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Silent progression of brain atrophy in aquaporin-4 antibody-positive neuromyelitis optica spectrum disorder

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ABSTRACT

Objective To investigate longitudinal brain atrophy in patients with neuromyelitis optica spectrum disorder (NMOSD).

Methods We investigated the longitudinal brain atrophy rate in patients with aquaporin-4 antibody-positive NMOSD (AQP4+NMOSD) and those with multiple sclerosis (MS) in a retrospective cohort study. Brain volume was calculated with statistical parametric mapping-12.

Results We enrolled 36 patients with AQP4+NMOSD and 60 with MS. Patients with NMOSD were older and had a higher Kurtzke's expanded disability status scale score at baseline MRI compared with those with MS. Disease duration, annual relapse rate and intervals from the last attack and from disease-modifying drugs initiation were not significantly different between the two groups. Lower normalised lesion volume and higher normalised white matter volume were found in patients with NMOSD compared with those with MS at baseline MRI. However, the annualised atrophy rate of normalised brain volume was similar between the NMOSD (median 0.47; IQR 0.75; p=0.49) and MS (median 0.46; IQR 0.84) groups. After adjustment of age and the presence of clinical relapse, no differences of the annualised atrophy rate of normalised brain volume also were found for NMOSD and MS. Patients with AQP4+NMOSD with long cord lesion showed higher annualised atrophy rate of normalised grey matter volume compared with those without long cord lesion.

Conclusions Silent progression of brain atrophy was present in patients with AQP4+NMOSD, as shown in patients with MS, even in the clinically inactive age-matched cases. Subclinical dying back degeneration may explain the brain atrophy in patients with AQP4+NMOSD.

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INTRODUCTION

Neuromyelitis optica spectrum disorder (NMOSD) is a severe inflammatory disease in the central nervous system (CNS). It mostly affects the optic nerve and spinal cord, but a brain lesion can occur. One feature of NMOSD is the positivity of antibodies against aquaporin-4 (AQP4).[1] Reportedly, 60%–90% of patients with NMOSD have serum anti-AQP4 antibodies (patients with AQP4+NMOSD).[2]

On the other hand, a feature of multiple sclerosis (MS), a demyelinating disease in the CNS, is the dissemination of demyelination over time and space.[3] Different from AQP4+NMOSD, MS has the progression phase in the disease course.[4 5] Brain atrophy, especially cortical and grey matter atrophy, is correlated with worsening of cognition and disability.[6–9] While patients with NMOSD reportedly have significantly reduced thalamic volumes compared with healthy controls in a cross-sectional study, patients with MS showed more reduced thalamic volumes than patients with NMOSD.[10] However, this issue has remained controversial.[11] On the other hand, another cross-sectional study showed no difference in the entire thalamic volumes between healthy controls and patients with NMOSD.[12] Another study also reported significantly lower grey matter volume (GMV) in patients with MS compared with those with NMOSD.[13] In addition, silent progression and longitudinal brain atrophy in patients with relapsing–remitting MS (RRMS) have been reported recently.[14] Meanwhile, the longitudinal brain atrophy in patients with NMOSD has not been fully investigated. To our best knowledge, only one study has reported the longitudinal brain atrophy in patients with NMOSD to date.[15]

We investigated longitudinal brain atrophy in patients with AQP4+NMOSD and RRMS in a retrospective cohort study.

MATERIALS AND METHODS
Study design and patient populations

The clinical records of 114 patients with AQP4+NMOSD and 283 with RRMS at Chiba University Hospital were reviewed retrospectively. The patient-enrolled process and study design are shown in figure 1. Patients who received two MRI scans using the same scanner at a>1 year interval were included. The two MRI scans (MRI-1 and MRI-2) were selected when the interval between MRI-1 and MRI-2 became larger as much as possible, as reported previously.[16] Because steroids can affect brain volume by causing steroid-related pseudo-atrophy, MRIs were excluded when steroid therapy was performed for MS attacks at 60 days before the brain MRI scan, according to previous reports.[17 18]

Because patients with AQP4+NMOSD receive prednisolone to prevent the attack, MRIs were excluded when prednisolone pulse therapy was initiated within 60 days before the brain MRI scan. All patients with AQP4 +NMOSD fulfilled the
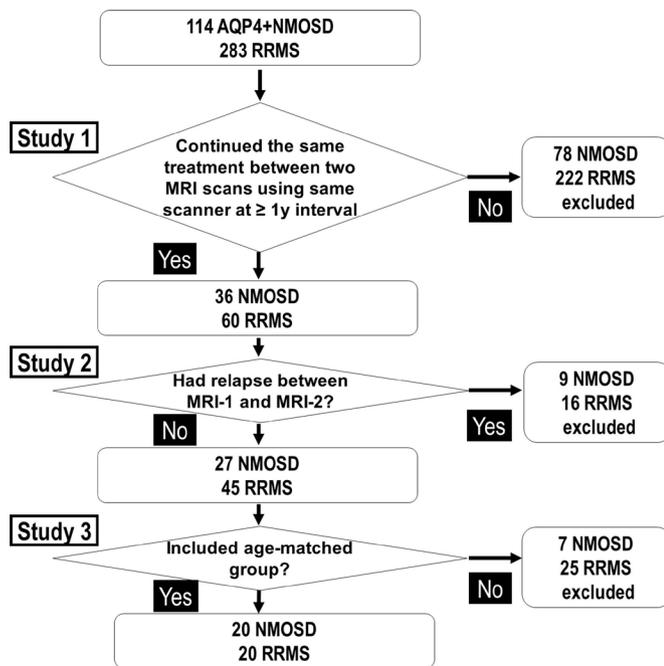
Figure 1 Flow chart and study design shows how we enrolled patients with AQP4+NMOSD and MS. AQP4+NMOSD, anti-aquaporin-4 antibody-positive neuromyelitis optica spectrum disorders; MS, multiple sclerosis; RRMS, relapsing–remitting multiple sclerosis.

2015 international diagnostic criteria for NMOSD[1] and were positive for AQP4 antibodies measured by cell-based assay as described previously.[19] Meanwhile, all patients with RRMS fulfilled the 2017 McDonald's diagnostic criteria and negative for myelin oligodendrocyte glycoprotein antibodies measured by cell-based assay as described previously.[19 20] To minimise the effect of disease-modifying drugs (DMDs) for the brain atrophy, patients with AQP4+NMOSD and RRMS who continued the same DMDs between MRI-1 and MRI-2 were included. We performed a main study and two substudies as below.

First, all patients who fulfilled the aforementioned inclusion criteria were included in study 1. In study 2, only patients without clinical relapses and disability progression between MRI-1 and MRI-2 were included to compare only clinically stable patients. Clinical relapse was defined as reported previously.[20] Disability progression also was defined as reported previously[14]; that is, if the baseline Kurtzke's expanded disability status scale (EDSS) score was 0, 1.0 to 5.0, and ≥5.5 at MRI-1, an EDSS score increase of 1.5, 1.0 and 0.5 was regarded as disability progression, respectively.[14] Finally, an age-matched study was performed as study 3. Age matching was performed as below; patients with AQP4+NMOSD and MS in study 2 were sorted by age. Younger patients with AQP4+NMOSD were matched by choosing younger patients with MS as their age difference became <5 years. The nearest patient in age was selected as the matched pair. If there were several candidates, a coin toss was performed to determine the age-matched patient.

Demographic characteristics, including sex ratio and age at MRI-1, and clinical features, including disease duration to MRI-1, EDSS score at MRI-1, annualised relapse rate (ARR) from disease onset to MRI-1, months from the last attack, months from the baseline treatment initiation, and the positivity of oligoclonal bands (OCB), were investigated. Positivity of cerebrospinal fluid (CSF) OCB was determined by isoelectric focusing.

To investigate the pathophysiology of the brain atrophy in patients with AQP4+NMOSD, the annualised atrophy rate of patients with AQP4+NMOSD was compared with or without some clinical features including the past history of the optic neuritis, myelitis, brain stem lesion, and long cord lesion. The association between relapse numbers and the annualised atrophy rate was also investigated. Long cord lesion was defined as >3 vertebral segments. The length of the spinal cord lesions (vertebral body segments) was measured from the image which showed the maximum spinal cord lesion length in all spinal cord images before MRI-1, and the correlation between the length and annualised atrophy rate was investigated.

Brain MRI scan
The brain MRI scan protocol included a conventional brain MRI, T1-weighted three-dimensional (3D) images, and fluid-attenuated inversion recovery (FLAIR) or multiplanar reconstruction from the 3D-FLAIR. The same MR scanner, a 1.5-Tesla Signa HDxT (GE Healthcare, Milwaukee, Milwaukee, USA), was used for all patients. Details of the MRI systems are shown in online supplemental table 1.

Brain volume measurements
The brain volumes of patients with AQP4+NMOSD and MS were calculated using statistical parametric mapping-12 (SPM12), which was implemented on MATLAB (V.R2016b; The MathWorks, Natick, Massachusetts, USA). A previously reported method was used to measure brain volume in each patient.[16 21] Briefly, intracranial volume (ICV) was calculated as the sum of the whole-brain grey matter volume (GMV) plus white matter volume (WMV) and CSF volumes, and this subsequently was treated to normalise for the brain and lesion volumes. Lesions were segmented by the lesion growth algorithm as implemented in the Lesion Segmentation Tool (LST) toolbox V.2.0.15 (available in the public domain at www.statisticalmodelling.de/lst.html) for SPM.[22] We used an initial threshold (κ) value of 0.30 in accordance with the recommendation of Schmidt *et al*.[22] The lesion volume filled by LST was expressed as the total lesion volume (TLV). GMV/ICV, WMV/ICV, (GMV +WMV)/ICV, and TLV/ICV were defined as normalised grey matter (NGV), normalised white matter (NWV), normalised brain (NBV) and normalised lesion (NLV) volumes, respectively.

The annualised atrophy rate of X was defined as follows after lesion filling was performed:

$$\frac{(\text{X at 1st MRI scan} - \text{X at 2nd MRI scan}) \times 12}{(\text{X at 1st MRI scan}) \times (\text{Months between 1st and 2nd MRI scan})}$$

where X=NGV, NWV, or NBV.

Statistical analysis
Statistical tests were conducted using SPSS V.26.0 (IBM Corporation). Continuous data were compared between patients with AQP4+NMOSD and RRMS using the Mann-Whitney U test. Categorical outcomes were evaluated using Fisher's exact test. An analysis of covariance (ANCOVA) was performed when the annual atrophy rate was determined using significant different items as covariates. A Spearman's rank correlation test was performed to analyse correlations. p<0.05 was considered statistically significant. Due to the exploratory nature of the study, no adjustment for multiple comparisons was made.

Table 1 Demographic and clinical characteristics in patients with AQP4+NMOSD and MS at MRI-1 in study1

	AQP4+NMOSD (n=36)	RRMS (n=60)	p value
Demographic			
Female (%)	32/36 (88.9%)	46/60 (76.7%)	0.18
Age (years)	54.5 (19.0) (34–77)	40.5 (13.0) (17–67)	<0.001*
Clinical			
Disease duration (years)	6.3 (14.9) (0.25–42.9)	8.3 (11.2) (0.42–34.7)	0.14
EDSS score	4.5 (4.0) (1.0–9.0)	2.0 (3.0) (0.0–7.5)	0.006*
ARR from disease onset	0.65 (0.59) (0.20–4.0)	0.54 (0.53) (0.06–2.8)	0.36
Months from last attack	20.6 (33.6) (2.4–48.0)	32.9 (44.3) (1.1–207)	0.81
Months from DMD initiation	12.6 (18.3) (1.2–68.1)	17.8 (34.1) (0–172)	0.29
Oligoclonal bands positivity	5/27 (18.5%)	33/49 (67.3%)	<0.001*
Number of patients with a history of			
Optic neuritis	25/36 (69.4%)		
Myelitis	30/36 (83.3%)		
Long cord lesion	21/36 (58.3%)		
Brain stem lesion	9/36 (25%)		
Area postrema syndrome	2/36 (5.6%)		
Cerebral syndrome	6/36 (16.7%)		
DMD			
Interferonβ–1a	0	16	
Interferonβ–1b	0	10	
Fingolimod	0	22	
Dimethyl fumarate	0	5	
Natalizumab	0	2	
Prednisolone	25	0	
Prednisolone+azathioprine	5	0	
Prednisolone+eculizumab	1	0	
None	5	5	

Data are presented as median number (%) or (IQR; range). *p < 0.05. Months from DMD initiation indicate period between the start of the same DMD given before MRI-1 and the date MRI-1 was performed.

AQP4+NMOSD, anti-aquaporin-4 antibody-positive neuromyelitis optica spectrum disorder disease; ARR, annual relapse rate; DMD, disease-modifying drug; EDSS, Kurtzke's Expanded Disability Status Scale.

RESULTS

Patient demographics and clinical characteristics at MRI-1 in study 1

The demographics and clinical characteristics of patients with AQP4+NMOSD and MS at MRI-1 are summarised in table 1. Age and EDSS score at MRI-1 were significantly higher in patients with AQP4 +NMOSD compared with those with MS. On the other hand, patients with AQP4+NMOSD showed lower OCB positivity compared with patients with MS (18.5% vs 67.3%, respectively). No other items, including female ratio, disease duration, ARR from disease onset, and months from DMD initiation, were different between the two groups. Of the patients with AQP4+NMOSD, 25 received prednisolone alone and six received prednisolone plus azathioprine (n=5) and prednisolone plus eculizumab (n=1). Among the treated patients with prednisolone, the median dose of prednisolone was 7.5 mg/day (IQR: 2.5, range: 5–15). On the other hand, 26, 22, 5 and 2 patients with MS received interferon-β, fingolimod, dimethyl fumarate or natalizumab, respectively. Five patients with AQP4 +NMOSD and five with MS received no treatment at MRI-1.

Annualised brain atrophy rates were not different between patients with AQP4+NMOSD and MS

The distribution of percentage brain volume change in each patient with AQP4+NMOSD and MS is shown in figure 2A.

The clinical characteristics and brain volumes between MRI-1 and MRI-2 in patients with AQP4+NMOSD and MS in study 1 are exhibited in table 2. The results showed no difference in ΔEDSS (EDSS at MRI-2 minus EDSS at MRI-1) and ARR between MRI-1 and MRI-2. Time between MRI-1 and MRI-2 was longer in patients with AQP4+NMOSD than in those with MS. ICV at MRI-1 and NLV at MRI-1 and MRI-2 were lower in AQP4+NMOSD compared to patients with MS. NWV at MRI-1 and MRI-2 were higher in patients with AQP4+NMOSD, but the annualised atrophy rate of NGV, NWM and NBV showed no difference between the two patient groups. No difference was found in the annualised atrophy rate of NBV, NGV and NWV after ANCOVA was performed using statistically significant different items in tables 1 and 2 as covariates (online supplemental table 2).

Annualised atrophy rates of NGV, NWV and NBV with or without continuous steroid in patients with AQP4+NMOSD

To investigate the pseudoatrophy effect of steroids, we have added the two analysis. First, we compared the annualised atrophy rate of NGV, NWV and NBV with or without continuous prednisolone at MRI-1 in study 1. The result showed the annualised atrophy rate of NBV and NWV was significantly higher in patients without continuous prednisolone (n=5) compared with patients with continuous prednisolone (n=31)
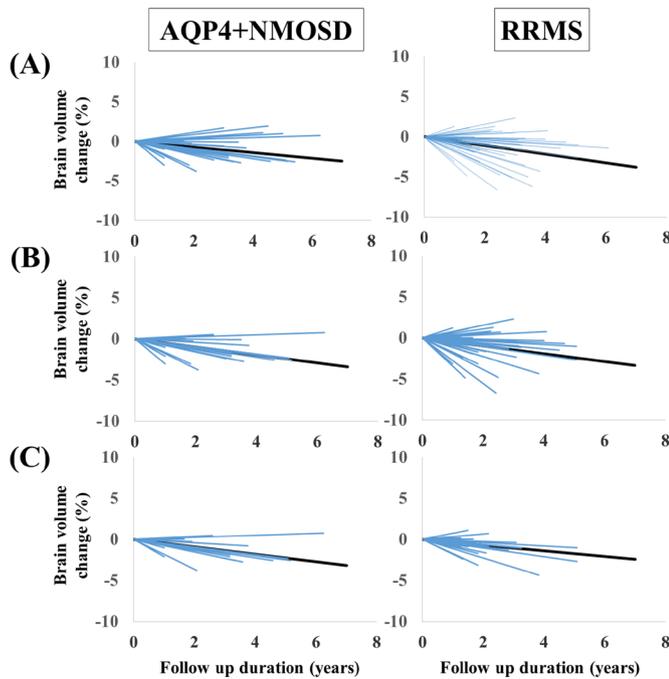
Figure 2 The percentage brain volume change between MRI-1 and MRI-2 in patients with AQP4+NMOSD and MS. (A) All patients with AQP4+NMOSD and MS (in study 1). (B) Patients without clinical relapse or disability progression (in study 2). (C) Age-matched patients without clinical relapse or disability progression (in study 3). The blue line shows the percentage brain volume change in each patient. The black line exhibits the fitted average slope in patients with AQP4+NMOSD and MS, respectively. AQP4+NMOSD, anti-aquaporin-4 antibody-positive neuromyelitis optica spectrum disorders; MS, multiple sclerosis; RRMS, relapsing–remitting multiple sclerosis.

(p=0.008 and 0.003, respectively). No difference was found in the annualised atrophy rate of NGV (p=0.45). We have tried the same analysis in study 2, but the number of patients without continuous prednisolone (n=3) was too small to perform the statistical analysis. Therefore, the same statistical analysis was not performed in study 2 and 3.

Next, we have analysed the correlation between the annualised atrophy rate of NBV, NGV and NWV and total steroid dose between MRI-1 and MRI-2. Total steroid dose tended to negatively correlate with the annualised atrophy rate of NBV (Spearman's rho=−0.33, p=0.052) and NWV (Spearman's rho=−0.31, p=0.063), but not with NGV (Spearman's rho=0.039, p=0.82) in study 1.

Patient demographics and clinical characteristics at MRI-1 only among patients without clinical relapse and disability progression between MRI-1 and MRI-2 (study 2)

Table 3 exhibits the demographics and clinical characteristics at MRI-1 in study 2. Age and EDSS score at MRI-1 were higher in patients with AQP4+NMOSD than in those with MS in study 1. Patients with MS showed higher OCB positivity compared with patients with AQP4+NMOSD. Female ratio, disease duration, ARR from disease onset, months from last attack, and months from DMD initiation were not different between the two groups at MRI-1. A total of 24 patients with AQP4 +NMOSD received treatment at MRI-1 (prednisolone alone, n=20; prednisolone plus azathioprine, n=3; and prednisolone plus eculizumab, n=1). On the other hand, 41 patients with MS received treatment at MRI-1 (interferon-β, n=17; fingolimod, n=18; dimethyl fumarate, n=4; and natalizumab, n=2). Three patients with AQP4+NMOSD and four with MS received no treatment at MRI-1.

Table 2 Clinical characteristics and brain volumes at MRI-1 and MRI-2 and the annualised atrophy rate in patients with MS and AQP4+NMOSD between MRI-1 and MRI-2 in study 1

	AQP4+NMOSD (n=36)	RRMS (n=60)	p value
ΔEDSS (MRI-2 − MRI-1)	0.0 (0.0) (−2.0–3.5)	0.0 (0.19) (−3.0–2.5)	0.81
Years from MRI-1 to MRI-2	3.1 (2.5) (1.0–6.3)	2.2 (1.8) (1.0–6.1)	0.045*
ARR between MRI-1 and MRI-2	0.0 (0.0) (0.0–0.88)	0.0 (0.0) (0.0–1.5)	0.56
At MRI-1			
ICV*10^{-3} (mL)	1.3 (0.12) (1.2–1.6)	1.4 (0.14) (1.1–1.7)	0.034*
NLV (mL)	1.0 (5.2) (0.0–33.2)	6.4 (10.3) (0.45–46.7)	<0.001*
NGV*10^{-3} (mL)	0.43 (0.063) (0.32–0.49)	0.44 (0.058) (0.32–0.50)	0.47
NWV*10^{-3} (mL)	0.31 (0.029) (0.22–0.34)	0.29 (0.033) (0.23–0.34)	0.007*
NBV*10^{-3} (mL)	0.75 (0.070) (0.62–0.81)	0.73 (0.085) (0.59–0.80)	0.61
At MRI-2			
ICV*10^{-3} (mL)	1.3 (0.12) (1.2–1.6)	1.4 (0.14) (1.1–1.7)	0.068
NLV (mL)	2.4 (9.0) (0.0–142)	6.6 (10.3) (0.0–187)	0.019*
NGV*10^{-3} (mL)	0.43 (0.053) (0.31–0.49)	0.44 (0.060) (0.32–0.50)	0.5
NWV*10^{-3} (mL)	0.31 (0.033) (0.22–0.34)	0.29 (0.029) (0.22–0.34)	0.007*
NBV*10^{-3} (mL)	0.74 (0.074) (0.60–0.80)	0.72 (0.088) (0.58–0.80)	0.43
Annualised atrophy rate			
NGV (%)	0.50 (1.1) (−2.4–4.9)	0.36 (1.4) (−2.5–5.7)	0.96
NWV (%)	0.21 (1.5) (−6.2–4.5)	0.33 (2.0) (−3.6–6.3)	0.72
NBV (%)	0.47 (0.75) (−0.57–3.0)	0.48 (0.85) (−1.3–3.4)	0.47

Data are presented as median number (%) or (IQR) (range). *p<0.05.

Annualised atrophy rate of X is defined as follows: $\frac{(\text{X at 1st MRI scan} - \text{X at 2nd MRI scan}) \times 12}{(\text{X at 1st MRI scan}) \times (\text{Months between 1st and 2nd MRI scan})}$, X= NGV, NWV or NBV.

ΔEDSS= EDSS at MRI-2 minus EDSS at MRI-1.

AQP4+NMOSD, anti-aquaporin-4 antibody-positive neuromyelitis optica spectrum disorder disease; EDSS, Kurtzke's Expanded Disability Status Scale; ICV, intracranial volume; NBV, normalised brain volume; NGV, normalised grey matter volume; NLV, normalised lesion volume; NWV, normalised white matter volume.

Table 3 Demographic and clinical characteristics in patients with MS and AQP4 +NMOSD without clinical relapses and disability progression at MRI-1

	AQP4+NMOSD (n=27)	RRMS (n=45)	p value
Demographic			
Female (%)	25/27 (92.6%)	34/45 (75.6%)	0.11
Age (years)	55.0 (16.0) (34–77)	42.0 (13.0) (19–67)	<0.001*
Clinical			
Disease duration (years)	6.7 (17.2) (0.25–41.2)	8.4 (12.8) (0.42–34.7)	0.15
EDSS score	5.0 (4.0) (1.0–9.0)	2.5 (3.0) (0.0–7.5)	0.020*
ARR from disease onset	0.55 (0.60) (0.20–4.00)	0.50 (0.45) (0.12–2.4)	0.24
Months from last attack	22.0 (35.5) (2.4–83)	35.8 (39.1) (3.1–149)	0.54
Months from DMD initiation	11.2 (20.3) (1.2–68.1)	22.2 (51.0) (1.4–172)	0.067
Oligoclonal bands positivity	5/19 (26.3%)	24/36 (66.7%)	0.006*
Number of patients with a history of			
Optic neuritis	18/27 (66.7%)		
Myelitis	23/27 (85.2%)		
Long cord lesion	16/27 (59.3%)		
Brain stem lesion	7/27 (25.9%)		
Area postrema syndrome	2/27 (7.4%)		
Cerebral syndrome	4/27 (14.8%)		
DMD			
Interferon β−1a	0	10	
Interferon β−1b	0	7	
Fingolimod	0	18	
Dimethyl fumarate	0	4	
Natalizumab	0	2	
Prednisolone	20	0	
Prednisolone+azathioprine	3	0	
Prednisolone+eculizumab	1	0	
None	3	4	

Data are presented as median number (%) or (IQR) (range). *p<0.05.
Months from DMD initiation indicate period between the start of the same DMD given before MRI-1 and the date MRI-1 was performed.
AQP4 +NMOSD, anti-aquaporin-4 antibody-positive neuromyelitis optica spectrum disorder disease; ARR, annualised relapse rate; DMD, disease modifying drug; EDSS, Kurtzke's Expanded Disability Status Scale; RRMS, relapsing-remitting multiple sclerosis.

Annualised brain atrophy rates were not different between patients with AQP4+NMOSD and MS without clinical relapse and disability progression

Figure 2B shows the distribution of the percentage brain volume change in each patient with AQP4+NMOSD and MS in study 2. Table 4 exhibits the clinical characteristics and annualised brain atrophy rate in study 2. The results showed lower ICV at MRI-1 and NLV at MRI-1 and MRI-2 in patients with AQP4 +NMOSD compared with those with MS. NWV was higher in AQP4+NMOSD than in patients with MS at MRI-1 and MRI-2. On the other hand, annualised atrophy rates of NGV, NWV and NBV were not different between the two groups. Other items, including ΔEDSS, years from MRI-1 to MRI-2, NGV at MRI-1 and MRI-2, NBV at MRI-1 and MRI-2, and ICV at MRI-2, were not different between the two groups. Annualised atrophy rate of NBV, NGV and NWV was not different after ANCOVA was performed using statistically significant different items in tables 3 and 4 as covariates (online supplemental table 2).

Patient demographics and clinical characteristics at MRI-1 only in age-matched patients without clinical relapse and disability progression (study 3)

Table 5 shows the demographic and clinical characteristics in age-matched patients with AQP4+NMOSD and MS without clinical relapses and progression at MRI-1. There was no difference in female ratio, age at MRI-1, EDSS score and months from last attack between the two groups. Disease duration at MRI-1 and months from DMD initiation were lower in patients with AQP4 +NMOSD than in those with MS. On the other hand, ARR from disease onset was higher in AQP4+NMOSD compared with patients with MS. OCB positivity was not different between the two groups. For treatment at MRI-1, 15, 2 and 1 patients with AQP4+NMOSD received prednisolone alone, prednisolone plus azathioprine, or prednisolone plus eculizumab, respectively. Two patients with AQP4+NMOSD received no treatment at MRI-1. Meanwhile, 9, 8 and 3 patients with MS received interferon-β, fingolimod or dimethyl fumarate, respectively.

Annualised brain atrophy rates were not different between age-matched patients with AQP4+NMOSD and MS without clinical relapse and disability progression

The percentage brain volume change in each patient with AQP4 +NMOSD and MS is shown in figure 2C. Clinical characteristics, brain volumes and the annualised brain atrophy rate in study 3 are demonstrated in table 6. NGV, NWV and NBV at MRI-1 and MRI-2 were higher in age-matched patients with AQP4+NMOSD than in age-matched patients with MS. On the other hand, NLV at MRI-1 and MRI-2 was lower in age-matched patients with AQP4+NMOSD compared with age-matched patients with MS. However, annualised atrophy rate of NGV, NWV and NBV were not different between the two groups. All other items, including ΔEDSS, years from MRI-1 to MRI-2, and

Table 4 Clinical characteristics and brain volumes at MRI-1 and MRI-2 and the annualised atrophy rate in patients with AQP4+NMOSD and MS without clinical relapses and disability progression between MRI-1 and MRI-2

	AQP4+NMOSD (n=27)	RRMS (n=45)	p value
ΔEDSS (MRI-2 − MRI-1)	0.0 (0.0) (−2.0–0.5)	0.0 (0.0) (−3.0–1.0)	0.83
Years from MRI-1 to MRI-2	3.0 (2.3) (1.0–6.3)	2.0 (1.8) (1.0–5.1)	0.12
At MRI-1			
ICV*10⁻³ (mL)	1.3 (0.12) (1.2–1.5)	1.4 (0.13) (1.2–1.7)	0.018*
NLV (mL)	0.96 (4.5) (0.0–33.2)	7.1 (12.9) (0.45–46.7)	<0.001*
NGV*10⁻³ (mL)	0.44 (0.047) (0.32–0.49)	0.44 (0.067) (0.32–0.50)	0.97
NWV*10⁻³ (mL)	0.31 (0.029) (0.24–0.33)	0.29 (0.028) (0.25–0.33)	0.001*
NBV*10⁻³ (mL)	0.75 (0.077) (0.62–0.81)	0.72 (0.086) (0.59–0.80)	0.19
At MRI-2			
ICV*10⁻³ (mL)	1.3 (0.12) (1.2–1.5)	1.4 (0.14) (1.2–1.7)	0.056
NLV (mL)	1.7 (8.7) (0.0–141)	8.0 (11.8) (0.0–187)	0.026*
NGV*10⁻³ (mL)	0.43 (0.061) (0.31–0.49)	0.42 (0.063) (0.33–0.50)	0.95
NWV*10⁻³ (mL)	0.31 (0.028) (0.22–0.34)	0.29 (0.028) (0.24–0.38)	0.001*
NBV*10⁻³ (mL)	0.75 (0.078) (0.60–0.80)	0.71 (0.094) (0.58–0.80)	0.21
Annualised atrophy rate			
NGV (%)	0.69 (1.2) (−1.7–3.0)	0.60 (1.7) (−2.5–4.5)	0.69
NWV (%)	0.16 (1.2) (−0.62–1.1)	0.19 (1.9) (−3.2–6.3)	0.81
NBV (%)	0.50 (0.75) (−0.20–0.77)	0.42 (0.85) (−1.3–3.4)	0.73

Data are presented as median number (%) or (IQR) (range). *p<0.05.

Annualised atrophy rate of X is defined as follows: $\frac{(\text{X at 1st MRI scan} - \text{X at 2nd MRI scan}) \times 12}{(\text{X at 1st MRI scan}) \times (\text{Months between 1st and 2nd MRI scan})}$, X= NGV, NWV or NBV.

ΔEDSS=EDSS at MRI-2 minus EDSS at MRI-1.

AQP4+NMOSD, anti-aquaporin-4 antibody-positive neuromyelitis optica spectrum disorder disease; EDSS, Kurtzke's Expanded Disability Status Scale; ICV, intracranial volume; NBV, normalised brain volume; NGV, normalised grey matter volume; NLV, normalised lesion volume; NWV, normalised white matter volume.

ICV at MRI-1 and MRI-2, were not different between the two groups. No significant difference was observed in the annualised atrophy rate of NBV, NGV and NWV after ANCOVA was performed using statistically significant different items in tables 5 and 6 as covariates (online supplemental table 2).

Patients with AQP4+NMOSD with long cord lesion showed higher annualised atrophy rate of NGV compared with those without long cord lesion

The result showed patients with AQP4+NMOSD demonstrated higher annualised atrophy rate of NGV compared with those without long cord lesion in study 1 (median; 0.79% vs 0.41%, p=0.046). On the other hand, annualised atrophy rate of NBV and NWV was not different with or without long cord lesion in patients with AQP4+NMOSD (p=0.47 for both). The annualised atrophy rate of NBV, NGV and NWV was not associated with relapse numbers or was not different in patients with AQP4+NMOSD with or without the history of the optic neuritis, myelitis, brain stem lesion in study 1. Patients with AQP4+NMOSD with long cord lesion also showed higher annualised atrophy rate of NGV compared with those without long cord lesion in study 2 (median; 0.92% vs 0.41%, p=0.005) and study 3 (median; 0.90% vs 0.41%, p<0.001). The annualised atrophy rate of NGV was positively correlated with the length of spinal cord lesion in study 2 (rho=0.52, p=0.005) and 3 (rho=0.63, p=0.003).

DISCUSSION

We found that the annualised atrophy rate of NBV was not different between patients with AQP4+NMOSD and MS even in the age-matched analysis. This finding is clinically important because longitudinal brain atrophy in patients with AQP4+NMOSD was shown for the first time to our knowledge.

Our result showed that the median value of the annualised atrophy rate of NBV in patients with MS was 0.46 in study 1, 0.42 in study 2, and 0.44 in study 3. These values are in the middle of the previously reported brain atrophy rate in healthy controls (0.1%–0.3% per year) and patients with MS (0.6%–1.35%), respectively.[23][24] However, another study reported that the pathological range of the brain atrophy rate in patients with MS was >0.46% with 90% specificity and >0.40% with 80% specificity.[25] The investigators reported that a 0.40% brain atrophy rate per year (with an error probability of 20%) showed high specificity and good sensitivity as the pathological cutoffs of brain atrophy rate in patients with MS.[25] In our study, more than half of the patients with MS in all studies exceeded the 0.40% brain atrophy rate per year (with an error rate of 20%). These findings indicated that our results were not contradictory to the previous report about longitudinal brain atrophy in patients with MS.

On the other hand, the brain atrophy rate was not different between patients with AQP4+NMOSD and MS. This result was not consistent with the previous study about the longitudinal brain atrophy rate in patients with NMOSD.[15] Our study included patients with longer disease duration compared with the previous study, which may result in the different result. The brain atrophy was also reported to be influenced by normal ageing,[26] but the similar slope of the brain volume change was observed even in age-matched patients with AQP4+NMOSD and MS without clinical relapses and disability progression in our study. This finding implied that patients with AQP4+N-MOSD could have similar subclinical pathologic brain atrophy as those with MS.

The result showed patients with AQP4+NMOSD with long cord lesion demonstrated the higher annualised atrophy rate of NGV compared with those without long cord lesion. Previous

Table 5 Demographic and clinical characteristics in age-matched patients with AQP4+NMOSD and MS without clinical relapses and disability progression at MRI-1

	AQP4+NMOSD (n=20)	RRMS (n=20)	p value
Demographic			
Female (%)	18/20 (90.0%)	15/20 (75.0%)	0.41
Age (years)	50.0 (16.5) (34–67)	48.0 (14.0) (35–67)	0.45
Clinical			
Disease duration (years)	2.9 (10.3) (0.25–36.7)	13.9 (13.5) (1.6–27.1)	0.004*
EDSS score	4.5 (4.1) (1.0–7.0)	3.5 (4.8) (0.0–7.0)	0.67
ARR from disease onset	0.73 (0.70) (0.32–4.0)	0.51 (0.38) (0.14–2.1)	0.045*
Months from last attack	17.7 (26.6) (2.4–61.9)	37.4 (56.7) (3.1–147.2)	0.19
Months from DMD initiation	9.7 (16.4) (1.2–68.1)	29.2 (61.2) (1.4–172.4)	0.009*
Oligoclonal bands positivity	3/14 (15%)	9/15 (60%)	0.06
Number of patients with a history of			
Optic neuritis	13/20 (65%)		
Myelitis	16/20 (80%)		
Long cord lesion	11/20 (55%)		
Brain stem lesion	5/20 (25%)		
Area postrema syndrome	1/20 (5%)		
Cerebral syndrome	4/20 (20%)		
DMD			
Interferon β–1a	0	5	
Interferon β–1b	0	4	
Fingolimod	0	8	
Dimethyl fumarate	0	3	
Natalizumab	0	0	
Prednisolone	15	0	
Prednisolone+azathioprine	2	0	
Prednisolone+eculizumab	1	0	
None	2	0	

Data are presented as median number (%) or (IQR) (range). *p<0.05.

Months from DMD initiation indicate period between the start of the same DMD given before MRI-1 and the date MRI-1 was performed.

AQP4+NMOSD, anti-aquaporin-4 antibody-positive neuromyelitis optica spectrum disorder disease; ARR, annualised relapse rate; DMD, disease-modifying drug; EDSS, Kurtzke's Expanded Disability Status Scale; PRMS, relapsing-remitting multiple sclerosis.

study showed mean upper cervical cord area was correlated with spinal cord lesion length in patients with NMO.[27] Another study reported associations between spinal cord atrophy with increased number of myelitis attacks.[28] Spinal cord injury was reported to lead to spinal cord atrophy and cortical atrophy.[29] Therefore, dying back degeneration as a result of long cord lesion could explain the subclinical brain atrophy in patients with AQP4+NMOSD in our study.

OCB positivity in patients with AQP4+NMOSD was 18.5% in study 1. A few studies reported OCB positivity in patients with AQP4+NMOSD, but the percentage in our study is higher compared with a previous report (8.8%),[30] but almost same as another study (16.4%).[31] On the other hand, OCB positivity in patients with MS in our study (67.3%) was lower than those in the Western countries (almost 90%), but OCB positivity of patients with MS in Asian countries was reported to be low compared with those in Western countries.[32][33] Our result showed the similar OCB positivity with another study in Japan (69%).[34]

There are some limitations in our study. First, we could not perform statistical analysis between patients and healthy controls since we had not enrolled healthy subjects as controls. However, as we discussed above, our data suggested that the annualised brain atrophy rate in patients with AQP4+NMOSD and MS was higher than the previously reported pathological brain atrophy rate in patients with MS. Second, most of the brain atrophy

studies we referenced were performed using another method, Structural Image Evaluation, using Normalisation, of Atrophy (SIENA). Recent studies showed that the method difference could cause the different results for measuring brain atrophy.[35][36] However, SPM and SIENA reportedly showed good agreement for whole-brain volume change in patients with MS in another study.[37] A validation study indicated that the brain atrophy rate calculated by SIENA tended to be higher than that calculated by SPM,[37] which means that the tendency for the annualised brain atrophy rate in our study to exceed the pathological cutoffs of brain atrophy rate in patients with MS reported by De Stefano et al could be increased. However, we cannot directly compare results of other studies due to differences in patient populations, differences in the way patients may have been treated with medications, and differences in MRI scanners, software and methods of calculation.

In conclusion, our study indicated that longitudinal brain atrophy could occur even in clinically stable patients with AQP4+NMOSD. A recent study reported that combined ganglion cell and inner plexiform layer loss was independent of optic neuritis attacks in AQP4+NMOSD.[38] Another study also showed subclinical neurodegeneration as revealed by optical coherence tomography in patients with AQP4+NMOSD.[39] Therefore, subclinical neurodegeneration may occur not only in the retina, but also in the brain in patients with AQP4+NMOSD. Our data suggest dying back degeneration after the long cord lesion

Table 6 Clinical characteristics and brain volumes at MRI-1 and MRI-2 and the annualised atrophy rate in age-matched patients with AQP4+NMOSD and MS without clinical relapses and disability progression between MRI-1 and MRI-2

	AQP4+NMOSD (n=20)	RRMS (n=20)	p value
ΔEDSS (MRI-2 − MRI-1)	0.0 (0.0) (-2.0–0.5)	0.0 (0.38) (-0.5 to 0.5)	0.69
Years from MRI-1 to MRI-2	3.1 (3.0) (1.0–6.3)	2.0 (1.8) (1.0–5.1)	0.27
At MRI-1			
ICV*10^{-3} (mL)	1.3 (0.12) (1.2–1.5)	1.4 (0.18) (1.2–1.7)	0.12
NLV (mL)	1.7 (4.4) (0.0–33.2)	11.1 (12.6) (1.8–46.7)	<0.001*
NGV*10^{-3} (mL)	0.44 (0.037) (0.39–0.49)	0.41 (0.059) (0.32–0.49)	0.006*
NWV*10^{-3} (mL)	0.31 (0.027) (0.24–0.33)	0.29 (0.031) (0.25–0.33)	0.002*
NBV*10^{-3} (mL)	0.75 (0.055) (0.66–0.81)	0.69 (0.062) (0.59–0.80)	0.001*
At MRI-2			
ICV*10^{-3} (mL)	1.3 (0.12) (1.2–1.5)	1.4 (0.18) (1.2–1.7)	0.17
NLV (mL)	2.8 (8.3) (0.0–81.0)	12.6 (17.0) (2.1–187)	0.001*
NGV*10^{-3} (mL)	0.44 (0.045) (0.37–0.49)	0.41 (0.054) (0.33–0.46)	0.004*
NWV*10^{-3} (mL)	0.31 (0.027) (0.22–0.33)	0.29 (0.035) (0.25–0.34)	0.010*
NBV*10^{-3} (mL)	0.76 (0.067) (0.64–0.80)	0.68 (0.067) (0.58–0.78)	0.003*
Annualised atrophy rate			
NGV (%)	0.68 (0.76) (−1.7 to 2.6)	0.76 (1.6) (−2.5 to 4.5)	0.82
NWV (%)	0.26 (0.79) (−1.4 to 4.2)	−0.087 (2.1) (−2.1 to 1.9)	0.2
NBV (%)	0.50 (0.69) (−0.20 to 2.1)	0.44 (0.63) (−0.75 to 1.8)	0.86

Data are presented as median number (%) or (IQR) (range). *p<0.05.
ΔEDSS= EDSS at MRI-2 minus EDSS at MRI-1.

Annualized atrophy rate of X is defined as follows: $\frac{(\text{X at 1st MRI scan} - \text{X at 2nd MRI scan}) \times 12}{(\text{X at 1st MRI scan}) \times (\text{Months between 1st and 2nd MRI scan})}$, X= NGV, NWV or NBV.

AQP4+NMOSD, anti-aquaporin-4 antibody-positive neuromyelitis optica spectrum disorder disease; EDSS, Kurtzke's Expanded Disability Status Scale; ICV, intracranial volume; NBV, normalised brain volume; NGV, normalised grey matter volume; NLV, normalised lesion volume; NWV, normalised white matter volume.

could cause the grey matter atrophy in the brain in patients with AQP4+NMOSD. Future studies should shed light on brain atrophy not only in patients with MS, but also in patients with AQP4+NMOSD. To exclude the treatment effect,[40] prospective studies should include longer follow-up MRI duration and HCs and compare the brain atrophy rate by the treatment difference between steroids and more efficacious medication in patients with AQP4+NMOSD.

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Supplemental material

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Supplemental table 1. Details of MRI.

	3DT1	FLAIR	MRP reconstructed from 3D-FLAIR
Tesla	1.5	1.5	1.5
FOV (mm×mm)	240 × 240	220 × 220	250 × 250
Number of sections	248	32	75–105
Section thickness (mm)	1.4	4	2
TR (ms)	7	11000–10002	6000
TE (ms)	2.9–3.0	120.2–128.6	131
TI (ms)	0–420	2400	1852
NSA	0.9921–1	1	1
Echo train length	1	1	160
FA	15°	90°	90°

*$P < 0.05$. 3D-FLAIR: fluid attenuated inversion recovery three-dimensional images; 3DT1: T1-weighted three-dimensional images; FA: flip angle; FLAIR: fluid attenuated inversion recovery; FOV: field of view; NSA: Number of signals acquired; MPR: multiplanar reconstruction; TR: repetition time; TE: echo time; TI: inversion time.

Supplemental table 2. Details of ANCOVA analysis in each study

	Number of patients (AQPAD+NMOSD vs MS)	*P* value	
		of the parallel slopes assumption	after ANCOVA
NBV in **study 1** using controlled for factors significant different			
OCB included	27 vs 49	0.80	0.24
OCB excluded	36 vs 60	0.64	0.45
NGV in **study 1** using controlled for factors significant different			
OCB included	27 vs 49	0.99	0.53
OCB excluded	36 vs 60	0.70	0.82
NWV in **study 1** using controlled for factors significant different			
OCB included	27 vs 49	0.24	0.061
OCB excluded	36 vs 60	0.30	0.091
NBV in **study 2** using controlled for factors significant different			
OCB included	19 vs 36	0.64	0.18
OCB excluded	27 vs 45	0.073	0.29
NGV in **study 2** using controlled for factors significant different			
OCB included	19 vs 36	0.93	0.25
OCB excluded	27 vs 45	0.19	0.34
NWV in **study 2** using controlled for factors significant different			
OCB included	19 vs 36	0.030*	Not obtained
OCB excluded	27 vs 45	0.002*	Not obtained

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NBV in **study 3** using controlled for factors significant different				
	OCB included	18 vs 20	0.31	0.31
	OCB excluded	20 vs 20	0.65	0.13
NGV in **study 3** using controlled for factors significant different				
	OCB included	18 vs 20	0.15	0.34
	OCB excluded	20 vs 20	0.63	0.79
NWV in **study 3** using controlled for factors significant different				
	OCB included	18 vs 20	0.80	0.96
	OCB excluded	20 vs 20	0.97	0.85

*$P < 0.05$.

ANCOVA: an analysis of covariance: AQP4+NMOSD: anti-aquaporin-4 antibody-positive neuromyelitis optica spectrum disorder disease; DMD: disease modifying drug; MS: multiple sclerosis. NBV: normalized brain volume; NGV: normalized grey matter volume; NLV: normalized lesion volume; NWV: normalized white matter volume; OCB: oligoclonal bands.

We performed with or without some significant different items because the number of samples were changed due to them.

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NSA	0.9921–1	1	1
Echo train length	1	1	160
FA	15°	90°	90°

*P < 0.05. 3D-FLAIR: fluid attenuated inversion recovery three-dimensional images; 3DT1: T1-weighted three-dimensional images; FA: flip angle; FLAIR: fluid attenuated inversion recovery; FOV: field of view; NSA: Number of signals acquired; MPR: multiplanar reconstruction; TR: repetition time; TE: echo time; TI: inversion time.

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NWV in **study 1** using controlled for factors significant different			
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OCB included	19 vs 36	0.64	0.18
OCB excluded	27 vs 45	0.073	0.29
NGV in **study 2** using controlled for factors significant different			
OCB included	19 vs 36	0.93	0.25
OCB excluded	27 vs 45	0.19	0.34
NWV in **study 2** using controlled for factors significant different			
OCB included	19 vs 36	0.030*	Not obtained
OCB excluded	27 vs 45	0.002*	Not obtained

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NBV in **study 3** using controlled for factors significant different				
	OCB included	18 vs 20	0.31	0.31
	OCB excluded	20 vs 20	0.65	0.13
NGV in **study 3** using controlled for factors significant different				
	OCB included	18 vs 20	0.15	0.34
	OCB excluded	20 vs 20	0.63	0.79
NWV in **study 3** using controlled for factors significant different				
	OCB included	18 vs 20	0.80	0.96
	OCB excluded	20 vs 20	0.97	0.85

*$P < 0.05$.

ANCOVA: an analysis of covariance: AQP4+NMOSD: anti-aquaporin-4 antibody-positive neuromyelitis optica spectrum disorder disease; DMD: disease modifying drug; MS: multiple sclerosis. NBV: normalized brain volume; NGV: normalized grey matter volume; NLV: normalized lesion volume; NWV: normalized white matter volume; OCB: oligoclonal bands.

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