antigen-specific immunoglobulin E (IgE) positivity or (2) coexistent atopic diseases [4], while the second survey also permitted IgE specific for other common environmental allergens besides mite antigens [5]. On the one hand, increasing numbers of AM cases have been reported outside Japan [2,3,6,8], while on the other hand, there is a need for early introduction of disease-modifying drugs for MS or even clinically isolated syndrome. Therefore, demand for more evidence-based criteria for AM is growing, to ensure the correct differentiation of AM from myelitis-onset MS as early as possible. In the present study, we first compared the clinical and laboratory features between patients with AM and those with myelitis-onset MS, which is usually the most difficult differential diagnosis for AM, especially in Asians. Second, we established the first diagnostic criteria for AM based on comparisons of the data, to achieve a reasonably high sensitivity and specificity for diagnosis of AM vs. myelitis-onset MS.

2. Subjects and methods

2.1. Subjects

The medical records of all patients who had been referred to the Department of Neurology, Kyushu University Hospital from January 1996 to September 2010 were reviewed. Consecutive patients with AM and MS who met the following criteria were enrolled. For AM patients, the former (2003) empirically defined AM inclusion criteria were used, which defined AM as myelitis of unknown cause, after exclusion of other diseases, with either (1) hyperIgEemia plus allergen-specific IgE positivity for any allergen, or (2) coexistent or past atopic diseases [5]. All MS patients met the 2005 McDonald criteria [9] and those presenting with myelitis at onset (myelitis-onset MS) who had been examined for allergen-specific IgE were exclusively used in the present study. All enrolled AM and MS patients were confirmed to be negative for anti-aquaporin 4 (AQP4) antibodies.

For both disease groups, AM and MS, the existence of myelitis was confirmed by spinal cord magnetic resonance imaging (MRI), motor-evoked potentials (MEPs), somatosensory-evoked potentials (SEPs), or the findings of neurological examinations in the absence of explainable brain MRI lesions, such as exaggerated deep tendon reflexes, motor weakness of the four limbs without involvement of the cranial region, sensory levels explainable for the spinal cord involvement, and Lhermitte's sign. Measurement of allergen-specific IgE and MRI data of the brain and spinal cord to judge dissemination in the space defined in the revised McDonald criteria [9] were mandatory for individuals in both enrolled groups. For all enrolled cases, the following diseases were considered exclusion criteria: collagen-vasculitis, HTLV-1-associated myelopathy, sarcoidosis, neuromyelitis optica, neurosyphilis, parasitic myelitis, cervical spondylotic myelopathy, spinal cord tumor, and spinal vascular malformation. For further discrimination of clinical and laboratory findings between AM and myelitis-onset MS, only AM patients who were followed up and evaluated by brain MRI more than 5 years from their disease onset, and who did not fulfill the Barkhof MRI criteria for MS [10], were used for comparison with myelitis-onset MS. AM and MS patients for whom there were available data for two or all of the three below-mentioned revised positive supporting criteria (1–3) and the negative supporting criterion (4) were used for sensitivity and specificity evaluation. Written informed consent for using clinical information was obtained from all the participants.

2.2. Clinical and immunological tests

Clinical data were collected from the hospital discharge records or the medical records of the outpatient clinic, which included age of onset, disease onset, and disease course. The severity of the clinical manifestation was evaluated at disease onset and at the latest examination using the Expanded Disability Status Scale (EDSS) of Kurtzke [11]. For measurement of allergen-specific IgE, the following allergens were included: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cedar pollen, *Candida*, egg white, milk, wheat, rice, soybean, mold, *Anisakis*, animal skins, house dust, and others according to the patients' atopic diseases. All of the enrolled patients were measured for at least nine common environmental allergens including mite antigens. Bronchial asthma, atopic dermatitis, allergic rhinitis, food allergy, and allergic conjunctivitis were regarded as atopic diseases in the present study. The serum level of IgE and the blood eosinophil count were examined, and 240 U/ml and 500/ml were used as the upper normal limits, respectively. The upper normal limits of IL9 and CCL11/eotaxin in the CSF, as measured by a fluorescent bead-based immunoassay, were 14.0 pg/ml and 2.2 pg/ml, respectively, based on a preliminary study of patients with non-inflammatory neurological disease [7]. Measurement of serum anti-AQP4 antibodies was conducted as previously described [12,13]. Any possibility of other diseases was excluded by comprehensive examination of serum antibodies for parasites, serum angiotensin-converting enzyme and lysozyme, serum anti-nuclear antigen antibodies, serum anti-SS-A/B antibodies, anti-HTLV-1 antibodies in serum and CSF, serologic tests for syphilis, *Treponema pallidum* hemagglutination, and by evaluation of chest X-rays, and brain and spinal cord MRI.

2.3. Electrophysiological tests

MEP, SEP, and visual-evoked potentials (VEP) were recorded as described previously [14].

2.4. Magnetic resonance imaging

Fulfillment of the Barkhof criteria [10], the criteria for dissemination in space, was judged for all the participants according to the distribution and number of T2 lesions. All MRI scans were performed as described previously [12,15]. For the evaluation of spinal cord lesions, the initial MRI scan was reviewed. For the evaluation of brain lesions, the brain MRI scan that was initially available and one conducted more than 5 years after disease onset were used.

2.5. Statistical analysis

Statistical analyses of the numerical variables among the patients' demographic features were performed using the Kruskal–Wallis H test. Comparison of ratios between the two groups was conducted using the χ^2 test or Fisher's exact probability test. We did not apply a logistic regression model for the selection of parameters to be included in the new AM diagnostic criteria due to the small sample size. Instead, those clinical or laboratory parameters that were statistically significantly distinct between AM and MS patients were manually included in the new AM diagnostic criteria to obtain high sensitivity and specificity. Sensitivity meant the probability of the new diagnostic criteria exclusively detecting AM cases from the mixed pool of both AM and myelitis-onset MS patients for whom there were sufficient data for evaluation. Specificity meant the probability that the new AM diagnostic criteria would exclude enrolled myelitis-onset MS cases. In addition, the positive predictive value was calculated by dividing the number of AM cases who also met the new AM criteria by the number of all the cases, including MS cases, who fulfilled the new criteria. The negative predictive value was calculated by dividing the number of MS cases who were successfully excluded by the new AM criteria by the number of all the cases who did not meet the new AM criteria. All analyses were performed using JMP 8.0 (SAS Institute, Cary, NC). Statistical significance was set at $p<0.05$.

3. Results

3.1. Comparison of the demographic features between AM and myelitis-onset MS patients

During the study period, there were 69 cases who fulfilled the former (2003) empirical definition of AM [5] (Fig. 1). Among MS cases who met the revised McDonald criteria [9], there were 90 cases whose disease started with spinal cord lesions; among them, 52 cases were measured for serum antigen-specific IgE for common environmental allergens. Because of insufficient data being available for enrollment, one case whose anti-AQP4 antibody status was unknown was removed; this left 51 MS cases for further analyses. Among the primary sorted 69 AM patients and 51 myelitis-onset MS patients, the baseline (initial) brain MRI scans in our department were available in 38 (55.1%) AM and 45 (88.2%) myelitis-onset MS patients (taken 1.3 ± 1.6 years and 2.7 ± 3.0 years from the disease onset, respectively, $p = 0.0808$); the frequency of fulfillment of the Barkhof criteria was significantly lower in AM patients than in myelitis-onset MS patients (0/38 (0.0%) vs. 11/45 (24.4%), respectively, $p < 0.0011$).

Among the 69 AM patients, 26 were followed up for more than 5 years. Eighteen of these patients underwent brain MRI after at least 5 years; one of these fulfilled the Barkhof criteria at that time. Among the other 17 AM patients, one was not eligible because of the lack of serum samples for anti-AQP4 antibody measurement, leaving 16 patients. Among these 16 AM patients, spinal cord involvement was confirmed by spinal cord MRI in 10 patients (62.5%), by MEP/SEP in four patients (25.0%), and by clinical evaluation in the other two patients (12.5%), one of whom had an exaggerated tendon reflex in four limbs, motor weakness of limb muscles without involvement of the cranial region, Lhermitte's sign and a sensory level, while the other had exaggerated tendon reflex in four limbs, motor weakness of limb muscles without involvement of the cranial region, and a sensory level.

The demographic features of the enrolled 16 AM and 51 MS patients are shown in Table 1. There was no patient who had undergone a spinal cord biopsy who was followed up for more than 5 years. Although the AM patients comprised both genders almost equally and the MS group comprised a larger percentage of females, there was no significant difference between the two. The age of onset for both disease groups was, on average, the early to middle fourth decade. AM patients were significantly more likely to have a current or past history of atopic disease at the time of disease onset compared with myelitis-onset MS patients. Chronic or step-wise onset of the disease was most common in AM, while acute or subacute onset was predominant in myelitis-onset MS. Patients with a monophasic disease course tended to occur more frequently in the AM group than in the myelitis-onset MS group, while those with a relapsing or fluctuating course were significantly more likely to have myelitis-onset MS. The disease duration and EDSS scores at disease onset or at the most recent examination were similar in the two groups. The serum level of total IgE was significantly higher in AM patients than in MS patients, while the blood eosinophil counts were not different between the two groups. For patients whose CSF was examined, the levels of IL9 and CCL11/eotaxin were significantly higher in AM patients than in MS patients. Oligoclonal IgG bands (OCB) were seen in 30.4% of myelitis-onset MS patients but none of the AM patients. There was no significant difference in the frequency of MEP central abnormalities in upper extremities and VEP abnormalities between the AM group and the myelitis-onset MS group. Spinal cord MRI revealed that posterior column lesions in the cervical spinal cord were detected at a similar frequency in both groups.

3.2. Establishment of the diagnostic criteria for AM

Based on the above-mentioned comparison data of the clinical, immunological, electrophysiological, and MRI parameters between the AM and MS groups, we have generated the first evidence-based diagnostic criteria for AM (Table 2). As absolute criteria, in addition to myelitis of unknown etiology excluding diseases mentioned in the footnote to Table 2, we adopted serum positivity for IgE specific to common environmental allergens, plus negativity for brain MRI lesions fulfilling the Barkhof criteria for MS, because these two items showed a statistically significant difference in frequency between AM patients and myelitis-onset MS patients. Although there was no patient with data from a spinal cord biopsy in the present series, we regarded the existence of perivascular lymphocyte cuffings with various degrees of eosinophil infiltration as the pathological criteria, according to previous pathological reports [16,17]. Our supporting

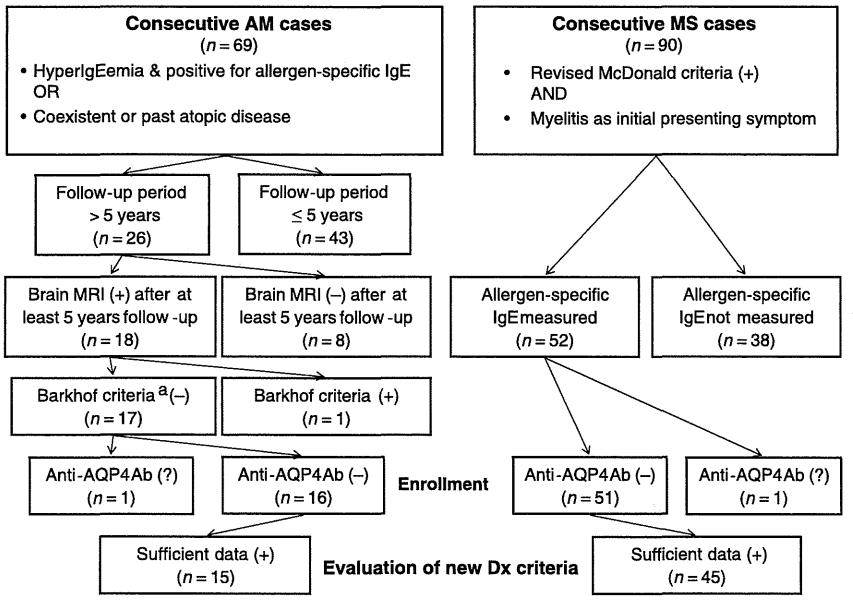

Fig. 1. Enrollment of patients with atopic myelitis (AM) and those with myelitis-onset multiple sclerosis (MS). From consecutive patients with these diseases, 16 AM cases and 51 myelitis-onset MS patients were enrolled for comparison of their demographic features. After establishing the new AM criteria, patients with sufficient data were selected to test the efficacy of the criteria. [a]Barkhof et al. [10]. Ab, antibodies; Dx, diagnostic.

Table 1
Demographic features of enrolled patients with either atopic myelitis or multiple sclerosis.

	AM[a]	Myelitis-onset MS[b]	*p* value
Number of patients	16	51	NA
Gender (male/female)	8/8 (1:1.00)	16/35 (1:2.19)	NS
Age of onset (years old)	35.3 ± 10.3	32.4 ± 11.5	NS
Disease duration (years)	9.1 ± 3.5	10.4 ± 8.1	NS
Present or past history of atopic diseases	14/16 (87.5%)	22/50 (44.0%)	0.0032
Clinical manifestations			
Disease onset			
Acute	2/16 (12.5%)	16/51 (31.4%)	NS
Subacute	2/16 (12.5%)	23/51 (45.1%)	0.0204
Chronic/step-wise	12/16 (75.0%)	12/51 (23.5%)	0.0006
Disease course			
Monophasic	2/15 (13.3%)	0/49 (0.0%)	0.0521
Relapsing/fluctuating	10/15 (66.7%)	44/49 (89.8%)	0.0456
Progressive	3/15 (20.0%)	5/49 (10.2%)	NS
EDSS score at the initial attack	4.1 ± 1.6	3.6 ± 2.1	NS
EDSS score at the final follow-up	3.1 ± 1.4	3.3 ± 2.6	NS
Blood or serum findings			
Blood eosinophil count (/ml)	383.4 ± 355.6	303.8 ± 395.6	NS
Hypereosinophilia[c] (+)	3/16 (18.8%)	9/46 (19.6%)	NS
Serum total IgE (U/ml)	1762 ± 3255	833 ± 4250	<0.0001
HyperIgEemia[d] (+)	12/16 (75.0%)	13/50 (26.0%)	0.0008
Allergen-specific IgE (+)	16/16 (100.0%)	27/51 (52.9%)	0.0003
CSF findings			
IL9 (pg/ml)	15.2 ± 4.7	9.8 ± 3.2	0.0357
Increased IL9[e] (+)	5/8 (62.5%)	1/8 (12.5%)	NS
CCL11/eotaxin (pg/ml)	4.1 ± 0.3	2.0 ± 0.5	0.0008
Increased CCL11/eotaxin[f] (+)	8/8 (100.0%)	3/8 (37.5%)	0.0256
OCB (+)	0/15 (0.0%)	14/46 (30.4%)	0.0137
Electrophysiological findings			
MEP central abnormalities in upper extremities	9/15 (60.0%)	19/36 (52.8%)	NS
VEP abnormalities	3/11 (27.3%)	16/39 (41.0%)	NS
MRI findings and others			
Cervical cord lesions in posterior column	4/16 (25.0%)	12/49 (24.5%)	NS

[a]Those who fulfilled the former (2003) empirical inclusion criteria for AM and were followed up for more than 5 years.
[b]MS patients presenting with myelitis as the initial symptom and who fulfilled the revised McDonald criteria [9].
[c]≥500/ml.
[d]≥240 U/ml.
[e]≥14.0 pg/ml.
[f]≥2.2 pg/ml.
AM = atopic myelitis; CSF = cerebrospinal fluid; EDSS = Expanded Disability Status Scale of Kurtzke; IgE = immunoglobulin E; IL = interleukin; MRI = magnetic resonance imaging; MEP = motor-evoked potential; MS = multiple sclerosis; NA = not applicable; NS = not significant; OCB = oligoclonal bands; VEP = visual-evoked potential.

criteria comprise the following three supporting positive findings: 1) present or past history of atopic disease; 2) serum hyperIgEemia; and 3) increased level of IL9 or CCL11/eotaxin in the CSF, and the following one supporting negative finding: no OCB in the CSF.

Definite AM is therefore defined as follows: (1) cases who meet the absolute criteria plus the pathological criteria, or (2) those who meet the absolute criteria plus two or all of the three supporting positive criteria plus the one supporting negative criterion. Probable cases of AM are defined in Table 2.

3.3. Sensitivity and specificity of the new diagnostic criteria for AM

To test the efficacy of the new AM diagnostic criteria, we selected the cases with sufficient data to judge the fulfillment of the new criteria from the same set of patients. As shown in Fig. 1, 15 AM cases and 45 myelitis-onset MS cases were enrolled. When we applied these AM diagnostic criteria to both the AM cases and myelitis-onset MS cases, 14 (93.3%) of the 15 AM cases met the definite criteria while three of the 45 myelitis-onset MS cases (6.7%) fulfilled the criteria (Table 3-1). Therefore, the sensitivity of this AM criteria was 93.3% and the specificity was 93.3%. Moreover, the positive predictive value was 82.4% while the negative predictive value was 97.7% (Table 3-2).

Table 2
New diagnostic criteria for atopic myelitis.

Criteria	
Absolute criteria	All three of the following are essential. 1) Myelitis with unknown etiology[a] 2) Positive for allergen-specific IgE 3) Negative for Barkhof brain MRI lesions[b]
Pathological criteria	Existence on spinal cord biopsy samples of perivascular lymphocyte cuffings with various degrees of eosinophil infiltration, sometimes accompanied by granuloma.
Supporting criteria Positive findings	1) Present and/or past history of atopic disease 2) Serum hyperIgEemia (≥240 U/ml) 3) Increased level of IL9 (≥14.0 pg/ml) or CCL11/eotaxin (≥2.2 pg/ml) in the CSF
Negative findings	4) No OCB in the CSF
Diagnosis	
Definite	1) Absolute criteria + Major pathological criteria OR 2) Absolute criteria + two or all of the Supporting criteria (1–3) + the Supporting criterion (4)
Probable	1) Absolute criteria + one of the Supporting criteria (1–3) + the Supporting criterion (4) OR 2) Absolute criteria + two or all of the Supporting criteria (1–3)

CSF = cerebrospinal fluid; IgE = immunoglobulin E; IL = interleukin; MEP = motor-evoked potential; MRI = magnetic resonance imaging; OCB = oligoclonal IgG bands; SEP = sensory-evoked potential.
[a]The presence of myelitis should be confirmed by neurologically abnormal sign(s) (limb hyperreflexia and/or sensory levels), MEP, and/or SEP abnormalities suggestive of central nervous system lesions, or spinal cord lesions on MRI. The following diseases should be excluded: parasitic myelitis, multiple sclerosis, collagen-vascular diseases, HTLV-1-associated myelopathy, sarcoidosis, neuromyelitis optica, neurosyphilis, cervical spondylotic myelopathy, spinal cord tumor, spinal vascular malformation.
[b]Barkhof et al. [10].

4. Discussion

This study is the first to compare clinical and laboratory findings between patients with AM and those with myelitis-onset MS, who were all seronegative for anti-AQP4 antibodies. The neurological features of the enrolled AM patients were similar to those in previous nationwide surveys [4,5]. In the present study, we found that,

Table 3-1
Application of the new diagnostic criteria for atopic myelitis.

	AM ($n=15$)	Myelitis-onset MS ($n=45$)
Fulfillment of the new AM diagnostic criteria		
(+)	14	3
(−)	1	42

Table 3-2
Utility of the new diagnostic criteria for atopic myelitis.

Sensitivity	93.3%
Specificity	93.3%
Positive predictive value	82.4%
Negative predictive value	97.7%

AM = atopic myelitis; MS = multiple sclerosis.

compared with myelitis-onset MS patients, AM patients were significantly more likely to have a present and/or past history of atopic disease, serum hyperIgEemia, and allergen-specific IgE, and showed significantly higher levels of IL9 and CCL11/eotaxin in the CSF. By contrast, OCB was significantly less frequent in AM patients than in myelitis-onset MS patients. Moreover, before filtering empirically diagnosed AM cases with the Barkhof criteria, the frequency of fulfillment of the Barkhof criteria at baseline (first available) MRI was found to be significantly lower in AM patients than in myelitis-onset MS patients (0.0% vs. 24.4%, $p<0.0011$). Therefore, it was considered reasonable to incorporate these items, reflecting the characteristic features of each condition, into the first evidence-based diagnostic criteria.

Although blood eosinophilia is one of the distinctive features of Churg–Strauss syndrome [5], the frequency of blood eosinophilia was similar between AM patients and myelitis-onset MS patients. Thus, this was not included in the present criteria. In the present study, we did not find a statistically significant difference for VEP between the two study groups. We consider that this is partly because myelitis-onset MS was used as a disease control, which was expected to have a relatively low frequency of optic nerve involvement early in the course of illness. In fact, we previously reported that the frequency of VEP abnormalities in our anti-AQP4 antibody-seronegative MS patients was around 60% when all cases were used, regardless of the onset sites [14], while in the present study only 41% of myelitis-onset MS patients had abnormal VEPs. In addition, Constantinescu et al. [18] reported a case of atopic optic neuritis, while we also previously reported that a significant fraction (21.7%) of AM patients had VEP abnormalities in the second nationwide survey [5]. Thus, the observation that 27.3% of AM patients had abnormal VEP findings might reflect such a clinically overt or subclinical involvement of optic nerve in this condition, thereby partly contributing to the absence of statistical significance in the comparison of abnormal VEP frequency between AM and myelitis-onset MS patients. For these reasons, we decided not to include the absence of VEP abnormality in the supporting negative criteria for AM. The frequency of posterior column lesions in the cervical cord on MRI was similar in both AM and myelitis-onset MS patients. This is probably explained by the fact that the cervical posterior column is also one of the preferential sites of spinal cord involvement in MS [12,19,20]. Therefore, we did not adopt cervical posterior column lesions as a supporting item in the present criteria.

These first criteria for AM achieved a relatively high sensitivity and specificity against myelitis-onset MS. Occasionally, spinal cord attacks in MS demonstrate neurological features indistinguishable from those of AM. Therefore, in the early stages of MS, especially myelitis-onset MS, it is critical to differentiate MS from AM using certain laboratory markers, because the early use of disease-modifying drugs, such as interferon-beta, is increasingly demanded. Interferon-beta or glatiramer acetate may worsen AM via induction of an immune shift toward a T helper (Th) type 2 cell response [21,22], which plays a key role in atopic disorders [7,23–25]. The high sensitivity and specificity of the present criteria may well facilitate the early discrimination of AM and myelitis-onset MS and contribute to better treatment for both diseases.

The present study has some limitations. First, because of the low prevalence of AM, it was difficult to obtain sufficient cases for enrollment. The paucity of AM patients enrolled in the present study might also have partly influenced the achievement of the surprisingly high negative predictive value and the relatively low positive predictive value of the new AM criteria. Second, for the same reason, we could not evaluate the efficacy of the new AM diagnostic criteria in a replicate population. In the future, the new AM criteria should be tested in other AM cohorts in the Japanese and other ethnic groups. Third, we did not apply a logistic regression model for the selection of parameters to be included in the new AM diagnostic criteria due to the small sample size. Multiple logistic analyses are needed in future large scale studies to identify more specific factors to be incorporated into the diagnostic criteria. Finally, the measurement of IL9 and CCL11/eotaxin in the CSF is not commonly undertaken. Thus, in the new AM criteria, diagnosis of definite AM is designed to be feasible without measuring CSF IL9 or CCL11/eotaxin; however, if measured, elevated levels of these cytokines in the CSF are strongly indicative of AM [7].

Because the prevalence of atopic diseases is rapidly increasing worldwide against a background of improved hygiene, more AM patients might emerge. The first diagnostic criteria for AM will encourage early differential diagnosis of AM and myelitis-onset MS.

Conflict of interest

T.M. received a grant and payment for manuscript preparation and development of educational presentations from Bayer Schering Pharma, and also received a payment for development of educational presentations from Mitsubishi Tanabe Pharma. J.K. is an advisory board member for Merck Serono and a consultant for Biogen Idec Japan. He has received payment for lectures from Bayer Schering Pharma, Cosmic Cooperation and Biogen Idec Japan. This work was supported in part by a Health and Labour Sciences Research Grant on Intractable Diseases (H22-Nanchi-Ippan-130 and H23- Nanchi-Ippan-017) from the Ministry of Health, Labour, and Welfare, Japan, and a Scientific Research B Grant (No. 22390178) and a Challenging Exploratory Research Grant (No. 23659459) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

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References

[1] Kira J, Yamasaki K, Kawano Y, Kobayashi T. Acute myelitis associated with hyper-IgEemia and atopic dermatitis. J Neurol Sci 1997;148:199–203.
[2] Zoli A, Mariano M, Fusari A, Bonifazi F, Antonicelli L. Atopic myelitis: first case report outside Japan? Allergy 2005;60:410–1.
[3] Gregoire SM, Mormont E, Laloux P, Godfraind C, Gilliard C. Atopic myelitis: a clinical, biological, radiological and histopathological diagnosis. J Neurol Sci 2006;247:231–5.
[4] Osoegawa M, Ochi H, Minohara M, Murai H, Umehara F, Furuya H, et al. Myelitis with atopic diathesis: a nationwide survey of 79 cases in Japan. J Neurol Sci 2003;209:5–11.
[5] Isobe N, Kira J, Kawamura N, Ishizu T, Arimura K, Kawano Y. Neural damage associated with atopic diathesis: a nationwide survey in Japan. Neurology 2009;73:790–7.
[6] Yoon JH, Joo IS, Li WY, Sohn SY. Clinical and laboratory characteristics of atopic myelitis: Korean experience. J Neurol Sci 2009;285:154–8.
[7] Tanaka M, Matsushita T, Tateishi T, Ochi H, Kawano Y, Mei FJ, et al. Distinct CSF cytokine/chemokine profiles in atopic myelitis and other causes of myelitis. Neurology 2008;71:974–81.
[8] Isaacs JD, Bodini B, Ciccarelli O, Scadding GK, Thompson AJ. Atopic myelitis in a European woman residing in Japan. J Neurol Neurosurg Psychiatry 2010.
[9] Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol 2005;58:840–6.
[10] Barkhof F, Filippi M, Miller DH, Scheltens P, Campi A, Polman CH, et al. Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. Brain 1997;120(Pt 11):2059–69.
[11] Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33:1444–52.
[12] Matsuoka T, Matsushita T, Kawano Y, Osoegawa M, Ochi H, Ishizu T, et al. Heterogeneity of aquaporin-4 autoimmunity and spinal cord lesions in multiple sclerosis in Japanese. Brain 2007;130:1206–23.
[13] Matsushita T, Matsuoka T, Isobe N, Kawano Y, Minohara M, Shi N, et al. Association of the HLA-DPB1*0501 allele with anti-aquaporin-4 antibody positivity in

Japanese patients with idiopathic central nervous system demyelinating disorders. Tissue Antigens 2009;73:171–6.
[14] Watanabe A, Matsushita T, Doi H, Matsuoka T, Shigeto H, Isobe N, et al. Multimodality-evoked potential study of anti-aquaporin-4 antibody-positive and -negative multiple sclerosis patients. J Neurol Sci 2009;281:34–40.
[15] Matsushita T, Isobe N, Matsuoka T, Shi N, Kawano Y, Wu XM, et al. Aquaporin-4 autoimmune syndrome and anti-aquaporin-4 antibody-negative opticospinal multiple sclerosis in Japanese. Mult Scler 2009;15:834–47.
[16] Kikuchi H, Osoegawa M, Ochi H, Murai H, Horiuchi I, Takahashi H, et al. Spinal cord lesions of myelitis with hyperIgEemia and mite antigen specific IgE (atopic myelitis) manifest eosinophilic inflammation. J Neurol Sci 2001;183:73–8.
[17] Osoegawa M, Ochi H, Kikuchi H, Shirabe S, Nagashima T, Tsumoto T, et al. Eosinophilic myelitis associated with atopic diathesis: a combined neuroimaging and histopathological study. Acta Neuropathol 2003;105:289–95.
[18] Constantinescu CS, Thomas M, Zaman AG. Atopic optic neuritis. Ocul Immunol Inflamm 2006;14:125–7.
[19] Tartaglino LM, Friedman DP, Flanders AE, Lublin FD, Knobler RL, Liem M. Multiple sclerosis in the spinal cord: MR appearance and correlation with clinical parameters. Radiology 1995;195:725–32.
[20] Pou Serradell A, Roquer Gonzalez J, Perich Alsina X. Acute posterior cord lesions in multiple sclerosis. An MRI study of the clinical course in 20 cases. Rev Neurol (Paris) 2000;156:1126–35.
[21] Qin Y, Zhang DQ, Prat A, Pouly S, Antel J. Characterization of T cell lines derived from glatiramer-acetate-treated multiple sclerosis patients. J Neuroimmunol 2000;108:201–6.
[22] Martin-Saavedra FM, Gonzalez-Garcia C, Bravo B, Ballester S. Beta interferon restricts the inflammatory potential of CD4+ cells through the boost of the Th2 phenotype, the inhibition of Th17 response and the prevalence of naturally occurring T regulatory cells. Mol Immunol 2008;45:4008–19.
[23] Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med 1992;326:298–304.
[24] van Reijsen FC, Bruijnzeel-Koomen CA, Kalthoff FS, Maggi E, Romagnani S, Westland JK, et al. Skin-derived aeroallergen-specific T-cell clones of Th2 phenotype in patients with atopic dermatitis. J Allergy Clin Immunol 1992;90:184–93.
[25] Ochi H, Osoegawa M, Murai H, Minohara M, Taniwaki T, Kira J. Presence of IgE antibodies to bacterial superantigens and increased IL-13-producing T cells in myelitic patients with atopic diathesis. Int Arch Allergy Immunol 2004;134:41–8.

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MINI-SYMPOSIUM: Neuromyelitis Optica (NMO), Part 2
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Cytokines and Chemokines in Neuromyelitis Optica: Pathogenetic and Therapeutic Implications

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Abstract

Neuromyelitis optica (NMO) is characterized by severe optic neuritis and longitudinally extensive transverse myelitis. The discovery of an NMO-specific autoantibody to the aquaporin-4 (AQP4) water channel has improved knowledge of NMO pathogenesis. Many studies have focused on inflammatory and pathological biomarkers of NMO, including cytokines and chemokines. Increased concentrations of T helper (Th)17- and Th2-related cytokines and chemokines may be essential factors for developing NMO inflammatory lesions. For example, interleukin-6 could play important roles in NMO pathogenesis, as it is involved in the survival of plasmablasts that produce anti-AQP4 antibody in peripheral circulation and in the enhancement of inflammation in the central nervous system. Therefore, assessment of these useful biomarkers may become a supportive criterion for diagnosing NMO. Significant advances in the understanding of NMO pathogenesis will lead to the development of novel treatment strategies. This review focuses on the current advances in NMO immunological research, particularly that of cytokines and chemokines.

INTRODUCTION

Neuromyelitis optica (NMO) is an autoimmune inflammatory disorder of the central nervous system (CNS), clinically presenting with longitudinally extensive transverse myelitis (LETM) and optic neuritis (54). The discovery of the disease-specific serum anti-aquaporin-4 (AQP4) antibody in NMO (25, 26) has dramatically changed the clinical definition of NMO, leading to recent advances in NMO research. The pathogenic role of anti-AQP4 antibody was demonstrated *in vivo* by passive transfer experiments in animal models (7, 22, 34). Several lines of evidence differentiating between NMO and multiple sclerosis (MS) have accumulated based on pathology (30, 33), neuroimaging (16), immunological findings (38) and responses to immunotherapies (24, 29, 39). On the basis of these extensive data, NMO is now considered an anti-AQP4 antibody-mediated astrocytopathy distinct from demyelinating disorders as represented by MS (12). Besides anti-AQP4 antibody, many additional biomarkers have proven useful for understanding the pathogenetic and immunological aspects of NMO (32, 36, 38). T and B cells may be implicated in the peripheral/CNS immune responses and pathogenesis of NMO, whereas various cytokines and chemokines have also been associated with the pathogenesis of NMO (38). Therefore, this review focuses on the current research on the roles of cytokines and chemokines in NMO pathogenesis and their therapeutic applications.

Cerebrospinal fluid (CSF) cytokines and chemokines in NMO patients

Many studies have analyzed CSF cytokine and chemokine levels in NMO patients (Table 1). Although some cytokines may increase nonspecifically because of CNS inflammation, several cytokines and chemokines are directly related to NMO pathogenesis. T helper (Th)17- and Th2-related cytokines are upregulated in the CSF of NMO patients (38). CSF interleukin (IL)-17 levels increase in patients with NMO (48) or opticospinal MS (OSMS; some of whom were considered to have NMO) (15, 37). Many studies have also shown increased CSF IL-6 levels in patients with NMO. Presumably, NMO expresses Th17 and Th2 axes in CNS (Figure 1) differently from MS, which is primarily a Th1-dominant disease. However, further studies are necessary to clarify the definite cytokine and chemokine profiles in NMO.

Th17-related cytokines and chemokines

IL-17 is involved in the development of autoimmune diseases and acts as a potent mediator in delayed-type inflammatory reactions by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the inflammation site. As described earlier, several reports have revealed elevated CSF IL-17 levels in NMO (48) or OSMS patients (15, 37). Our own study could not confirm such an elevation, but levels of some Th17-related cytokines and chemokines are reportedly increased in NMO patients (38).

Elevated CSF IL-6 levels in NMO have also been reported. IL-6 is a proinflammatory cytokine with a wide variety of functions. Secreted by immunocytes and activated astrocytes (11), it promotes immunoglobulin (Ig) synthesis in activated B cells and differentiation of naïve T cells into Th17 cells or cytotoxic T cells (6, 23). Among the several CSF cytokines and chemokines elevated in NMO, IL-6 shows the strongest correlation with clinical variables in NMO; these include CSF glial fibrillary acidic

Table 1. Cerebrospinal fluid (CSF) cytokine/chemokine levels in NMO patients.

CSF cytokines/chemokines	Axis	Change	Reference	Correlation
IL-17	Th17	↑ (vs. MS, ONNDs)	(48)	CSF HMGB1
IL-6	Th17	↑ (vs. MS, HC)	(9)	
		↑ (vs. MS, ONNDs)	(14)	EDSS and AQP4 ab positivity
		↑ (vs. MS)	(55)	Definite form > limited form
		↑ (vs. MS, ONNDs)	(41)	CSF cells, CSF proteins and AQP4 ab positivity
		↑ (vs. MS, ONNDs)	(38) (44)	CSF cells, CSF GFAP, AQP4 ab, recovery from relapse and relapse duration
		↑ (vs. MS, ONNDs)	(43)	CSF HMGB1
		↑ (vs. MS, ONNDs)	(49)	EDSS
		↑ (vs. MS, ONNDs)	(48)	CSF HMGB1
IL-1ra	Th17	↑ (vs. MS, ONNDs)	(38)	CSF cells and CSF GFAP
G-CSF	Th17	↑ (vs. MS, ONNDs)	(38)	CSF cells and CSF GFAP
IL-8	Th17	↑ (vs. MS, ONNDs)	(38)	CSF cells, CSF GFAP and EDSS
		→ (vs. MS)	(55)	
IL-13	Th2	↑ (vs. MS, ONNDs)	(38)	CSF cells and CSF GFAP
IL-5	Th2	↑ (vs. MS, HC)	(9)	
Eotaxin-2, -3	Th2	↑ (vs. MS, HC)	(9)	
Eotaxin	Th2	→ (vs. MS, ONNDs)	(38)	
		→ (vs. MS, ONNDs)	(31)	
TARC	Th2	↑ (vs. ONNDs)	(31)	
IL-10	Treg	↑ (vs. ONNDs) → (vs. MS)	(38)	CSF cells, AQP4 ab and CSF GFAP
		→ (vs. MS)	(55)	
IL-12	Th1	↑ (vs. HC)	(9)	
		→ (vs. MS, ONNDs)	(38)	
IL-1β	Th1	↑ (vs. MS)	(55)	Definite form > limited form
		→ (vs. MS, ONNDs)	(38)	
CXCL10 (IP-10)	Th1	↑ (vs. ONNDs)	(31)	
		↑ (vs. ONNDs)	(38)	CSF cells and CSF GFAP
CXCL13	B cell	↑ (vs. MS, ONNDs)	(57)	ARR and EDSS
IFN-γ, G-CSF, IL-17		↑ (vs. CMS, ONNDs)	(37)*	
IL-17, MIP-1β, IL-1β, IL-13,		↑ (vs. ONNDs)	(15)*	IL-8: EDSS, albumin quotient and length of spinal cord lesion
IL-8, IL-10, TNF-α, IL-5		↑ (vs. CMS)		IL-17: albumin quotient and length of spinal cord lesion

*Cytokine analyses were performed in opticospinal MS patients.

AQP4 ab = aquaporin-4 antibody; ARR = annualized relapse rate; CMS = conventional multiple sclerosis; CXCL = (C-X-C motif) ligand; EDSS = Expanded Disability Status Scale; G-CSF = granulocyte colony-stimulating factor; GFAP = glial fibrillary acidic protein; HC = healthy controls; HMGB1 = high mobility group box 1; IFN-γ = interferon-gamma; IL = interleukin; IP-10 = interferon gamma-induced protein 10; MIP = macrophage inflammatory protein; MS = multiple sclerosis; NMO = neuromyelitis optica; ONNDs = other noninflammatory neurological disorders; TARC = thymus and activation-regulated chemokine; Th = T helper; TNF-α = tumor necrosis factor-alpha; Treg = regulatory T cell.

↑ = upregulation; → = unchanged.

protein (GFAP) levels, CSF cell counts and anti-AQP4 antibody titers (38). Içöz *et al* reported that patients with NMO have higher CSF IL-6 levels than those with optic neuritis, relapsing–remitting MS or healthy control (HC). Further, CSF IL-6 levels in NMO patients correlate with anti-AQP4 antibody titers and the Expanded Disability Status Scale (EDSS) score (14). Wang *et al* found that CSF IL-6 and soluble IL-6 receptor levels are significantly higher in patients with NMO than in those with MS and other noninflammatory neurological disorders (ONNDs) (49). Yanagawa *et al* reported elevated CSF IL-6 levels in patients with definite NMO compared with those with limited NMO (anti-AQP4-positive myelitis without optic neuritis) (55). The CSF/serum ratio of IL-6 is significantly higher in NMO than in ONNDs, suggesting that IL-6 is mainly produced in the CNS of NMO patients (38). Although IL–6-producing cells in CNS have not yet been identified, activated or damaged, astrocytes by anti-AQP4 antibody may produce IL-6 in the CNS of NMO patients. Of note, high CSF IL-6 levels have been found in 82.3% of NMO patients, but no such increase has been observed in MS patients (38). CSF IL-6 levels are also markedly high not only during relapse, but also during the initial attacks in NMO patients (45). Interestingly, CSF IL-6 levels can predict recovery from NMO relapses and relapse-free duration (44). NMO patients who relapse with optic neuritis exhibit high CSF IL-6 levels, similar to NMO patients who relapse with myelitis (38, 45); nevertheless, optic neuritis lesions are usually much smaller than myelitis lesions in NMO patients. These data suggest that CSF IL-6 is not a product of NMO inflammation, but an important molecule in the pathology of this disease. We have recently shown that CSF IL-6 levels correlate with CSF levels of high mobility group box 1 (HMGB1), a proinflammatory mediator (43), and with CSF-soluble intercellular adhesion molecule 1 levels, one of the markers of blood–brain barrier disruption

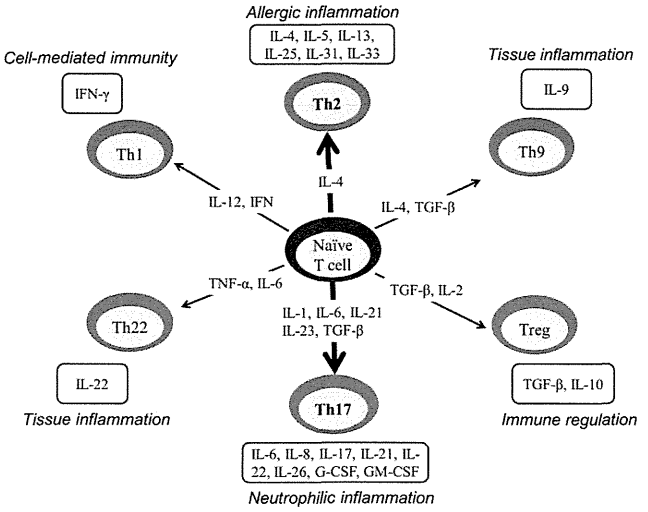

Figure 1. *Differentiation pathways of naïve CD4⁺ T cells.* CD4⁺ T cells can differentiate into T helper (Th)1, Th2, Th9, Treg, Th17 or Th22 by the actions of differentiation cytokines. These T-cell subsets promote different inflammatory responses based on their respective cytokine profiles, responses to chemokines and interactions with other cells. The Th17 and Th2 axes may be mainly upregulated in neuromyelitis optica. IL = interleukin; TGF = transformaing growth factor; TNF = tumor necrosis factor.

(42). Accumulated evidence suggests important roles for CSF IL-6 in NMO pathogenesis; these could include CNS inflammation, astrocytic damage and blood–brain barrier disruption. In addition, CSF IL-6 may serve as a biomarker to diagnose NMO and differentiate it from MS. It remains unclear whether astrocytic damage releases IL-6, or IL-6 directly contributes to astrocytic damage and CNS inflammation in NMO.

IL-1ra is a member of the IL-1 cytokine family. CSF IL-1ra levels are significantly elevated in NMO compared with MS or ONNDs and correlate with CSF cells and CSF GFAP levels (38). Granulocyte colony-stimulating factor (G-CSF) stimulates survival, proliferation and differentiation of neutrophils. CSF G-CSF levels are higher in NMO than in MS and ONNDs and correlate with CSF GFAP levels and CSF cell counts (38). Tanaka *et al* reported significantly elevated CSF G-CSF levels in patients with OSMS (some of whom were considered to have NMO) compared with conventional MS (CMS) and ONNDs, and found correlations with the albumin quotient, length of spinal magnetic resonance image (MRI) lesions and EDSS score (37). IL-8 is known as a neutrophil chemotactic factor. CSF IL-8 levels are significantly elevated in NMO compared with MS and ONNDs, and correlate with the CSF GFAP levels, CSF cell counts and EDSS score (38). However, Yanagawa *et al* reported no difference between CSF IL-8 levels in NMO and MS patients (55). Ishizu *et al* found that significantly elevated CSF IL-8 levels in OSMS patients (some of whom were considered to have NMO) compared with CMS and ONNDs. These levels correlate with the albumin quotient, length of spinal MRI lesion and EDSS score (15). They speculated that the markedly increased IL-8 in CSF may be relevant to neutrophil infiltration in CNS.

Th2-related cytokines and chemokines

Although IL-4, a representative Th2-related cytokine, has not been elevated in CSF (38), other Th2-related cytokines and chemokines are upregulated in the CSF of NMO patients. The effects of IL-13 on immune cells are similar to those of IL-4. CSF IL-13 levels are elevated in NMO compared with MS or ONNDs, and their levels correlate with CSF cells and CSF GFAP levels (38). IL-5 stimulates B-cell growth and increases Ig secretion; it is also a key mediator in eosinophil activation. CSF IL-5 levels are significantly higher in NMO patients than in MS patients or HC (9). Eotaxin is an eosinophil-selective chemokine. CSF eotaxin levels in NMO patients are similar to those in MS or ONNDs patients (31, 38). However, Correale and Fiol reported significant increases in CSF eotaxin-2 and eotaxin-3 levels in NMO patients compared with MS patients or HC (9). The chemokine thymus and activation-regulated chemokine (TARC) specifically binds and induces chemotaxis in T cells. CSF TARC levels are significantly higher in NMO than in ONNDs (31).

Th1-related cytokines and chemokines

Interferon-gamma (IFN-γ), a representative Th1-related cytokine, has not been elevated in the CSF of NMO patients (38). (C-X-C motif) ligand (CXCL10) (interferon gamma-induced protein 10) is secreted by several cells in response to IFN-γ. CSF CXCL10 levels are significantly higher in NMO than in ONNDs (31, 38), and correlate with CSF GFAP levels and CSF cell counts (38). IL-12 is involved in the differentiation of naïve T cells into Th1 cells and plays an important role in the activities of natural killer cells and T lymphocytes. CSF IL-12 levels are significantly elevated in NMO patients compared with HC (9). However, some studies report no differences in CSF IL-12 levels between NMO, MS or ONNDs (38). IL-1β is an important mediator of the inflammatory response. CSF IL-1β levels are elevated in patients with definite NMO compared with those with limited NMO (55). However, no differences have been found between NMO, MS or ONNDs patients (38).

Other cytokines and chemokines

IL-10, a regulatory T (Treg)-related cytokine with pleiotropic effects in immunoregulation and inflammation, is capable of inhibiting proinflammatory cytokine synthesis. CSF IL-10 levels are significantly elevated in NMO compared with ONNDs (38), but no difference is observed in MS patients (38, 55). CSF IL-10 levels correlate with CSF GFAP levels, CSF cell counts and anti-AQP4 antibody titers (38).

CXCL13 is selectively chemotactic for B cells. CSF CXCL13 levels are significantly higher in NMO than in MS or ONNDs and correlate with the annualized relapse rate and EDSS score (57). Alvarez *et al* reported elevated CSF CXCL13 levels in NMO and MS patients compared with ONNDs, which correlate with CSF cell counts in NMO patients (1).

Serum/plasma cytokines and chemokines in NMO patients

Serum/plasma cytokine and chemokine levels in NMO patients are summarized in Table 2. As with CSF analyses, Th17- and

Table 2. Serum cytokine/chemokine levels in NMO patients.

Serum cytokines/ chemokines	Axis	Change	Reference	Correlation
IL-17	Th17	↑ (vs. HC)	(51)	
		↑ (vs. HC)	(52)	HMGB1
		↑ (vs. HC)	(27)	
		↑ (vs. MS, HC)	(50)	
		↑ (vs. NMO without LSCL)	(13)	Length of spinal cord lesion
IL-6	Th17	↑ (vs. HC)	(28)	EDSS
		↑ (vs. MS, HC)	(9)	
		↑ (vs. MS, ONNDs)	(14)	
		↑ (vs. ONNDs)	(38)	
		↑ (vs. HC)	(47)	IL-32
		↑ (vs. HC)	(46)	
		↑ (vs. HC)	(51)	
IL-23	Th17	↑ (vs. HC)	(27)	
		↑ (vs. HC)	(50)	
		↑ (vs. HC)	(28)	
IL-21	Th17	↑ (vs. HC)	(50)	
		↑ (vs. HC)	(28)	EDSS
IL-4	Th2	↑ (vs. HC)	(2)	
		↑ (vs. MS, HC)	(51)	
IL-10	Treg	↑ (vs. MS, HC)	(51)	
IL-2	Treg	↑ (vs. MS, HC)	(51)	
		↓ (vs. HC)	(28)	
IFN-γ	Th1	↑ (vs. HC)	(52)	HMGB1
		↑ (vs. MS, HC)	(51)	
		↓ (vs. HC)	(28)	
TNF-α	Th1	↑ (vs. HC)	(52)	HMGB1
		↑ (vs. HC)	(51)	
IL-32		↑ (vs. MS, HC)	(47)	IL-6, EDSS

EDSS = Expanded Disability Status Scale; HC = healthy controls; HMGB1 = high mobility group box 1; IFN-γ = interferon-gamma; IL = interleukin; LSCL = long spinal cord lesions greater than three vertebral segments; MS = multiple sclerosis; NMO = neuromyelitis optica; ONNDs = other noninflammatory neurological disorders; Th = T helper; TNF-α = tumor necrosis factor-alpha; Treg = regulatory T cell.
↑ = upregulation; ↓ = downregulation.

Th2-related cytokines and chemokines are predominantly upregulated in the serum/plasma of NMO patients (Figure 1).

Serum/plasma IL-17 levels increase in NMO patients compared with HC or MS patients (27, 50–52). Plasma IL-17 levels correlate with plasma HMGB1 levels (52). NMO patients with LETM (more than three vertebral segments) have higher serum IL-17 levels than NMO patients without LETM (13). NMO patients in the relapse phase have significantly higher serum IL-6 levels than ONND patients (38). Içöz *et al* also reported that patients with NMO, particularly those who are anti-AQP4 antibody positive, have higher serum IL-6 levels than those with optic neuritis, relapsing–remitting MS or HC (14). Wang *et al* found that plasma IL-6 levels are higher in NMO patients than in HC and are positively correlated with IL-32 levels (47). AQP4-specific T-cell responses are amplified in NMO patients and exhibit a Th17 bias, and intracellular IL-6 production increases after lipopolysaccharide stimulation in monocytes from NMO patients (46). The number of anti-myelin oligodendrocyte glycoprotein IL-6- and IL-12-secreting cells in the peripheral blood and CSF of NMO patients is higher than that of MS, ONNDs or HC (9). The release of IL-6, IL-21 and IL-23 from activated peripheral blood mononuclear cells is significantly higher in NMO patients than in controls, and IL-6 and IL-21 levels positively correlate with the EDSS score in NMO patients (28). Although the role of IL-6 in peripheral blood is unclear, Chihara *et al* recently reported that the population of plasmablasts exhibiting the $CD19^{int}CD27^{high}CD38^{high}CD180^{-}$ phenotype selectively increases in the peripheral blood of NMO patients, and that these plasmablasts are major producers of anti-AQP4 antibodies (8). IL-6 enhances plasmablast survival and anti-AQP4 antibody production in these cells, whereas anti-IL-6 receptor antibody lessens their survival. IL-6 in the peripheral blood of NMO patients is implicated in the peripheral immune response and anti-AQP4 antibody production. Serum IL-23 and IL-21 levels are also elevated in NMO patients compared with HC (27, 50).

The Th2 cytokine IL-4 is upregulated in the serum of NMO patients compared with HC and MS patients (2, 51). Other Th2-related cytokines and chemokines have not been analyzed.

Studies of Treg-related cytokines show that IL-10 and IL-2 levels increase significantly in NMO patients compared with MS patients and HC (51), but Linhares *et al* reported that IL-2 levels decrease significantly in NMO patients compared with controls (28).

The levels of the Th1-related cytokines IFN-γ and tumor necrosis factor-alpha (TNF-α) increase in NMO patients compared with HC and MS patients (51, 52), and are correlated with plasma HMGB1 levels (52).

Chemokine receptor expression on peripheral blood T cells in NMO patients

CD8+CXCR3+T cells might affect the pathogenesis of both NMO and MS, and could be an important marker of disease activity. The CD8+CXCR3+/CD8+CCR4+ ratio, which reflects immune and inflammatory activities, is higher in NMO than in MS patients (35). Th1 dominance of chemokine receptors on blood T cells and the correlation between CXCR3+ T cells and disease activity have been confirmed by analyzing chemokine receptors on peripheral blood lymphocytes during the relapse phase in MS patients. However, such deviations in the Th1/Th2 balance have not been observed in NMO patients (40).

Pathogenic role of cytokines and chemokines

IL-6 infusion into the spinal subarachnoid space of rats induces progressive weakness with CNS inflammation, axonal degeneration and myelin loss (18). CSF IL-6 is mainly produced by astrocytes in transverse myelitis patients, and its levels correlate with astrocytic expression and disease severity (18). IFN-β treatment is effective in reducing experimental autoimmune encephalomyelitis (EAE) symptoms induced by Th1 cells, but exacerbates disease induced by Th17 cells (4). The Th17 EAE model represents several aspects of NMO, suggesting that Th17 cells may play a pathogenic role in NMO pathogenesis. *Ex vivo* experiments performed on murine spinal cords have revealed that slices exposed to NMO IgG and human complement exhibit NMO-like lesions. These lesions increase in severity with the addition of neutrophils, natural killer cells, macrophages or cytokines (such as TNF-α, IL-6, IL-1β or IFN-γ), implicating specific immune cells and cytokines may amplify tissue damage in NMO (56).

Therapeutic implications of cytokine blockade in NMO patients

Low-dose oral corticosteroids, azathioprine, mitoxantrone, cyclophosphamide, mycophenolate mofetil and rituximab are used as maintenance treatments to prevent NMO relapses (10, 17, 20, 21, 53). Novel treatments using the IL-6 pathway blocker tocilizumab, a recombinant humanized monoclonal antibody against the IL-6 receptor, may be useful for suppressing relapses in NMO patients who cannot tolerate standard immunosuppression therapy (3, 5, 19). Treatment with tocilizumab rapidly reduces the number of elevated plasmablasts and anti-AQP4 antibody titers in NMO patients. Furthermore, neuropathic pain and disability scores improve gradually (3). Patients with highly active anti-AQP4 antibody-positive NMO, in whom numerous immunosuppressive interventions had failed, exhibited improved EDSS scores and annualized relapse rates after initiating tocilizumab. Tocilizumab significantly reduces CSF IL-6 levels, signal transducer and activation of transcription 3 (STAT3) activation (19). Three female patients with anti-AQP4 antibody-positive NMO, who were resistant to rituximab treatment, exhibited a decrease in the median annualized relapse rate from 3.0 to 0.6 after treatment with tocilizumab (5). IL-6 receptor-blocking therapy can be effective against NMO even in patients who fail to respond to conventional therapy. This direct clinical evidence suggests that IL-6 may be a critical molecule in NMO immunopathogenesis. In the future, other cytokine-blocking therapies may also be applied clinically.

CONCLUSIONS

A growing number of recent immunological studies have supported the important role of cytokines and chemokines in NMO pathogenesis. Although many cytokines and chemokines are upregulated in both the peripheral and CNS of NMO patients, Th17- and Th2-related cytokines and chemokines, particularly Th17-related cytokines, may be key players in NMO inflammation. IL-6 in the peripheral blood is implicated in anti-AQP4 antibody production in NMO patients, and IL-6 in CSF plays important roles in CNS inflammation, astrocytic damage and blood–brain barrier disruption. Thus, IL-6-blocking therapy with tocilizumab may be a promising treatment option for NMO patients. New treatments need to be developed to prevent severe relapses in these patients. A better understanding of the role of cytokines and chemokines in NMO pathogenesis is critical to developing effective treatments.

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REFERENCES

1. Alvarez E, Piccio L, Mikesell RJ, Klawiter EC, Parks BJ, Naismith RT, Cross AH (2013) CXCL13 is a biomarker of inflammation in multiple sclerosis, neuromyelitis optica, and other neurological conditions. *Mult Scler* **19**:1204–1208.
2. Alves-Leon SV, Pimentel ML, Sant'Anna G, Malfetano FR, Estrada CD, Quirico-Santos T (2008) Immune system markers of neuroinflammation in patients with clinical diagnose of neuromyelitis optica. *Arq Neuropsiquiatr* **66**:678–684.
3. Araki M, Aranami T, Matsuoka T, Nakamura M, Miyake S, Yamamura T (2013) Clinical improvement in a patient with neuromyelitis optica following therapy with the anti-IL-6 receptor monoclonal antibody tocilizumab. *Mod Rheumatol* **23**:827–831.
4. Axtell RC, de Jong BA, Boniface K, van der Voort LF, Bhat R, De Sarno P *et al* (2010) T helper type 1 and 17 cells determine efficacy of interferon-beta in multiple sclerosis and experimental encephalomyelitis. *Nat Med* **16**:406–412.
5. Ayzenberg I, Kleiter I, Schröder A, Hellwig K, Chan A, Yamamura T, Gold R (2013) Interleukin 6 receptor blockade in patients with neuromyelitis optica nonresponsive to anti-CD20 therapy. *JAMA Neurol* **70**:394–397.
6. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M *et al* (2006) Reciprocal developmental pathways for the generation of pathogenic effector Th17 and regulatory T cells. *Nature* **441**:235–238.
7. Bradl M, Misu T, Takahashi T, Watanabe M, Mader S, Reindl M *et al* (2009) Neuromyelitis optica: pathogenicity of patient immunoglobulin *in vivo*. *Ann Neurol* **66**:630–643.

8. Chihara N, Aranami T, Sato W, Miyazaki Y, Miyake S, Okamoto T *et al* (2011) Interleukin 6 signaling promotes anti-aquaporin 4 autoantibody production from plasmablasts in neuromyelitis optica. *Proc Natl Acad Sci U S A* **108**:3701–3706.
9. Correale J, Fiol M (2004) Activation of humoral immunity and eosinophils in neuromyelitis optica. *Neurology* **63**:2363–2370.
10. Costanzi C, Matiello M, Lucchinetti CF, Weinshenker BG, Pittock SJ, Mandrekar J *et al* (2011) Azathioprine: tolerability, efficacy, and predictor of benefit in neuromyelitis optica. *Neurology* **77**:659–666.
11. Farina C, Aloisi F, Meinl E (2007) Astrocytes are active players in cerebral innate immunity. *Trends Immunol* **28**:138–145.
12. Fujihara K, Misu T, Nakashima I, Takahashi T, Bradl M, Lassmann H *et al* (2012) Neuromyelitis optica should be classified as an astrocytopathic disease rather than a demyelinating disease. *Clin Exp Neuroimmunol* **3**:58–73.
13. Herges K, de Jong BA, Kolkowitz I, Dunn C, Mandelbaum G, Ko RM *et al* (2012) Protective effect of an elastase inhibitor in a neuromyelitis optica-like disease driven by a peptide of myelin oligodendroglial glycoprotein. *Mult Scler* **18**:398–408.
14. Içöz S, Tüzün E, Kürtüncü M, Durmuş H, Mutlu M, Eraksoy M, Akman-Demir G (2010) Enhanced IL-6 production in aquaporin-4 antibody positive neuromyelitis optica patients. *Int J Neurosci* **120**:71–75.
15. Ishizu T, Osoegawa M, Mei FJ, Kikuchi H, Tanaka M, Takakura Y *et al* (2005) Intrathecal activation of the IL-17/IL-8 axis in opticospinal multiple sclerosis. *Brain* **128**:988–1002.
16. Ito S, Mori M, Makino T, Hayakawa S, Kuwabara S (2009) "Cloud-like enhancement" is a magnetic resonance imaging abnormality specific to neuromyelitis optica. *Ann Neurol* **66**:425–428.
17. Jacob A, Matiello M, Weinshenker BG, Wingerchuk DM, Lucchinetti C, Shuster E *et al* (2009) Treatment of neuromyelitis optica with mycophenolate mofetil: retrospective analysis of 24 patients. *Arch Neurol* **66**:1128–1133.
18. Kaplin AI, Deshpande DM, Scott E, Krishnan C, Carmen JS, Shats I *et al* (2005) IL-6 induces regionally selective spinal cord injury in patients with the neuroinflammatory disorder transverse myelitis. *J Clin Invest* **115**:2731–2741.
19. Kieseier BC, Stüve O, Dehmel T, Goebels N, Leussink VI, Mausberg AK *et al* (2013) Disease amelioration with tocilizumab in a treatment-resistant patient with neuromyelitis optica: implication for cellular immune responses. *JAMA Neurol* **70**:390–393.
20. Kim SH, Kim W, Li XF, Jung IJ, Kim HJ (2011) Repeated treatment with rituximab based on the assessment of peripheral circulating B cells in patients with relapsing neuromyeltis optica. *Arch Neurol* **68**:1412–1420.
21. Kim SH, Kim W, Park MS, Sohn EH, Li XF, Kim HJ (2011) Efficacy and safety of mithoxantrone in patients with highly relapsing neuromyelitis optica. *Arch Neurol* **68**:473–479.
22. Kinoshita M, Nakatsuji Y, Kimura T, Moriya M, Takata K, Okuno T *et al* (2009) Neuromyelitis optica: passive transfer to rats by human immunoglobulin. *Biochem Biophys Res Commun* **386**:623–627.
23. Kishimoto T (2005) Interleukin-6: from basic science to medicine—40 years in immunology. *Annu Rev Immunol* **23**:1–21.
24. Kleiter I, Hellwig K, Berthele A, Kümpfel T, Linker RA, Hartung HP, *et al*, for the Neuromyelitis Optica Study Group (2012) Failure of natalizumab to prevent relapses in neuromyelitis optica. *Arch Neurol* **69**:239–245.
25. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR (2005) IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med* **202**:473–477.
26. Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K *et al* (2004) A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* **364**:2106–2112.
27. Li Y, Wang H, Long Y, Lu Z, Hu X (2011) Increased memory Th17 cells in patients with neuromyelitis optica and multiple sclerosis. *J Neuroimmunol* **234**:155–160.
28. Linhares UC, Schiavoni PB, Barros PO, Kasahara TM, Teixeira B, Ferreira TB *et al* (2013) The *ex vivo* production of IL-6 and IL-21 by CD4(+) T cells is directly associated with neurological disability in neuromyelitis optica patients. *J Clin Immunol* **33**:179–189.
29. Min JH, Kim BJ, Lee KH (2012) Development of extensive brain lesions following fingolimod (FTY720) treatment in a patient with neuromyelitis optica spectrum disorder. *Mult Scler* **18**:113–115.
30. Misu T, Fujihara K, Kakita A, Konno H, Nakamura M, Watanabe S *et al* (2007) Loss of aquaporin-4 in lesions of neuromyelitis optica: distinction from multiple sclerosis. *Brain* **130**:1224–1234.
31. Narikawa K, Misu T, Fujihara K, Nakashima I, Sato S, Itoyama Y (2004) CSF chemokine levels in relapsing neuromyelitis optica and multiple sclerosis. *J Neuroimmunol* **149**:182–186.
32. Okada K, Matsushita T, Kira J, Tsuji S (2010) B-cell activating factor of the TNF family is upregulated in neuromyelitis optica. *Neurology* **74**:177–178.
33. Roemer SF, Parisi JE, Lennon VA, Benarroch EE, Lassmann H, Bruck W *et al* (2007) Pattern-specific loss of aquaporin-4 immunoreactivity distinguishes neuromyelitis optica from multiple sclerosis. *Brain* **130**:1194–1205.
34. Saadoun S, Waters P, Bell BA, Vincent A, Verkman AS, Papadopoulos MC (2010) Intra-cerebral injection of neuromyelitis optica immunoglobulin G and human complement produces neuromyelitis optica lesions in mice. *Brain* **133**:349–361.
35. Shimizu Y, Ota K, Kubo S, Kabasawa C, Kobayashi M, Ohashi T, Uchiyama S (2011) Association of Th1/Th2-related chemokine receptors in peripheral T cells with disease activity in patients with multiple sclerosis and neuromyelitis optica. *Eur Neurol* **66**:91–97.
36. Takano R, Misu T, Takahashi T, Sato S, Fujihara K, Itoyama Y (2010) Astrocytic damage is far more severe than demyelination in NMO: a clinical CSF biomarker study. *Neurology* **75**:208–216.
37. Tanaka M, Matsushita T, Tateishi T, Ochi H, Kawano Y, Mei FJ *et al* (2008) Distinct CSF cytokine/chemokine profiles in atopic myelitis and other causes of myelitis. *Neurology* **71**:974–981.
38. Uzawa A, Mori M, Arai K, Sato Y, Hayakawa S, Masuda S *et al* (2010) Cytokine and chemokine profiles in neuromyelitis optica: significance of interleukin-6. *Mult Scler* **16**:1443–1452.
39. Uzawa A, Mori M, Hayakawa S, Masuda S, Kuwabara S (2010) Different responses to interferon beta-1b treatment in patients with neuromyelitis optica and multiple sclerosis. *Eur J Neurol* **17**:672–676.
40. Uzawa A, Mori M, Hayakawa S, Masuda S, Nomura F, Kuwabara S (2010) Expression of chemokine receptors on peripheral blood lymphocytes in multiple sclerosis and neuromyelitis optica. *BMC Neurol* **10**:113.
41. Uzawa A, Mori M, Ito M, Uchida T, Hayakawa S, Masuda S, Kuwabara S (2009) Markedly increased CSF interleukin-6 levels in neuromyelitis optica, but not in multiple sclerosis. *J Neurol* **256**:2082–2084.
42. Uzawa A, Mori M, Masuda S, Kuwabara S (2011) Markedly elevated soluble intercellular adhesion molecule 1, soluble vascular cell adhesion molecule 1 levels, and blood–brain barrier breakdown in neuromyelitis optica. *Arch Neurol* **68**:913–917.
43. Uzawa A, Mori M, Masuda S, Muto M, Kuwabara S (2013) CSF high-mobility group box 1 is associated with intrathecal

inflammation and astrocytic damage in neuromyelitis optica. *J Neurol Neurosurg Psychiatry* **84**:517–522.
44. Uzawa A, Mori M, Sato Y, Masuda S, Kuwabara S (2012) CSF interleukin-6 level predicts recovery from neuromyelitis optica relapse. *J Neurol Neurosurg Psychiatry* **83**:339–340.
45. Uzawa A, Mori M, Sawai S, Masuda S, Muto M, Uchida T *et al* (2013) Cerebrospinal fluid interleukin-6 and glial fibrillary acidic protein levels are increased during initial neuromyelitis optica attacks. *Clin Chim Acta* **421**:181–183.
46. Varrin-Doyer M, Spencer CM, Schulze-Topphoff U, Nelson PA, Stroud RM, Cree BA, Zamvil SS (2012) Aquaporin 4-specific T cells in neuromyelitis optica exhibit a Th17 bias and recognize Clostridium ABC transporter. *Ann Neurol* **72**:53–64.
47. Wang H, Wang K, Wang C, Xu F, Qiu W, Hu X (2013) Increased plasma interleukin-32 expression in patients with neuromyelitis optica. *J Clin Immunol* **33**:666–670.
48. Wang H, Wang K, Wang C, Xu F, Zhong X, Qiu W, Hu X (2013) Cerebrospinal fluid high-mobility group box protein 1 in neuromyelitis optica and multiple sclerosis. *Neuroimmunomodulation* **20**:113–118.
49. Wang H, Wang K, Zhong X, Dai Y, Qiu W, Wu A, Hu X (2012) Notable increased cerebrospinal fluid levels of soluble interleukin-6 receptors in neuromyelitis optica. *Neuroimmunomodulation* **19**:304–308.
50. Wang HH, Dai YQ, Qiu W, Lu ZQ, Peng FH, Wang YG *et al* (2011) Interleukin-17-secreting T cells in neuromyelitis optica and multiple sclerosis during relapse. *J Clin Neurosci* **18**:1313–1317.
51. Wang KC, Lee CL, Chen SY, Chen JC, Yang CW, Chen SJ, Tsai CP (2013) Distinct serum cytokine profiles in neuromyelitis optica and multiple sclerosis. *J Interferon Cytokine Res* **33**:58–64.
52. Wang KC, Tsai CP, Lee CL, Chen SY, Chin LT, Chen SJ (2012) Elevated plasma high-mobility group box 1 protein is a potential marker for neuromyelitis optica. *Neuroscience* **226**:510–516.
53. Watanabe S, Misu T, Miyazawa I, Nakashima I, Shiga Y, Fujihara K, Itoyama Y (2007) Low-dose corticosteroids reduce relapses in neuromyelitis optica: a retrospective analysis. *Mult Scler* **13**:968–974.
54. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG (2006) Revised diagnostic criteria for neuromyelitis optica. *Neurology* **66**:1485–1489.
55. Yanagawa K, Kawachi I, Toyoshima Y, Yokoseki A, Arakawa M, Hasegawa A *et al* (2009) Pathologic and immunologic profiles of a limited form of neuromyelitis optica with myelitis. *Neurology* **73**:1628–1637.
56. Zhang H, Bennett JL, Verkman AS (2011) *Ex vivo* spinal cord slice model of neuromyelitis optica reveals novel immunopathogenic mechanisms. *Ann Neurol* **70**:943–954.
57. Zhong X, Wang H, Dai Y, Wu A, Bao J, Xu W *et al* (2011) Cerebrospinal fluid levels of CXCL13 are elevated in neuromyelitis optica. *J Neuroimmunol* **240–241**:104–108.

INTERNAL MEDICINE

□ CASE REPORT □

Anti-Aquaporin-4 Antibody-Seronegative NMO Spectrum Disorder with Baló's Concentric Lesions

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Abstract

A 34-year-old woman developed simultaneous bilateral severe optic neuritis and subsequent myelitis. Two months after the first attack, she developed a headache and dysesthesia in the left arm. Brain magnetic resonance imaging revealed multiple hyperintense lesions in the white matter of the right hemisphere, some of which were Baló-like concentric lesions. Our diagnosis was neuromyelitis optica spectrum disorder with Baló's concentric sclerosis (BCS), although the patient was negative for anti-aquaporin-4 (anti-APQ4) antibodies. Our case suggests that Baló's concentric sclerosis overlaps with neuromyelitis optica spectrum disorder and that this overlapping is caused by a mechanism that does not involve anti-AQP4 antibodies.

Key words: Baló's concentric sclerosis, neuromyelitis optica, aquaporin-4, multiple sclerosis, myelitis, optic neuritis

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Introduction

Neuromyelitis optica (NMO) is a severe demyelinating disease defined principally by its tendency to selectively affect optic nerves and the spinal cord causing recurrent attacks of blindness and paralysis. Anti-aquaporin-4 (anti-APQ4) antibodies have been found to be a specific biomarker for NMO, and it was also discovered that NMO-IgG recognizes the astrocytic water channel aquaporin-4 [AQP4 (1, 2)]. Some clinically limited forms of this disorder, such as bilateral simultaneous or recurrent optic neuritis, are included in its pathogenetic spectrum and are classified as NMO spectrum disorder (NMOsd) (3).

Meanwhile, Baló's concentric sclerosis (BCS) is a rare demyelinating disorder pathologically characterized by alternating layers of myelinated and demyelinated tissue (4). It has been pointed out that NMO and BCS have some features in common (5), and cases of NMO (Devic's syndrome) with Baló's concentric lesions (BCLs) have been reported (6). However, such cases were reported a long time before the disease entity of NMO was established (1, 7, 8).

Recently, a case of NMO with a BCL in the brainstem was reported (9). In addition, a loss of APQ4 in BCSLs and a lack of anti-APQ4 antibodies were reported in a pathological study (10, 11), thus resulting in more attention to the associations between NMO and BCL and between BCS and APQ4. We herein describe a case of NMOsd without anti-APQ4 antibodies with BCLs. Our case may help to further understanding of the associations between NMO, BCS/BCL and APQ4.

Case Report

A 34-year-old Japanese woman with no prior neurologic history presented with bilateral simultaneous optic neuritis in April 2010. The patient was admitted one week after symptom onset. A neurological examination revealed no abnormalities, except for bilateral visual acuity loss. The next day, the patient developed sensory disturbances at the T7 spinal segment. Brain magnetic resonance imaging (MRI) revealed bilateral tortuous swelling of the optic nerves on fluid-attenuated inversion recovery (FLAIR) images (Figure A). No abnormalities fulfilling Barkhof's criteria (12)

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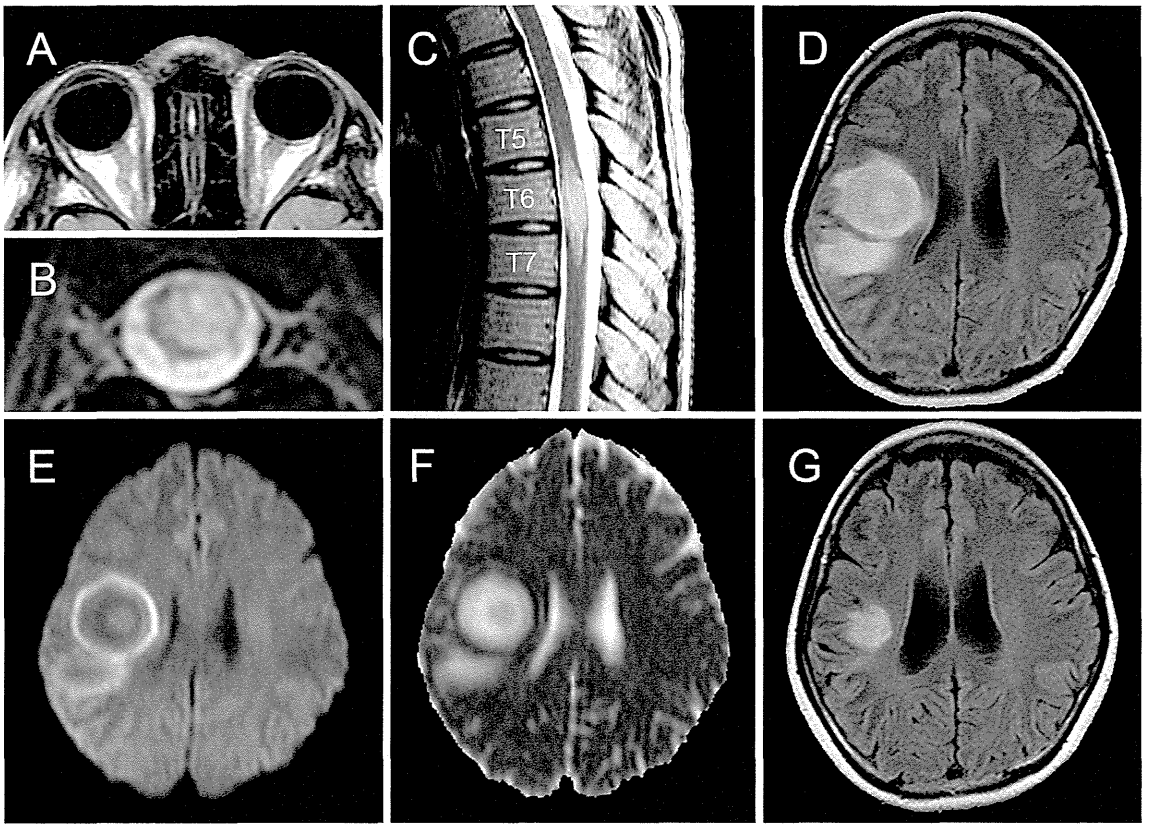

Figure. **MRI of the present patient with neuromyelitis optica spectrum disorder and Baló's concentric lesions. Brain MRI fluid-attenuated inversion recovery (FLAIR) imaging performed after the first episode revealed bilateral tortuous swelling of the optic nerves (A). Spinal cord MRI performed after the first episode revealed centrally located, concentric lesions at the T6 vertebral segment on axial T2*-weighted imaging (B) and longitudinal lesions extending more than two vertebral segments on sagittal T2-weighted imaging (C). Brain MRI performed after the second episode revealed concentric lesions comprising the central core and various rings in the white matter on FLAIR imaging (D), diffusion-weighted imaging (E) and apparent diffusion coefficient imaging (F). MRI FLAIR imaging revealed a remarkable reduction in the size of the lesions following steroid treatment compared with that observed before treatment (G).**

were observed. MR T2-weighted imaging of the spinal cord revealed centrally located and longitudinal lesions extending from the T5 to T7 vertebral segments (Figure B, C). Axial T2*-weighted MRI of the T6 vertebral segment also revealed Baló-like lesions with concentric rings (Figure B). The patient was also diagnosed with transverse myelitis because she exhibited bilateral symptoms at a clearly defined sensory level (13). Common biochemical laboratory tests were normal. The level of anti-APQ4 antibodies was tested as previously reported (14), and a serum sample obtained nine days after the neurologic onset was negative. Antinuclear, antineutrophil cytoplasmic, anti-SSA/RO, anti-SSB/LA and antiphospholipid antibodies were also negative. The patient was administered two courses of intravenous methylprednisolone pulse therapy at a dose of 1,000 mg for three consecutive days followed by oral prednisolone at a dose of 15 mg on alternate days, after which her symptoms improved.

In June 2010, she was readmitted to the hospital due to a headache and left dysesthesia in the left arm. A neurological examination revealed left facial weakness, hemiparesis and sensory disturbances on the left side of the body. T2-weighted MRI and FLAIR imaging of the brain revealed multiple hyperintense lesions in the white matter of the right hemisphere, some of which were Baló-like concentric lesions with various rings (Figure D). The central cores of these lesions demonstrated hyperintensity on diffusion-weighted imaging (DWI) (Figure E); however, no abnormalities in the apparent diffusion coefficient (ADC) were observed (Figure F). The intervening rings exhibited relatively unrestricted diffusion. As previously reported (15), the outermost layer demonstrated hyperintensity on DWI with a restricted ADC and revealed an active demyelination site on gadolinium enhancement. A cerebrospinal fluid analysis revealed a normal white blood cell count and protein level with mild elevation of the level of myelin basic proteins (183 pg/mL; normal limit, <102 pg/mL). No oligoclonal bands were observed and the IgG index was normal. A test for anti-APQ4 antibodies performed one day after the onset of the second neurologic episode was also negative. Intravenous methylprednisolone pulse therapy at a dose of 1,000

Table. **Cases of Neuromyelitis Optica Spectrum Disorder with Baló's Concentric Lesion**

Authors	Graber et al. (2009)[9]	Kreft et al. (2009)[20]	Marti et al. (2010)[21]	Our case (2013)
Age at the onset of neurological disorder	29	57	34	34
Sex	woman	woman	man	woman
Disease duration (Observation period)	> 8M	> 5Y	> 8M	
Disease course	multiphasic	multiphasic	multiphasic	multiphasic
Duration from the neurological disorder onset to the BCS onset	6M	>2Y	5M	2M
Initial symptom	myelitis	myelitis	optic neuritis, encephalopathy	optic neuritis
Neurological system involved clinically				
Cerebrum	NA	+	+	-
Brainstem	+	NA	+	-
Cerebellum	NA	NA	-	-
Optic nerve	right	bilateral	bilateral	bilateral
Spinal cord	3 VS, single	2VS, multiple	>3 VS, single	2VS, single
NMO-IgG / Anti-AQP4 Ab	+	NA	-	-
CSF OCB	-	-	-	-
IgG index	NA	0.47	NA	0.44
Numbers of BCL	one	multiple	multiple	multiple
Localization of BCL	brainstem	cerebrum	cerebrum	cerebrum , spine
Fulfillment of Barkhoff's MRI criteria for MS on initial MRI	No	No	Yes	No
Fulfillment of Barkhoff's MRI criteria for MS on the last MRI	No	Yes	Yes	Yes
Diagnosis	NMO+BCS	NMOsd+BCS	NMOsd+BCS	NMOsd+BCS
Response to steroid therapy	modest	no clinical effect	good→not improved	good

NMO: neuromyelitis optica, NMOsd: NMO spectrum disorder, BCS: Baló's concentric sclerosis, BCL: Baló's concentric lesion, VS: vertebral segments, AQP4: aquaporin-4, OCB: oligoclonal band, NA: not available, M: months, Y: years

References are indicated by the superscript numbers in parentheses.

mg for three consecutive days was reinitiated. Following the administration of pulse therapy, oral prednisolone at a dose of 40 mg daily was given, reduced by 5 mg every four weeks, then stopped. The patient's symptoms remarkably improved, with the exception of residual, mild left hemiparesis, left facial weakness, sensory disturbances of the left side of the body and decreased visual acuity. MRI revealed a remarkable reduction in the size of the lesions following steroid therapy (Figure G). The patient remains free of relapse more than nine months after the onset of the second episode.

Discussion

This case report describes a patient with simultaneous bilateral optic neuritis, transverse myelitis and Baló's concentric lesions.

NMO-IgG and anti-APQ4 antibodies, biomarkers of NMO (1, 2), are also detected in the serum of patients with NMO-related disorders (3). Any syndrome that includes recurrent or simultaneous bilateral optic neuritis or single or recurrent myelitis associated with longitudinally extensive myelitis of more than three vertebral segments is referred to as NMOsd in the context of this study (3). The patient did not fulfill the criteria for NMO due to the negative test results for NMO-IgG/anti-APQ4 antibodies and the presence of myelitis shorter than three vertebral segments. However, she originally presented with simultaneous bilateral severe optic neuritis, immediately followed by transverse myelitis, the typical clinical course of NMO. In addition, brain MRI showed no abnormalities fulfilling Barkhof's criteria (12), and spinal cord MRI revealed long lesions extending more than two vertebral segments. The spinal cord lesions observed in patients with MS are rarely longer than a single vertebral segment (16). Hence, the patient was diagnosed with NMOsd, although the differential diagnoses, including acute disseminated encephalomyelitis and tumefactive MS, could not be completely excluded.

The association between BCS and NMO has hardly been discussed due to differences in the clinical and laboratory

features of these disorders. Poser and Brinar classified BCS and NMO into one group based on purely clinical considerations of chronicity and severity (5). Although it was pointed out in the review of BCS conducted by Kuroiwa in 1985 that BCLs occasionally coexist with Devic's syndrome (6), these cases (16-18) were reported long before the discovery of NMO-IgG and the establishment of the current concept of NMO (1, 2, 7, 8).

Recently, a single case of comparatively and longitudinally extensive spinal cord lesions in a patient with BCS (20) and a single case of NMO with a BCL in the brainstem were reported (9). The clinical and laboratory characteristics of these cases are summarized in Table. Almost all of the patients presented with bilateral optic neuritis. The spinal cord lesions extended from two vertebral segments to more than three vertebral segments. NMO-IgG and anti-APQ4 antibodies were negative in the case reported by Marti et al. (21).

The extensive loss of APQ4 in the BCLs of four BCS patients was recently reported in a pathological study (10). The loss occurred in both demyelinated and myelinated layers of the BCLs, and the authors concluded that APQ4 loss can occur in patients with NMO and BCS. Furthermore, the same group showed that none of the Baló's disease patients were positive for anti-APQ4 antibodies (11). Although a current dominant hypothesis of the origins of concentric demyelination in BCS patients involves distal oligodendrogliopathy mediated by hypoxia-like tissue injury and tissue preconditioning (22), it was recently proposed that antibody-independent astrocytopathy with APQ4 loss may cause tissue destruction via prolongation of vasogenic edema and amelioration of tissue damage due to reduced cytotoxic edema, thereby resulting in alternating bands of demyelination and preserved myelin (10, 11, 23). Our BCS case, which was characterized by negative test results for anti-APQ4 antibodies, may be in line with the antibody-independent hypothesis.

In conclusion, our case suggests that an immune mechanism other than anti-APQ4 antibodies may cause NMOsd with BCS. If loss of APQ4 is a common pathologic feature in the brains of patients with BCS, pathogenetic factors other than anti-APQ4 antibodies may cause BCL formation with a loss of APQ4. This case report may help to further understanding of the associations and pathogeneses of these disorders.

The authors state that they have no Conflict of Interest (COI).

Authors' contributions

HM drafted the first manuscript and made a contribution to acquiring the data. MM revised the manuscript, leading to the final approval of the current submission. KK and YK revised the draft. SK revised the manuscript, leading to the final approval of the current submission. All authors read and approved the final manuscript.

References

1. Lennon VA, Wingerchuk DM, Kryzer TJ, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. Lancet **364**: 2106-2112, 2004.
2. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. J Exp Med **202**: 473-477, 2005.
3. Wingerchuk DM, Lennon VA, Lucchinetti CF, Pittock SJ, Weinshenker BG. The spectrum of neuromyelitis optica. Lancet Neurol **6**: 805-815, 2007.
4. Baló J. Encephalitis periaxialis concentrica. Arch Neurol **19**: 242-264, 1928.
5. Poser CM, Brinar VV. The nature of multiple sclerosis. Clin Neruol Neurosurg **106**: 159-171, 2004.
6. Kuroiwa Y. Concentric sclerosis. In: Handbook of Clinical Neurology. Volume 3. Koetsier JC, Ed. Elsevier, Amsterdam, 1985: 409-417.
7. Wingerchuk DM, Hogancamp WF, O'Brien PC, Weinshenker BG. The clinical course of neuromyelitis optica (Devic's syndrome). Neurology **53**: 1107-1114, 1999.
8. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. Neurology **66**: 1485-1489, 2006.
9. Graber JJ, Kister I, Geyer H, Khaund M, Herbert J. Neuromyelitis optica and concentric rings of Baló in the brainstem. Arch Neurol **66**: 274-275, 2009.
10. Matsuoka T, Suzuki SO, Iwaki T, Tabira T, Ordinario AT, Kira JI. Aquaporin-4 astrocytopathy in Baló's disease. Acta Neuropathol **120**: 651-660, 2010.
11. Masaki K, Suzuki SO, Matsushita T, et al. Extensive loss of connexins in Baló's disease: evidence for an auto-antibody-independent astrocytopathy via impaired astrocyte-oligodendrocyte/myelin interaction. Acta Neuropathol **123**: 887-900, 2012.
12. Barkhof F, Filippi M, Miller DH, et al. Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. Brain **120**: 2059-2069, 1997.
13. Scott TF, Frohman EM, De Seza J, Gronseth GS, Weinshenker BG. Evidence-based guideline: clinical evaluation and treatment of transverse myelitis: report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology **77**: 2128-2134, 2011.
14. Hayakawa S, Mori M, Okuta A, et al. Neuromyelitis optica and anti-aquaporin-4 antibodies measured by an enzyme-linked immunosorbent assay. J Neuroimmunol **196**: 181-187, 2008.
15. Kavanagh EC, Heran MK, Fenton DM, Lapointe JS, Nugent RA, Graeb DA. Diffusion-weighted imaging findings in Balo concentric sclerosis. Br J Radiol **79**: e28-e31, 2006.
16. Kidd D, Thorpe JW, Thompson AJ, et al. Spinal cord MRI using multi-array coils and fast spin echo. II. Findings in multiple sclerosis. Neurology **43**: 2632-2637, 1993.
17. Field EJ, Miller H, Russel D. Observations on glial inclusion bodies in a case of acute disseminated sclerosis. J Clin Pathol **15**: 278-284, 1962.
18. Courville CB. Concentric sclerosis. In: Handbook of Neurology Volume 9. Multiple Sclerosis and Other Demyelinating Diseases. Vinken PJ, Bruyn GW, Eds. North-Holland Publishing Co., Amsterdam, 1970: 437-451.
19. Kuroiwa Y, Tateishi J, Tabira T. Concentric sclerosis--with special reference to the high incidence of this unusual demyelinating disease in the Philippines. [in Japanese] Rinsho Shinkeigaku **24**: 1217-1220, 1984.
20. Kreft KL, Mellema SJ, Hintzen RQ. Spinal cord involvement in Balo's concentric sclerosis. J Neurol Sci **279**: 114-117, 2009.
21. Marti A, Nociti V, Frisullo G, et al. Demyelinating encephalo-

myeloradiculitis with Balò-like lesions. J Neurol **257**: 1566-1567, 2010.

22. Moore GR, Berry K, Oger JJ, Prout AJ, Graeb DA, Nugent RA. Baló's concentric sclerosis: surviving myelin in a patient with a relapsing remitting course. Mult Scler **7**: 375-382, 2001.

23. Kira J. Astocytopathy in Baló's disease. Mult Scl **17**: 771-779, 2011.

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Cerebrospinal fluid interleukin-6 and glial fibrillary acidic protein levels are increased during initial neuromyelitis optica attacks

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ABSTRACT

Background: The current 2006 neuromyelitis optica (NMO) criteria is useful for diagnosing NMO, however this criteria seemed to be insufficient at early stage of NMO. Hence, the development of diagnostic marker besides anti-aquaporin 4 antibody at early stage of NMO may be required. Our main aim of this study is to test the usefulness of measuring cerebrospinal fluid (CSF) interleukin (IL)-6 and glial fibrillary acidic protein (GFAP) concentrations as early diagnostic markers during initial NMO attacks.
Methods: We investigated CSF IL-6 and GFAP concentrations in 13 NMO spectrum disorder (NMOSD) patients at initial attacks, 24 idiopathic central nervous system inflammatory disease patients (9 optic neuritis, 9 myelitis and 6 encephalitis) and 20 other non-inflammatory neurological disorders (ONNDs) patients, retrospectively.
Results: The mean CSF IL-6 and GFAP concentrations during the initial NMOSD attack were 91.4 pg/ml and 369.3 ng/ml, respectively, and were significantly higher than in ONNDs, idiopathic optic neuritis and myelitis patients ($P < 0.01$). The sensitivity of high CSF IL-6 during initial NMO attack was 76.9% and that of high CSF GFAP was 84.6%, respectively.
Conclusion: Our data suggests that CSF IL-6 and GFAP may be useful early diagnostic markers of NMOSD.

1. Introduction

Neuromyelitis optica (NMO) is a severe autoimmune-mediated central nervous system (CNS) inflammatory disease that preferentially affects the optic nerves and spinal cord [1]. Anti-aquaporin-4 (AQP4) antibody is a disease-specific autoantibody found in NMO patient sera, and is considered to target the astrocytic foot processes [2]. Although NMO patients require early diagnosis for preventing relapses by intensive immunosuppressive treatments, the diagnostic capacity of the 2006 NMO criteria [1] may be insufficient during early NMO stages [3]. Measuring anti-AQP4 antibody titres aids in the early diagnosis of NMO; however, 20%–30% of NMO patients are anti-AQP4 antibody-negative [4]. Therefore, alternative early diagnostic markers are required. Elevated cerebrospinal fluid (CSF) interleukin (IL)-6 and glial fibrillary acidic protein (GFAP) concentrations have been reported in NMO [5,6], which could be useful NMO biomarkers. However, these increased concentrations were confirmed during relapses and not during initial attacks. Therefore, we retrospectively investigated CSF IL-6 and GFAP concentrations during the initial attack in NMO patients to test the usefulness of these molecules as early NMO diagnostic markers.

2. Methods

2.1. Patients

CSF samples were obtained from 13 NMO spectrum disorder (NMOSD) patients (10 women and 3 men; mean age, 50.4 y), including 2 patients with definite NMO who met the 2006 Wingerchuk's criteria [1] and 11 patients with partial NMO defined as anti-AQP4 antibody-positive optic neuritis or myelitis [7], within 1 month of the initial attack before treating the acute exacerbations with high-dose intravenous methylprednisolone or plasmapheresis, 9 idiopathic optic neuritis patients (3 women and 6 men; 48.6 y), 9 idiopathic myelitis patients (5 women and 4 men; 51.6 y) and 6 idiopathic encephalitis patients (1 woman and 5 men; 34.7 y). All idiopathic optic neuritis, myelitis and encephalitis patients showed monophasic disease course and did not present anti-AQP4 positivity or clinical/laboratory findings that suggest multiple sclerosis (MS) or NMO at final follow-up. CSF samples from 20 patients (8 women and 12 men; 58.8 y) with other non-inflammatory neurological disorders (ONNDs), including 5 with amyotrophic lateral sclerosis, 5 with spinocerebellar degeneration, 4 with Parkinson's disease, 4 with multiple system atrophy and 2 with

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progressive supranuclear palsy, were used as controls. All patients involved in this study visited Chiba University Hospital between 2003 and 2012. CSF samples were preserved at −80 °C until use.

The expanded disability status scale (EDSS) scores, lesion localisations, length of spinal cord lesion on MRI, CSF variables and positivity for serum anti-AQP4 antibody using an enzyme-linked immunosorbent assay (ELISA) [8] at the time of CSF sampling were recorded. All subjects gave informed consent and ethics approval was granted by the Ethics Committee of Chiba University School of Medicine, Chiba, Japan.

2.2. CSF IL-6 and GFAP measurement

CSF IL-6 concentrations were measured at our clinical laboratory by chemiluminescent enzyme immunoassay (CLEIA) using a two-step sandwich method with an IL-6 measurement cartridge (Human IL-6 CLEIA Fujirebio, Fujirebio, Tokyo) that was developed specifically for the fully automated CLEIA system (Lumipulse f®, Fujirebio, Tokyo) reported previously [9]. The chemiluminescence intensity was measured using a luminometer to determine IL-6 concentrations. These procedures required approximately 30 min.

CSF GFAP concentrations were measured using a human GFAP ELISA kit (BioVendor, Minneapolis, MN). The optical density was measured at 450 nm, and measurements were repeated at 405 nm if the microplate read did not record absorbances above the highest standard. All procedures followed the manufacturer's instructions.

2.3. Statistics

The groups were compared using the Mann–Whitney *U*-test for unpaired continuous measures. A $P < 0.05$ was considered statistically significant.

3. Results

3.1. Clinical profiles of patients

Ten NMOSD patients presented with myelitis, while 3 presented with optic neuritis during the initial attack. None received immunosuppressive treatments, and 12 (92%) of 13 were anti-AQP4 antibody-positive during initial attacks. The median EDSS was 5.0 (range, 1.5–8.5). The interval between the initial NMOSD attack and CSF sampling was 14.5 ± 9.8 days (mean ± SD). The follow-up period after the primary inflammatory attack was 49.5 ± 35.9 months. Median EDSS, median functional status (FS) score of visual function of EDSS, median length of spinal cord lesion on MRI and mean CSF cell counts of all patients at CSF sampling were summarised in Table 1.

Table 1
Clinical profiles of patients.

	EDSS, median (range)	FS score of visual functions, median (range)	Length of spinal cord lesion, median vertebral segments (range)	CSF cell count (/mm^3), mean ± SD
NMOSD	5.0 (1.5–8.5)	5 (5–6)	4.5 (3–10)	15.0 ± 15.0
i-ON	–	5 (3–6)	–	4.8 ± 7.5
i-myelitis	2.5 (2.0–7.0)	–	1.0 (1–12)	7.2 ± 7.2
i-encephalitis	–	–	–	16.7 ± 11.7
ONNDs	–	–	–	1.0 ± 1.1

EDSS, expanded disability status scale; FS, functional system; NMOSD, neuromyelitis optica spectrum disorder; i-ON, idiopathic optic neuritis; i-myelitis, idiopathic myelitis; i-encephalitis, idiopathic encephalitis; ONNDs, other non-inflammatory neurological disorders.

3.2. CSF IL-6 and GFAP concentrations in patients

CSF IL-6 concentrations were 91.4 ± 143.0 (mean ± SD) pg/ml and CSF GFAP concentrations were 369.3 ± 523.7 ng/ml in NMOSD patients during the initial attack. These CSF IL-6 concentrations in NMOSD patients were significantly higher than those in ONNDs patients ($P < 0.001$), idiopathic optic neuritis patients ($P = 0.003$) or myelitis patients ($P = 0.007$) and CSF GFAP concentrations in NMOSD patients were higher than those in ONNDs patients ($P < 0.001$), idiopathic optic neuritis patients ($P < 0.001$), idiopathic myelitis patients ($P < 0.001$) or idiopathic encephalitis patients ($P < 0.001$). The CSF IL-6 and GFAP concentrations did not differ between optic neuritis and myelitis in NMOSD patients. The cut-off concentrations of CSF IL-6 and GFAP were 4.93 pg/ml and 2.67 ng/ml, respectively, which were defined as mean + 3SD of the concentrations in ONNDs patients (Fig. 1). The sensitivity of CSF IL-6 positivity for diagnosing NMO was 76.9%, while that for CSF-GFAP was 84.6%. One (11.1%) of the 9 idiopathic optic neuritis patients (mean CSF IL-6 concentration was 2.7 pg/ml), 3 (33.3%) of the 9 idiopathic myelitis patients (mean CSF IL-6 concentration was 3.8 pg/ml) and 3 (50.0%) of the 6 idiopathic encephalitis patients (mean CSF IL-6 concentration was 16.7 pg/ml) showed high CSF IL-6 concentrations above the cut-off concentration, while 1 (11.1%) of the 9 idiopathic optic neuritis patients (mean CSF GFAP concentration was 3.2 ng/ml), 1 (11.1%) of the 9 idiopathic myelitis patients (mean CSF GFAP concentration was 0.9 ng/ml) and 1 (16.7%) of the 6 idiopathic encephalitis patients (mean CSF GFAP concentration was 0.9 ng/ml) showed high CSF GFAP concentrations above the cut-off concentration (Fig. 1).

4. Discussion

Evidence of the efficacy of oral prednisolone and azathioprine therapy in preventing NMO relapses has been reported [10]. Therefore, early diagnosis is essential for preventing NMO relapses using immunosuppressive treatments, but the early diagnostic capacity of the 2006 NMO criteria [1] is relatively weak (the median interval from disease onset to fulfilment of the criteria was 28 months) [3]. Although, measurement of anti-AQP4 antibody titres is crucially important in differentiating NMO, MS and other inflammatory diseases, anti-AQP4 antibody is often negative in NMO patients and the autoantibody detection usually requires several days. Markedly increased CSF IL-6 [5] and GFAP concentrations [5,6] have been established during NMO relapses, and 77.4% and 83.9% of NMO patients showed high CSF IL-6 and GFAP, respectively [5]; however, no similar analyses during initial NMO attacks have been performed. CSF IL-6 plays important roles in CNS inflammation and could be a useful NMO activity biomarker [5,11], while increased CSF GFAP concentrations indicate astrocytic damage as a primary pathological process. The current study demonstrates that CSF IL-6 and GFAP concentrations in NMOSD patients were significantly increased during initial attacks. The high sensitivities (75–85%) of these molecules for NMO diagnosis were comparable to those of serum anti-AQP4 antibody in NMO patients [3]. Therefore, CSF IL-6 and GFAP may be useful early diagnostic markers and measuring CSF IL-6 could be valuable because of its easy availability and rapid measurement.

One of the 13 NMOSD patients was anti-AQP4 antibody-negative and was initially diagnosed with viral myelitis. However, 4 months later, the patient was diagnosed with a NMOSD after having a myelitis relapse and a high serum anti-AQP4 antibody titre. Analysis of CSF samples obtained during the initial attack revealed increased CSF IL-6 and GFAP concentrations. The possibility of NMO would have been considered if we had known the increased CSF IL-6 and GFAP concentrations at the initial myelitis episode.

We showed the clear differences in CSF IL-6 and GFAP concentrations between NMOSD patients during their first event and ONNDs patients. CSF IL-6 concentration is also increased in other CNS

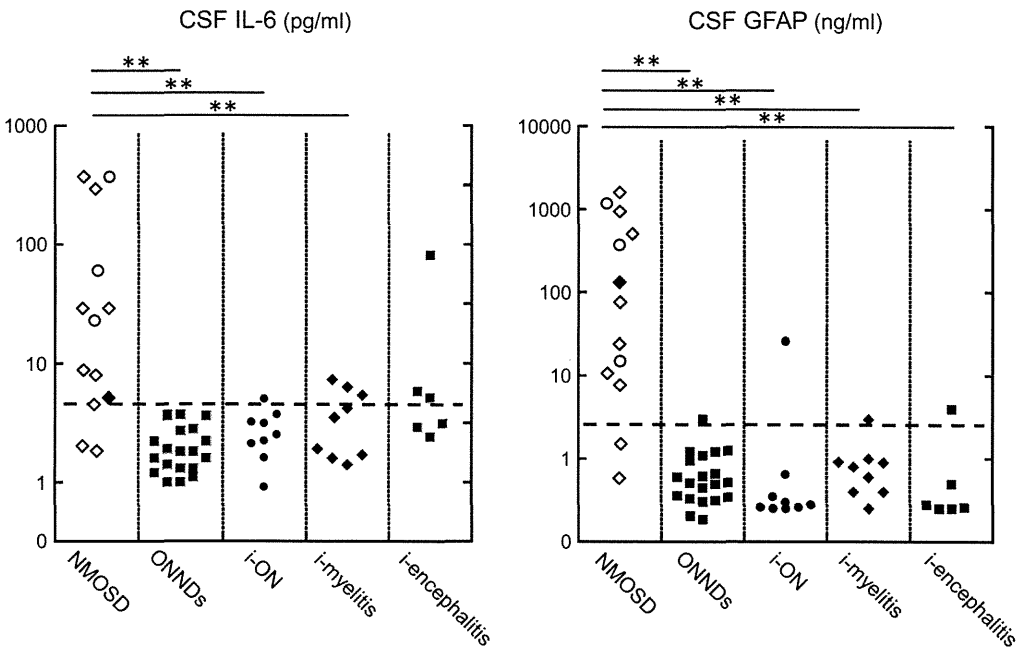

Fig. 1. CSF IL-6 and GFAP levels. CSF interleukin (IL)-6 levels were significantly higher in neuromyelitis optica spectrum disorder (NMOSD) during the initial attacks compared with other non-inflammatory neurological disorders (ONNDs) ($P < 0.001$), idiopathic optic neuritis (i-ON) ($P = 0.003$) or idiopathic myelitis (i-myelitis) ($P = 0.007$). CSF glial fibrillary acidic protein (GFAP) levels in NMOSD during the initial attacks were also significantly higher compared with ONNDs ($P < 0.001$), i-ON ($P < 0.001$), i-myelitis ($P < 0.001$) or idiopathic encephalitis (i-encephalitis) ($P < 0.001$). Diamonds indicate myelitis patients, while circles indicate optic neuritis patients. White diamonds and circles indicate anti-aquaporin-4 (AQP4) antibody-positive patients. Black diamonds, squares and circles indicate anti-AQP4 antibody-negative patients. The dashed lines are the cut-off levels for CSF IL-6 and GFAP.

inflammatory diseases, such as CNS lupus, neuro-Behçet's disease, meningitis, acute disseminated encephalomyelitis and herpetic encephalitis, while CSF-GFAP concentration is also increased in spinal infarction and acute disseminated encephalomyelitis. In this study, some of the idiopathic optic neuritis, myelitis and encephalitis patients showed high CSF IL-6 and GFAP concentrations. However, mean concentrations and the sensitivities of CSF IL-6 and GFAP in these patients were obviously low compared with those in NMOSD patients. Although anti-AQP4 antibody is an established validated biomarker and measuring anti-AQP4 antibody is needed for the accurate diagnosis of NMO, practical application for CSF IL-6 and GFAP measurements are also helpful for early diagnosis of NMO.

5. Conclusions

CSF IL-6 and GFAP were increased during initial NMO attack with high sensitivities. Measuring these molecules may be valuable for early diagnosis in NMO patients. We propose that CSF IL-6 and GFAP concentrations, besides serum anti-AQP4 antibody titres should be actively measured in patients with CNS inflammation of undetermined aetiology.

Conflict of interest

None.

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References

[1] Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. Neurology 2006;66:1485–9.
[2] Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic–spinal multiple sclerosis binds to the aquaporin-4 water channel. J Exp Med 2005;202:473–7.
[3] Uzawa A, Mori M, Muto M, Masuda S, Kuwabara S. When is neuromyelitis optica diagnosed after disease onset? J Neurol 2012;259:1600–5.
[4] Waters PJ, McKeon A, Leite MI, et al. Serologic diagnosis of NMO: a multicenter comparison of aquaporin-4-IgG assays. Neurology 2012;78:665–71.
[5] Uzawa A, Mori M, Arai K, et al. Cytokine and chemokine profiles in neuromyelitis optica: significance of interleukin-6. Mult Scler 2010;16:1443–52.
[6] Takano R, Misu T, Takahashi T, Sato S, Fujihara K, Itoyama Y. Astrocytic damage is far more severe than demyelination in NMO: a clinical CSF biomarker study. Neurology 2010;75:208–16.
[7] Mandler RN. Neuromyelitis optica—Devic's syndrome, update. Autoimmun Rev 2006;5:537–43.
[8] Hayakawa S, Mori M, Okuta A, et al. Neuromyelitis optica and anti-aquaporin-4 antibodies measured by an enzyme-linked immunosorbent assay. J Neuroimmunol 2008;196:181–7.
[9] Oda S, Hirasawa H, Shiga H, Nakanishi K, Matsuda K, Nakamua M. Sequential measurement of IL-6 blood levels in patients with systemic inflammatory response syndrome (SIRS)/sepsis. Cytokine 2005;29:169–75.
[10] Carroll WM, Fujihara K. Neuromyelitis optica. Curr Treat Options Neurol 2010;12:244–55.
[11] Uzawa A, Mori M, Sato Y, Masuda S, Kuwabara S. CSF interleukin-6 level predicts recovery from neuromyelitis optica relapse. J Neurol Neurosurg Psychiatry 2012;83:339–40.