 (4.2%)	
Medulla	-0.02 (0.4%)		-0.37 (8.5%)	*	+0.35 (8.1%)	*

Abbreviations: MS= multiple sclerosis; NMOSD= neuromyelitis optica spectrum disorders; HC= healthy controls; NBV= normalized brain volume; GM= gray matter; WM= white matter; NStBV= normalized supratentorial brain volume; NBsV= normalized brainstem volume
* p <0.05; ** p <0.01; *** p <0.001

Table 3. Partial correlations between normalized regional volumes and other disease parameters after correction for age and sex in MS subjects

	WB	Brainstem	Midbrain	Pons	Medulla	Cb
NStBV	–	0.628*	0.448^	0.602*	NS	NS
Normalized total T2LV	-0.447^	NS	NS	-0.464^	NS	NS
Cerebral	-0.460^	NS	NS	-0.480^	NS	NS
Infratentorial	NS	NS	NS	NS	NS	NS
Disease duration	NS	NS	NS	NS	-0.487^	NS
EDSS	NS	-0.461^	-0.444^	NS	NS	NS
FSS-brainstem	NS	NS	NS	NS	NS	NS
FSS-cerebellar	-0.603 *	NS	NS	NS	NS	NS

Abbreviations: MS= multiple sclerosis; WB= whole brain; Cb= cerebellum; NStBV= normalized supratentorial brain volume; T2LV= T2 lesion volume; EDSS= expanded disability status scale; FSS= functional system score; NS= not significant

^ $p<0.1$, * $p<0.05$

Table 4. Multiple regression models for prediction of EDSS and FSS in MS subjects

	R^2	Adjusted R^2	p-value
FSS-cerebellar			
Age, sex, NBV	0.693	0.480	0.032*
EDSS; FSS-brainstem			
(No significant model was found)			

Abbreviations: MS= multiple sclerosis; EDSS= expanded disability status scale; FSS= functional system score; R^2=squared multiple correlation coefficient of the model; NBV= normalized brain volume

* p<0.05

Table 5. Partial correlations between normalized regional volumes and other disease parameters after correction for age and sex in NMOSD subjects

	WB	Brainstem	Midbrain	Pons	Medulla	Cb
NStBV	–	NS	NS	NS	NS	NS
Normalized total T2LV	NS	NS	NS	NS	NS	NS
Cerebral	NS	NS	NS	NS	NS	NS
Infratentorial	NS	-0.605*	NS	-0.625*	NS	NS
Disease duration	NS	NS	NS	NS		-0.573^
EDSS	NS	-0.571^	NS	-0.541^	-0.534^	NS
FSS-brainstem	NS	-0.829**	-0.778**	-0.755**	-0.757 **	-0.615*
FSS-cerebellar	NS	-0.819**	-0.681*	-0.779**	-0.739 **	-0.766**

Abbreviations: NMOSD= neuromyelitis optica spectrum disorder; WB= whole brain; Cb= cerebellum; NStBV= normalized supratentorial brain volume; T2LV= T2 lesion volume; EDSS= expanded disability status scale; FSS= functional system score; NS= not significant
^ $p<0.1$, * $p<0.05$, ** $p<0.01$

Table 6. Multiple regression models for prediction of EDSS and FSS in NMOSD subjects

	R^2	Adjusted R^2	*p*-value
EDSS			
Age, sex, NBsV	0.574	0.432	0.045 *
FSS-brainstem			
Model 1: Age, sex, NBsV	0.710	0.614	0.009 **
Model 2: Age, sex, NMbV, NPonsV, NMedV	0.786	0.633	0.027 *
FSS-cerebellar			
Model 1: Age, sex, NBsV, NStV	0.893	0.840	0.001 **
Model 2: Age, sex, NPonsV, NMedV, NStV	0.907	0.841	0.002 **

Abbreviations: NMOSD= neuromyelitis optica spectrum disorders; EDSS= expanded disability status scale; FSS= functional system score; R^2=squared multiple correlation coefficient of the model; NBsV= normalized brainstem volume; NMbV= normalized midbrain volume; NPonsV= normalized pons volume; NMedV= normalized medulla volume; NStV= normalized supratentorial brain volume

* *p*<0.05, ** *p*<0.01
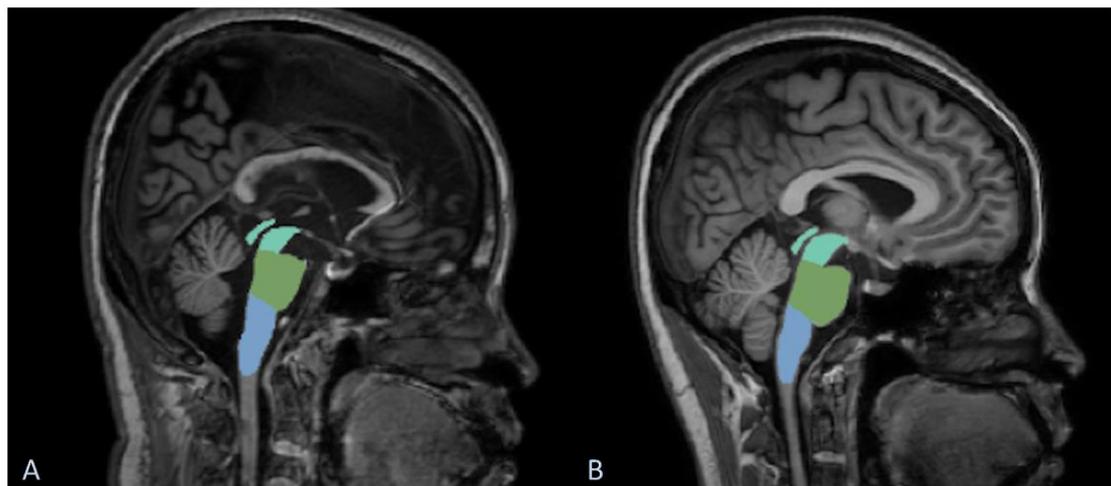
Figure Legends

Figure 1. Typical sagittal T1w MR images of an MS subject (A) with midbrain atrophy, and an NMOSD subject (B) with medulla oblongata atrophy, processed with Freesurfer and corrected manually. Color code: light green= midbrain, green= pons, pale blue= medulla oblongata

	MS subject	**NMOSD Subject**
Age and gender	32 years old female	48 years old female
Disease duration	2 years	3 years
EDSS	2.0	2.0
FSS (brainstem, cerebellar)	1, 3	0, 0
Normalized VOIs (mL)		
Whole brainstem	21.14	25.78
Midbrain	**5.09**	**6.16**
Pons	11.42	15.36
Medulla oblongata	**4.40**	**3.77**