Different patterns of longitudinal brain and spinal cord changes and its association with disability progression in NMO and MS

Yaou Liu[1,2,3,4]* MD, PhD, Yunyun Duan[1] MD, Jing Huang[1] MD, Zhuoqiong Ren[1] MD, Zheng Liu[5]MD, Huiqing Dong[5] MD, Florian Weiler[6] PhD, Horst K. Hahn[6] PhD, Fu-Dong Shi[4] MD, PhD, Helmut Butzkueven[7] MD,PhD, Frederik Barkhof[3,8] MD,PhD , Kuncheng Li[1,2]* MD,PhD

[1]Department of Radiology, Xuanwu Hospital, Capital Medical University, Beijing 100053, P. R. China

[2]Beijing Key Lab of MRI and Brain Informatics ， Beijing 100053, P. R. China

[3]Department of Radiology and Nuclear Medicine, Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam 1007 MB, The Netherlands

[4]Department of Neurology and Tianjin Neurological Institute, Tianjin Medical University General Hospital, Tianjin, 300052, P. R. China

[5]Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing 100053, P. R. China

[6]Fraunhofer MEVIS, Institute for Medical Image Computing, Universitätsallee 29, Bremen 28359, Germany

[7]Department of Medicine, University of Melbourne, Parkville 3010, Australia

[8] Institutes of Neurology and Healthcare Engineering, UCL, London, UK

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***Correspondence to:** Yaou Liu, MD, PhD, Department of Radiology, Xuanwu Hospital, Capital Medical University, Beijing 100053, China. Tel: 86 13810345782; Fax: 86 10 83198376. E-mail: asiaeurope80@gmail.com or Kuncheng Li, MD, Department of Radiology, Xuanwu Hospital, Capital Medical University, Beijing 100053, China. Tel: 86 13911099059; Fax: 86 10 83198376. E-mail: kunchengli55@gmail.com

Author contributions:

1 guarantor of integrity of the entire study: Yaou Liu; Kuncheng Li

2 study concepts: Yaou Liu; Frederik Barkhof

3 study design: Yaou Liu; Yunyun Duan; Fu-Dong Shi; Helmut Butzkueven

4 definition of intellectual content: Yaou Liu; Kuncheng Li

5 literature research: Yaou Liu; Yunyun Duan;

6 clinical studies: Huiqing Dong; Zheng Liu; Yunyun Duan

7 experimental studies: Jing Ye; Yaou Liu; Yunyun Duan

8 data acquisition: Yunyun Duan; Yaou Liu; Jing Huang; Zhuoqiong Ren

9 data analysis: Yaou Liu; Florian Weiler; Horst K. Hahn;

10 statistical analysis: Yaou Liu; Yunyun Duan

11 manuscript preparation: Yaou Liu; Helmut Butzkueven

12 manuscript editing: Yaou Liu; Helmut Butzkueven; Frederik Barkhof

13 manuscript review: Fu-Dong Shi; Helmut Butzkueven; Frederik Barkhof

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Abstract

Objective

To investigate the longitudinal spinal cord and brain changes in neuromyelitis optica (NMO) and multiple sclerosis (MS), and its association with disability progression.

Patients and Methods

We recruited 28 NMO, 22 MS and 20 healthy controls (HC), who underwent both spinal cord and brain MRI at baseline. 25 NMO and 20 MS completed 1-year follow up. Baseline spinal cord and brain lesion loads, mean upper cervical cord area (MUCCA), brain, and thalamus volume and their changes during a 1-year follow-up were measured and compared between groups. All the measurements were also compared between progressive and non-progressive groups in NMO and MS.

Results

MUCCA decreased significantly during the 1-year follow-up in NMO not in MS. Percentage brain volume changes (PBVC) and thalamus volume changes in MS were significantly higher than NMO.MUCCA changes were significantly different between progressive and non-progressive groups in NMO, while baseline brain lesion volume and PBVC were associated with disability progression in MS. MUCCA changes during 1-year follow-up showed association with clinical disability in NMO.

Conclusion

Spinal cord atrophy changes were associated with disability progression in NMO, while baseline brain lesion load and whole brain atrophy changes were related to disability progression in MS.

Key words: multiple sclerosis; neuromyelitis optica; mean upper cervical cord area; brain; MRI

Key point 1: Spinal cord atrophy progression was observed in NMO.

Key point 2: Spinal cord atrophy changes were associated with disability progression in NMO.

Key point 3 Brain lesion and atrophy were related to disability progression in MS.

Abbreviations: HC: healthy control; MUCCA: mean upper cervical cord area; NBV: normalized brain volume; NMO: neuromyelitis optica; NTV: normalized thalamic volume; PBVC: percentage brain volume changes; PVVC: percentage ventricular volume change; RRMS: relapsing remitting MS

Introduction

Neuromyelitis optica (NMO) is an autoimmune inflammatory disease of the central nervous system. This syndrome is characterized clinically by recurrent attacks of optic neuritis and myelitis [1-3] and now considered as a primary astrocytopathy [4; 5]caused by pathogenic antibodies to aquaporin-4 (AQP-4) water channels that are concentrated on the foot processes of astrocytes[6]. Clinically, accurate diagnosis of MS and NMO is critical especially in the early stage, since the prognosis and treatments of these disorders differ[1], and some MS treatments (including interferon-β and Fingolimod) may exacerbate NMO[7-9].

The major MRI feature of NMO is spinal cord pathology, particularly longitudinally extensive lesions and atrophy[1; 3]. Subtle, regional brain atrophy has also been reported in NMO [10-12]. A recent cross-sectional study showed association between spinal cord but not brain atrophy and clinical disability in NMO[13]. However, longitudinal changes of brain and spinal cord atrophy, and their correlations with clinical worsening in NMO are unknown.

In contrast to NMO, MRI has been used extensively to measure longitudinal changes in global and regional brain volumes in multiple sclerosis (MS)[14]. Whole brain and thalamic atrophy, as well as spinal cord atrophy progression have been found to correlate with clinical worsening[15-17]. We therefore investigated these MRI biomarkers as predictors of disability worsening in NMO patients.

This study evaluated the longitudinal changes of spinal cord MRI measurements including cord lesions and cord atrophy, using the mean upper cervical cord area (MUCCA) [18], together with brain MRI measurements including white matter lesions, whole brain and thalamus volume in AQP4 positive NMO patients over 1 year and correlated these changes with clinical disability changes. A cohort of MS patients served as a comparator.

Materials and Methods

Standard protocol approvals, registrations, and patient consents

The institutional review board of *BLINDED* approved the study and written informed consent was obtained from each participant prior to participation.

Participants

Twenty-eight relapsing NMO patients were recruited from *BLINDED*. The diagnosis of NMO was determined according to the revised diagnostic criteria[2]. All patients had optic neuritis and myelitis, and met 2 of the 3 supporting criteria including AQP4 antibody positivity, T2 weighted brain MRI non-diagnostic for MS, and a spinal cord lesion involving at least three vertebral segments. For comparison with the NMO patients, we recruited 22 relapsing-remitting MS (RRMS) patients fulfilling the McDonald criteria [19], matched for age, sex, disease duration and EDSS score to the NMO group. To exclude the confounding effect of edema or medication on the spinal cord measurements, all of the participating patients had been relapse-free and without treatment with disease-modifying medications or steroid within 4 weeks before the MRI scans being obtained. Twenty-five NMO patients and 20 MS patients completed a 1-year follow-up. During the 1-year follow-up, three NMO patients and five MS patients had at least one relapses, the maximal number of relapses in a single patient was two. The main demographic and clinical characteristics of the patients were shown in Table 1.

We also recruited 20sex- and age-matched healthy controls (HC) with no current or previous history of neurological dysfunction and no visible abnormalities on T2 weighted MRI (Table 1).

To exclude the possible diagnostic confounders of AQP4 negative NMO patients, all included patients with NMO were anti- AQP4 antibody positive (using an indirect immunofluorescence method[20]).At follow-up, patients were classified as clinically worse if they had an EDSS score increase $\geq$1.0, when baseline EDSS was <6.0 or an EDSS score increase$\geq$0.5, when baseline EDSS was $\geq$6.0.[21]The median follow-up length was 1.2 years (range 0.9 –3.0 years).

MRI Acquisition

Imaging was performed on a 1.5 T scanner (Sonata; Siemens Medical Systems, Erlangen, Germany) at *BLINDED*. MRI scans of the brain and cervical cord from all patients were obtained at study entry and follow-up, using the same MRI machine, which did not undergo any upgrade during the study period and was under a regular program of maintenance.
At follow-up, patients were repositioned following published guidelines[22]. During each session, the following sequences were acquired:

Brain MRI:

Axial slices were positioned parallel to line that joins the AC-PC line, with in plane resolution of $1\times1mm^2$, 30 slices, 4 mm slice thickness (10% gap) and included T2-weighted turbo spin-echo (repetition time[TR]=5500ms, echo time[TE]=94ms, number of signals acquired=3, echo train length=11) and FLAIR (TR/TE=8500/150, inversion time[TI]=2200ms, number of signals acquired=3, echo train length=8). Sagittal 3D T1-weighted magnetization-prepared rapid acquisition gradient echo (MPRAGE) (TR/TE = 1970/3.9ms, TI = 1100ms, flip angle (FA) = 15°,

slice thickness= 1.7mm, with in plane resolution of 1×1mm^2) images were also obtained to measure brain volume and MUCCA measurement.

Spinal cord MRI:

Cervical and thoracic spinal cord imaging included 3-mm-thick sagittal sections and 4-mm-thick axial sections using T2-weighted and proton density (PD) sequences (in-plane resolution 1 mm^2).

Image Analysis

Marking and measurement of hyperintense brain lesions (T2LV) on baseline and one year follow up T2-weighted was performed by an experienced neuroradiologist (*BLINDED*) using MRIcro software (http://www.mccauslandcenter.sc.edu/mricro/mricro/mricro.html), using FLAIR images for reference.

As the NMO patients had no lesions or only non-specific white matter abnormalities, T2LV was not measured in NMO patients. Using T2-weighted spinal cord images, the number and total length of spinal cord lesions were marked and counted in all patients. The total lesion length in each NMO and MS patient was calculated by adding the cranio-caudal length of each spinal cord lesion on sagittal MR images.

Baseline and longitudinal whole brain, thalamus volume measures

Baseline normalized whole-brain (NBV) were computed using the 3D T1-weighted images and SIENAX (part of FSL 5.0.4, http://www.fmrib.ox.ac.uk/fsl)[23]. Thalamus volumes were quantified by FMRIB Integrated Registration and Segmentation Tool (FIRST) [24](also part of FSL). The left and right thalamic volumes were averaged and normalized by the V-scaling from SIENAX.

Percentage whole brain change (PBVC) was analyzed using the semi-automated software SIENA[25; 26]. Using an adaptation of SIENA, we calculated central atrophy rate as percentage ventricular volume change by VIENA[26], where only brain/non-brain edge points on the ventricular edges were selected and their mean edge displacement was calculated. The percentage change of thalamus volumes between two time points was numerically determined.

Baseline and longitudinal MUCCA measurements

For each time point, MUCCA was measured by the same neuroradiologist (*BLINDED*), blinded to the date of the MRI, using the sagittal 3D T1-weighted brain images (with upper cervical cord coverage). MUCCA was measured using a semi-automated segmentation method over a predefined 30mm section length (NeuroQLab; Fraunhofer MEVIS, Bremen, Germany). The workflow and the reliability of the software were described previously[18; 27; 28]. Overview of the image-processing pipeline of MUCCA measurement was illustrated in figure 1. The percentage change of MUCCA between two time points was calculated using two cross-sectional scans.

Statistical Analysis

Analyses were performed using SPSS software (Version 18; SPSS, Chicago, Ill). Kolmogorov-Smirnov tests were performed together with visual inspection of histograms to assess normality of the variables, and Levene's test was performed to test homogeneity of the variables. For normally distributed variables with homogeneity of variance, one-way analysis of covariance (Bonferroni test used for post-hoc comparisons) or a two-sample t test was performed to examine group effects. For variables that were not normally distributed, the Wilcoxon rank sum test or Kruskal-Wallis test was used. A binary logistic regression model was used to investigate the role of clinical and MRI measurements as independent predictors of EDSS worsening at follow-up.

Results

Baseline demographics, clinical and MRI characteristics

As shown in Table 1, no significant differences were observed in age and sex among the 3 groups (NMO, MS and HC). EDSS, number of relapses, and disease duration showed no significant differences between MS and NMO groups.

The median cord lesion number in NMO patients was 1 (range 1-3), and the median total lesion length was 4vertebral segments (range 3-10). In MS patients, spinal cord lesions were present in 19 patients (19/20, 95%), with a median of 2 lesions per patient (range 0-6), and a median total lesion length of 1 vertebral segment (range 0-4). The median brain T2LV in MS patients was 98ml.

There were no significant differences between baseline NBV between the three study groups, however normalized thalamic volume (NTV) was significantly lower in MS patients when compared to controls ($p=0.04$).No difference of NTV was observed between MS and NMO($p>0.1$). NMO patients showed a lower MUCCA than HC ($p<0.01$) and a trend for lower MUCCA compared to MS ($p=0.05$). No significant difference was identified between MS and HC in MUCCA ($p=0.28$).

Longitudinal brain and spinal cord lesion load changes

Both in MS and NMO, no significant changes were observed in spinal cord or brain lesion loads between baseline and follow-up.

Longitudinal brain volume and MUCCA changes

Within the NMO cohort, the baseline (0.65±0.10 cm^2) MUCCA decreased slightly atone year follow-up(0.64±0.08 cm^2, p=0.003), while NBV and NTV did not change significantly (p>0.05).

In MS, only NTV significantly decreased during follow-up (baseline:10.28±1.38 ml; follow-up9.98±1.42 ml; p<0.001), while the whole brain volume and MUCCA measures did not change significantly (p>0.1).

The PBVC and NTV changes were significantly greater in MS compared to NMO (p=0.03, p=0.02 respectively), while no significant differences in MUCCA changes were observed between NMO and MS.(Table2).

Association between brain lesions, volume, MUCCA and clinical measurements

No significant correlations between longitudinal changes of brain measurement and MUCCA were observed in either NMO or MS. We haven't found We found a significant correlation between PVVC (percentage ventricular volume change) and disease duration in MS (r=-0.478, p=0.033). No correlations were identified between other clinical measurements and baseline values or changes of brain volume or MUCCA in both NMO and MS. (all p>0.1).

Determinants of disability progression

Within the NMO cohort, the only significant difference between progression and non-progression groups was the magnitude of change in MUCCA. In MS, PBVC and baseline lesion volume were significantly different between progressive and non-progressive groups (Table 3 and Figure 2). In a multivariable logistic regression with disease progression over 1 year as a binary dependent variable, percentage changes of MUCCA in NMO (Odd ratio 0.671 95%CI:0.458-0.982, p=0.04) remained significant. After adding the number of relapse as a covariant in the analysis, the results of differences between MS and NMO, and between progression and non-progression groups were unchanged.

Discussion

In this study, we demonstrated significant longitudinal changes of MUCCA during one year of follow up in NMO, while significant thalamic volume changes were identified in MS.

In NMO, spinal cord atrophy progression was associated with clinical worsening, while baseline WM lesion volume and whole brain atrophy changes assessed by PBVC were associated with disability progression in MS. In a binary logistic multivariable model predicting EDSS progression over the course of 1 year, MUCCA remained a significant variable in NMO, whereas no imaging variables remained significant in MS.

At baseline, atrophy patterns of brain and spinal cord differed between NMO and MS patients. NMO patients showed spinal cord atrophy without brain atrophy, while MS patients exhibited whole brain and thalamus atrophy, but no significant spinal cord atrophy compared to HC, consistent with previous studies [13; 28].

For the longitudinal results, no significant whole brain or thalamic atrophy changes were identified in NMO, implying that rate of brain neurodegeneration is very limited in NMO during short-term follow-up, in line with a previous longitudinal NMO study [29]. However, this finding need to be confirmed in a larger sample with longer follow-up, since pathology studies[30] reveal pathological processes consisting not only of inflammatory lesions characterized by loss of astrocytic AQP4 immunoreactivity, but also cortical degeneration in NMO brains. Significant MUCCA changes were observed during 1 year follow-up, implying spinal cord atrophy

measurement is more sensitive, and probably also much more consistent, than brain atrophy measurement in NMO. The likely pathological mechanisms of upper cervical spinal cord volume loss is axonal degeneration, demyelination, necrosis and cavitation[1].In contrast, MS patients showed progressive thalamic atrophy, but not whole brain atrophy or spinal cord atrophy between baseline and follow-up, suggesting thalamus volume as a more sensitive measurement than whole brain volume or spinal cord volume to detect neurodegeneration in a relatively small sample of MS patients. This finding is consistent with previous studies showing thalamic atrophy as a key marker of disease progression[31].

The role of spinal cord atrophy in predicting the accumulation of disability in NMO patients has been already suggested by previous cross-sectional study[13]. The current longitudinal study extends these findings in an independent dataset, with the only difference between disability progression and non-progressive group in NMO being MUCCA changes. In the binary logistic regression model, MUCCA was the only variable to predict clinical worsening. Together with the results from the prior cross sectional study, our finding emphasizes the role of spinal cord atrophy in disability worsening in NMO, suggesting that MUCCA change could serve as a biomarker to predict rate of disability worsening. In this short-duration study we did not find any association between MUCCA changes and disability change in MS, in line with other studies in patients with RRMS showing no association between MUCCA change and clinical progression[16]. Spinal cord and brain atrophy changes were not correlated with each other in either NMO or MS patients, suggesting cord atrophy may occur partly independently from brain volume change during the disease progression in both diseases. No significant differences in

demographic and clinical measurements were observed between progression and non-progression groups in either NMO or MS.

Some limitations of our study have to be mentioned. First, we didn't follow up the HC, which prevented any comparison of brain and spinal cord changes between patients and HC. However the observed decrease of MUCCA and brain atrophy over time in patients is highly likely to be a disease related effect, as in the normal population significant spinal cord atrophy were not observed in young subjects, and the mean age of the healthy subjects in the current study is 35 years[32]. Second, MUCCA and thalamic atrophy rates were derived from two separated segmentations. Optimal registration-based methods may lead to a more precise estimation of longitudinal changes in future studies. Third, we did not normalize the MUCCA measurements since there is no consensus on spinal cord atrophy normalization. Fourth, this study is a preliminary study with a relatively small sample size and short follow-up period, and no correction for multiple testing was performed; additionally a small proportion of the patients had relapse during the follow-up, we can't fully exclude the effect of relapse on the results; therefore further studies with larger sample sizes and longer term follow up are warranted to confirm our results. Finally, to further improve the clinical correlation or future clinical trials in NMO and MS, other MRI measurements should be explored and integrated such as diffusion metrics [33], functional MRI [34] in both brain and spinal cord, as well as structural and diffusion measurements of the optic nerve [35].

Conclusion

Different patterns of longitudinal brain and spinal cord changes were identified in NMO and MS. Spinal cord atrophy progression was observed in NMO, while thalamus atrophy progression was identified in MS. Spinal cord atrophy changes were associated with disability progression in NMO, while baseline brain lesion load and whole brain atrophy changes were related to disability progression in MS.

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Tables and figures

Table 1. Baseline, Demographic, clinical characteristics and brain, spinal cord volume measurement

	NMO	MS	HC	p value
N	25	20	20	/
Sex	4M/21F	5M/15F	5M/15F	0.46 [a]
Age (years)	35.6±9.9 (22-56)	35.5±10.6 (18-54)	34.6±10.0 (22-59)	0.93 [b]
EDSS	3.5 (0.5-7)	2.5 (0-6.5)	/	0.06[c]
Disease Duration (years)	4.0±2.8 (0.60-16.00)	3.7±2.5 (0.60-15.00)	/	0.51[d]
Number of relapses	3 (2-9)	3 (1-7)		0.45[c]
T2LV (ml)	/	98 (1.3; 1943)	/	/
NBV(L)	1.54±0.75 (1.31-1.67)	1.51±0.76* (1.40-1.67)	1.59±0.18 (1.34-1.88)	**<0.01**[b]
Thalamus volume (ml)	10.5±1.2 (8.4-12.8)	10.3±1.4* (8.3-13.7)	11.2±1.0 (8.3-13.7)	**0.03**[b]
Total spinal cord lesion number	1 (1-3)	2 (0-6)	/	0.145[c]
Total spinal cord lesion length	4 (3-10)	1 (0-4)	/	**<0.001**[c]
MUCCA (cm^2)	0.69±0.07* (0.54-0.84)	0.72±0.07 (0.60-0.86)	0.78±0.07 (0.65-0.93)	**<0.001**[b]

Data are presented as mean±SD (range) or median (range) except for gender.

Abbreviations: EDSS: Expanded Disability Status Scale; MS=multiple sclerosis; MUCCA=mean upper cervical cord area; T2LV =T2 lesion volume; NBV = normalized brain volume; NMO = neuromyelitis optica

[a]*p*-value was obtained by chi-square test.

[b]*p*-value was obtained by one-way ANOVA. Post hoc analysis was performed using Bonferroni correction.

[c]*p*-value was obtained by Kruskal-Wallis test.

[d]*p*-value was obtained by two-sample t-test.

**p*<0.05 compared to healthy controls

Table 2. Longitudinal changes brain, spinal cord lesions and volume measurements

	NMO	MS	P value
Brain			
T2LV changes (%)	/	1.36±20.43 (-69.15;51.56)	/
PBVC (%)	-0.15±0.97 (-2.08;2.15)	-1.09±1.85 (-6.20;1.19)	**0.03**
PVVC (%)	3.66±3.13 (-1.69;10.9)	5.56±7.34 (-1.29;27.2)	0.24
Thalamus changes (%)	-0.75±1.96 (-5.58;4.44)	-2.99±3.29 (-7.68;4.51)	**0.01**
Spinal cord			
Total Lesion number changes	0 (-2;1)	1 (-2;2)	0.59
Total Lesion length changes	-0.5 (-2;2)	0.5 (-1;2)	0.08
MUCCA changes(%)	-1.92±3.00 (-8.35;2.10)	-0.83±2.04 (-4.85;2.19)	0.18

Data are presented as mean±SD (range) or median (range) depending on the normality of the data

Abbreviations: EDSS: Expanded Disability Status Scale; MS=multiple sclerosis; MUCCA=mean upper cervical cord area; T2LV = T2 lesion volume; NBV = normalized brain volume; NMO = neuromyelitis optica

Table 3. Clinical and MRI characteristics of patients with NMO and MS, separated according to disease progression after 1 year follow up

	NMO			**MS**		
	No progression N=18	**Progression N=7**	**p value**	**No progression N=15**	**Progression N=5**	**p value**
Age	36.0±10.7 (24-56)	34.4±8.0 (22-48)	0.73	37.3±10.7 (24-56)	30.0±9.6 (24-56)	0.19
Gender	2/16	2/5	0.29	3/12	1/4	0.72
EDSS	3.3±1.1 (1-5.5)	3.7±1.6 (2-6)	0.50	2.6±1.2 (0.5-5.5)	3.2±0.3 (2-6)	0.30
Disease duration (years)	4.3±3.1 (0.6-11.0)	3.6±2.0 (2.0-7.0)	0.59	3.3±2.1 (1.0-8.0)	4.5±2.6 (2.0-6.0)	0.30
Number of relapses	3.6±2.0 (2-9)	4.4±2.1 (2-8)	0.34	3.2±1.5 (1-5)	3.8±2.2 (2-7)	0.49
Brain						
T2LV baseline (ml)				75 (1.3-288)	170 (117-1943)	**0.03**
T2LV changes (%)				-2.08±19.29 (-69.19-9.35)	11.68±22.43 (-1.68-51.56)	0.20
NBV(baseline)	1.53±0.08 (1.31-1.63)	1.56±0.06 (1.48-1.67)	0.39	1.52±0.08 (1.40-1.67)	1.47±0.06 (1.40-1.56)	0.25
PBVC (%)	-0.15±0.73 (-2.08-0.87)	-0.14±1.49 (-2.08-2.15)	0.98	-0.51±1.42 (-4.99-1.19)	-2.81±2.10 (-6.20;-0.94)	**0.01**

PVVC(%)	3.31±2.96 (-1.69-10.96)	4.55±3.61 (-0.71-9.13)	0.38	6.39±7.86 (-1.29-27.28)	3.06±4.68 (-0.86-9.32)	0.39
Thalamus Baseline (ml)	10.5±1.3 (8.5-12.8)	10.5±1.0 (8.4-11.5)	0.92	10.5±1.4 (8.6-13.8)	9.7±1.6 (8.3-11.7)	0.32
Thalamus volume changes	-0.67±2.28 (-5.58;4.44)	-0.96±0.75 (-3.34;-0.1)	0.75	-2.27±3.35 (-7.32;4.51)	-5.12±2.14 (-7.68;-2.67)	0.09
Spinal cord						
Total Lesion number(baseline)	1 (1;3)	1 (1;2)	0.59	2 (0;5)	2 (0;4)	0.58
Total Lesion number(changes)	0 (-1;1)	0 (-1;1)	0.50	0 (-2;3)	0 (0;3)	0.16
Total Lesion length (baseline)	6 (3-12)	5 (3;11)	0.64	1 (0;3)	1.5 (0;3)	0.66
Total Lesion length(changes)	-1 (-4;2)	-1 (-3;2)	0.82	0 (-2-3)	1 (0-5)	0.11
MUCCA(baseline)	0.68±0.07 (0.53;0.79)	0.72±0.08 (0.61;0.84)	0.17	0.72±0.06 (0.60-0.83)	0.75±0.09 (0.64-0.86)	0.42
MUCCA(changes)	-1.10±2.41 (-8.35;2.10)	-4.02±3.51 (-7.86;1.97)	**0.03**	-0.80±2.24 (-4.85-2.19)	-0.94±1.49 (-3.23-0.29)	0.90

Data are presented as mean±SD (range) or median (range) depending on the normality of the data

Abbreviations: EDSS: Expanded Disability Status Scale; MS=multiple sclerosis; MUCCA=mean upper cervical cord area; T2LV = T2 lesion volume; NBV = normalized brain volume; NMO = neuromyelitis optica

Figure Legends:

Figure 1. Overview of the image-processing pipeline of upper spinal cord area measurement

Figure 2: The significant differences of MRI measurements between progression and no progression groups in NMO and MS.

Abbreviations: KDE=kernel density estimation; MS=multiple sclerosis; MUCCA=mean upper cervical cord area; T2LV = T2 lesion volume; NMO = neuromyelitis optica; PBVC=percentage brain volume changes.