European Journal of Radiology 94 (2017) 25–30

Contents lists available at ScienceDirect

European Journal of Radiology

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

Analysis of brain and spinal cord lesions to occult brain damage in seropositive and seronegative neuromyelitis optica

Jie Sun[a,1], Xianting Sun[a,1], Ningnannan Zhang[a], Qiuhui Wang[a], Huanhuan Cai[a], Yuan Qi[b], Ting Li[b], Wen Qin[a], Chunshui Yu[a,*]

[a] Department of Radiology and Tianjin Key Laboratory of Functional Imaging, Tianjin Medical University General Hospital, Tianjin 300052, China
[b] Department of Neurology, Tianjin Medical University General Hospital, Tianjin 300052, China

ARTICLE INFO

Keywords:
Neuromyelitis optica
Aquaporin-4 antibody
Magnetic resonance imaging
Lesions
Occult damage
Brain

ABSTRACT

Objectives: According to aquaporin-4 antibody (AQP4-Ab), neuromyelitis optica (NMO) can be divided into seropositive and seronegative subgroups. The purpose of this study was to a) compare the distribution of spinal cord and brain magnetic resonance imaging (MRI) lesions between seropositive and seronegative NMO patients; b) explore occult brain damage in seropositive and seronegative NMO patients; and c) explore the contribution of visible lesions to occult grey and white matter damage in seropositive and seronegative NMO patients.

Materials and methods: Twenty-two AQP4-Ab seropositive and 14 seronegative NMO patients and 30 healthy controls were included in the study. Two neuroradiologists independently measured the brain lesion volume (BLV) and the length of spinal cord lesion (LSCL) and recorded the region of brain lesions. The normal-appearing grey matter volume (NAGM-GMV) and white matter fractional anisotropy (NAWM-FA) were calculated for each subject to evaluate occult brain damage.

Results: The seropositive patients displayed more extensive damage in the spinal cord than the seronegative patients, and the seronegative group had a higher proportion of patients with brainstem lesions (28.57%) than the seropositive group (4.55%, $P = 0.064$). Both NMO subgroups exhibited reduced NAGM-GMV and NAWM-FA compared with the healthy controls. NAGM-GMV was negatively correlated with LSCL in the seropositive group ($r_s = -0.444$, $P = 0.044$) and with BLV in the seronegative group ($r_s = -0.768$, $P = 0.002$). NAWM-FA was also negatively correlated with BLV in the seropositive group ($r_s = -0.682$, $P < 0.001$).

Conclusion: Our findings suggest that the occult brain damage in these two NMO subgroups may be due to different mechanisms, which need to be further clarified.

1. Introduction

Neuromyelitis optica (NMO) is an acquired inflammatory disorder characterized by recurrent optic neuritis and longitudinally extensive spinal cord lesions [1]. An antibody against aquaporin-4 (AQP4-Ab) has been identified as a highly specific serum marker [2], and this protein is thought to play a pathogenic role in NMO [3–5]. However, not all NMO patients are seropositive for AQP4-Ab, and seronegative NMO patients are also observed [6–9]. This suggests that seropositive and seronegative NMO patients may exhibit different mechanisms of demyelination.

Although brain lesions are commonly observed in both subgroups [10], recent studies have shown that seropositive NMO patients tend to present significantly longer spinal cord lesions [11,12]. The occult damage in normal-appearing grey matter (NAGM) and in normal-appearing white matter (NAWM) of the brain has frequently been reported in NMO [13–16]. Axonal degeneration secondary to visible lesions in the spinal cord and the brain has been considered as important mechanism for occult brain damage [13,17,18]. However, the relationships between these visible lesions and occult brain damage in these two subgroups remain largely unknown. Our objectives were to assess differences in brain and spinal cord lesions in seropositive and seronegative NMO patients and to test possible differences in NAGM and NAWM in these groups.

2. Materials and methods

2.1. Subjects

This study included 22 AQP4-Ab seropositive NMO patients, 14

* Corresponding author at: Department of Radiology, Tianjin Medical University General Hospital, No. 154, Anshan Road, Heping District, Tianjin 300052, China.
E-mail addresses: chunshuiyu@vip.163.com, chunshuiyu@tijmu.edu.cn (C. Yu).
[1] These authors contributed equally to the manuscript.

http://dx.doi.org/10.1016/j.ejrad.2017.07.002
Received 20 August 2016; Received in revised form 29 April 2017; Accepted 7 July 2017

seronegative patients and 30 sex- and age-matched healthy controls (HCs). All subjects signed a written, informed consent form that was approved by the Medical Research Ethics Committee of Tianjin Medical University. NMO patients were diagnosed according to the revised Wingerchuk criteria [19]. Moreover, a retrospective evaluation confirmed that they also fulfilled the 2015 criteria for NMO spectrum disorder (NMOSD) [20]. All patients were relapsing form of NMO and were recruited during chronic stage without taking corticosteroid within 1 month. For each patient, the following information was collected: age, gender, age of onset, disease duration, annualized relapses rate, Expanded Disability Status Scale score (EDSS), AQP4-Ab status and brain and spinal MRI scans. Patients were excluded from the study if they met any of the following criteria: (1) acute stage; (2) complication by other autoimmune disorders; (3) MRI abnormalities other than NMO in the brain; or (4) bad image quality.

2.2. AQP4 antibody testing

The AQP4 antibody was tested using a cell-based array through quantitative flow cytometry. For each NMO patient, a serum sample was isolated from whole blood and stored in a −80 °C freezer. A sample of 293 human embryonic kidney (HEK) cells expressing AQP4-M23-EGFP-fused genes was fixed. After incubation with Alexa Fluor 568–conjugated anti-human IgG, an Olympus IX-71 fluorescence microscope was used to detect the binding of the sera to the cells [21]. The results were independently scored by two investigators.

2.3. MRI data acquisition

MRI data were acquired using a 3.0-T MR system (Discovery MR750, General Electric, Milwaukee, WI, USA). Axial brain T2-weighted images were acquired using a fast spin-echo sequence with the following parameters: repetition time (TR)/echo time (TE) = 6816/103 ms; flip angle = 142°; field of view (FOV) = 240 mm × 240 mm; matrix = 512 × 512; slice thickness = 6 mm; gap = 1.5 mm; and 20 axial slices. Sagittal and axial spinal T2-weighted images were acquired for spinal cord lesion analysis. Sagittal spinal T2-weighted images were acquired using a fast recovery fast spin-echo sequence with the following parameters: TR/TE = 2500/120 ms; number of excitation (NEX) = 4; echo train length = 21; FOV = 240 mm × 240 mm; matrix = 320 × 224; slice thickness = 3 mm; gap = 0.3 mm; and 9 slices. Axial spinal T2-weighted images were acquired using a fast spin-echo sequence with the following parameters: TR/TE = 3520/120 ms; NEX = 2; echo train length = 21; FOV = 200 mm × 200 mm; matrix = 320 × 256; slice thickness = 5 mm; gap = 3.5 mm; and 15 slices. Sagittal brain 3D T1-weighted images were acquired through a brain volume sequence with the following parameters: TR/TE = 8.2/3.2 ms; inversion time = 450 ms; flip angle = 12°; FOV = 256 mm × 256 mm; matrix = 256 × 256; slice thickness = 1 mm; no gap; and 188 sagittal slices. Diffusion MRI data for the brain were acquired using a spin-echo single-shot echo planar imaging (EPI) sequence with the following parameters: TR/TE = 5800/77 ms; FOV = 256 mm × 256 mm; matrix = 128 × 128; slice thickness = 3 mm with no gap; 48 axial slices; 25 encoding diffusion directions with two values of b (b = 1000 and 2000 s/mm^2) for each direction; and 10 non-diffusion-weighted images (b = 0 s/mm^2). Only diffusion images of b = 0 and 1000 s/mm^2 were used for diffusion fitting. All images were visually inspected to ensure that only images without visible artifacts were included in the subsequent analyses.

2.4. Image analysis

The brain lesion volume (BLV) and the length of spinal cord lesion (LSCL) were independently measured by two neuroradiologists. For each patient, each brain lesion was manually outlined in each slice in T2-weighted images using MRIcron software (http://www.mccauslandcenter.sc.edu/CRNL/). Brain lesions were classified according to their location as subcortical lesions, periventricular lesions, basal ganglia lesions, brainstem lesions or cerebellum lesions. LSCL (vertebral segments) was estimated in sagittal T2-weighted images with reference to axial T2-weighted images. Inter-observer agreement for BLV was assessed using an intraclass correlation coefficient (ICC). Inter-observer agreement for spinal lesion segments was assessed based on weighted k values. The mean values of BLV and spinal lesion length in each patient obtained from the two investigators were considered to be the values for that patient.

The T2-weighted images of each patient were registered at the Montreal Neurological Institute (MNI) space using a linear transformation method (FLIRT [FMRIB's linear image registration tool]). The linear transformation matrix was then applied to each patient's lesion mask to create standardized individual lesion masks, which were then summed to generate a normalized general lesion mask.

The structural MR images were segmented into grey matter (GM), white matter (WM), and CSF using the standard unified segmentation model with the voxel-based morphometry (VBM8) toolbox (http://dbm.neuro.uni-jena.de/vbm/) in Statistical Parametric Mapping (SPM8, http://www.fil.ion.ucl.ac.uk/spm/). NAGM images for each subject were acquired by removing the general lesion from GM images, and we then determined NAGM-GMV by summing the intensity.

Eddy current distortions and motion artifacts from DTI data were corrected using FMRIB's Diffusion Toolbox (FDT; FSL 4.1.4, www.fmrib.ox.ac.uk/fsl). Then, DTIfit was used to fit a diffusion tensor to each voxel independently and generate a fraction anisotropy (FA) map for each subject. The FA image of each subject was aligned to a target FA image (FMRIB58_FA) via nonlinear registration and then transformed into the MNI152 space via affine registration using the tract-based spatial statistics (TBSS) method [22,23]. Based on the mean FA map of all subjects, a WM mask was created by collecting voxels with FA > 0.2. The NAWM mask was acquired by removing the general lesion mask from the WM mask. The NAWM mask in the standard space was transferred to the individual space, and the NAWM-FA was extracted from each subject.

2.5. Statistics

Statistical analyses were performed using the Statistical Package for the Social Sciences version 19.0 (SPSS, Chicago, IL, USA). Variables with a normal distribution (age, age of onset and EDSS) are presented as the mean ± standard deviation; variables without a normal distribution are presented as the median (quartile range). The Mann-Whitney *U* test was used to detect differences in the disease course, relapse frequency, BLV and LSCL between the seropositive and seronegative NMO groups. Fisher's exact test was employed to compare the ratios of patients with brain lesions in each location between the seropositive and seronegative NMO groups. Student's *t*-test was used to detect significant differences in the age of onset and EDSS scale between the two NMO groups. A One-way analysis of variance (ANOVA) was used to detect differences in NAGM-GMV and mean NAWM-FA values between the seropositive NMO patients, the seronegative NMO patients and the HC group. Spearman correlation was applied to explore the association between brain or spinal lesions and NAGM or NAWM damage in each NMO subgroup. $P < 0.05$ was considered statistically significant.

3. Results

The demographic, clinical and MRI features of the subjects are summarized in Table 1. There were no significant differences in gender (chi-square test, $\chi^2 = 3.939$, $P = 0.140$), age (ANOVA, F = 0.015, $P = 0.985$) and educational years (Kruskal-Wallis test, $\chi^2 = 3.367$, $P = 0.186$) among the three groups. Among the NMO patients, 61.11% (22/36) were seropositive for AQP4-Ab. The age of onset did not significantly differ between the seropositive (41.3 ± 14.4 years) and