Research article

Anti-AQP4 antibody in idiopathic acute transverse myelitis with recurrent clinical course: frequency of positivity and influence in prognosis

Marina Papais Alvarenga[1], Regina Maria Papais Alvarenga[1,2], Marcos Papais Alvarenga[1,2], Adriano Miranda Santos[1], Luiz Claudio Santos Thuler[1,3]

[1]Pós graduação em Neurologia, Universidade Federal do Estado do Rio de Janeiro, Brazil, [2]Hospital da Lagoa (Ministério da Saúde), Rio de Janeiro, Brazil, [3]Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil

Background: The anti-aquaporin4 (anti-AQP4) antibody is specific for neuromyelitis optica (NMO), but is also found in limited forms. The presence of this antibody in acute transverse myelitis (ATM) has been associated with recurrence and conversion to NMO, but the influence on disability has not yet been described.
Objective: To describe the frequency of anti-AQP4 in ATM and analyze the influence in long-term prognosis.
Design: Cross-sectional and retrospective study.
Methods: Consecutive ATM cases in a multiple sclerosis center in Rio de Janeiro, Brazil, from 2000 through 2009 were reviewed. Recurrent cases tested for anti-AQP4 were selected. ATM with magnetic resonance imaging spinal cord lesions extending over three or more vertebral segments was classified as longitudinally extensive transverse myelitis (LETM); Kurtzke scale was applied at last evaluation.
Outcome measures: Frequency of anti-AQP4; severity of spinal cord dysfunction at last follow-up.
Results: Twenty six patients (21 female:5 male; 17 white:9 African descent) were studied. The first ATM occurred at 38.04 ± 12.7 years. The interval between the first and the second ATM was eight months (1–150) and the number of ATM varied from two to seven. After 40.5 months (12–192) of disease, the median Expanded Disability Status Scale (EDSS) score was three (0–9). Anti-AQP4 antibody was positive in 26.9%. LETM was found in 65.4%. LETM presented later onset, higher disability and higher positivity to anti-AQP4 (LETM 41.2% versus no-LETM 0%, $P = 0.024$). Dysfunction at long-term follow-up was similar in anti-AQP4 positive and negative cases.
Conclusion: The frequency of anti-AQP4 in recurrent ATM was 26.9%, increasing to 41.2% among LETM. Presence of the antibody had no influence on morbidity.

Keywords: Transverse myelitis, Demyelinating diseases, Neuromyelitis optica, Anti-AQP4 antibody, Disability, Multiple sclerosis, Disability, Paresis

Introduction

Idiopathic acute transverse myelitis (ATM) is a rare demyelinating, inflammatory condition of the central nervous system. It is clinically characterized by different degrees of motor, sensory and/or autonomic dysfunction, variable remission rates and a monophasic or recurrent clinical course.[1] According to the Transverse Myelitis Consortium Work Group,[2] diagnosis requires the identification of acute spinal cord inflammation as shown by areas of gadolinium uptake on magnetic resonance imaging (MRI) or pleocytosis and/or a high-IgG index at cerebrospinal fluid (CSF) evaluation and following exclusion of secondary causes of acute myelopathy and other idiopathic inflammatory demyelinating diseases such as relapsing–remitting multiple sclerosis (RRMS) and neuromyelitis optica (NMO). Recently, the acute transverse spinal cord syndromes were classified from a clinical viewpoint into partial (APTM) or complete (ACTM) with probable different

Correspondence to: Regina Maria Papais Alvarenga, Rua Mariz e Barros 775, 2o andar, Tijuca, CEP 20270-004, Rio de Janeiro, Brazil.
Email: regina_alvarenga@hotmail.com

DOI 10.1179/2045772312Y.0000000019

etiopathogeneses and prognoses.[3] APTM is frequently associated with small spinal cord lesions on MRI and has a high risk to conversion to RRMS when associated with inflammatory brain lesions on MRI.[4] ACTM, however, is usually associated with longitudinally extensive transverse myelitis (LETM) and is being studied within the spectrum of NMO, another rare inflammatory condition that is more severe than multiple sclerosis (MS), and is clinically characterized by ATM and optic neuritis (ON), occurring simultaneously or within days, months, or years of each other with monophasic or recurrent clinical course.[5]

A milestone in the study of demyelinating inflammatory diseases of the central nervous system (CNS) was the discovery in 2004 by investigators at the Mayo Clinic of a serum autoantibody with a high specificity for NMO using indirect immunofluorescence (IIF) on a substrate of nerve tissue from rats. This antibody was also identified in partial syndromes (50% of the patients with acute LETM and in 25% of those with recurrent ON), as well as in 54% of a group of Japanese patients tested, who had the optic-spinal form of Asian MS.[6] The presence of a circulating antibody confirmed the participation of humoral mechanisms in the immunological pathogenesis of these syndromes and heralded the possibility of its use as a biological marker to aid diagnosis. Identification of this antibody in the serum of patients with NMO, ATM, ON, and OS–MS (optic spinal-MS) led to the creation of the term *syndromes of the NMO complex*.[5]

The frequency of positivity for the NMO-IgG antibody in patients with different phenotypes of acute myelitis has been reported in descriptive cross-sectional studies carried out from 2004 in Western[7–15] and Asian populations.[16–18]

In the present study, the frequency of positivity to the anti-AQP4 antibody was described in patients with idiopathic recurrent ATM from Rio de Janeiro (Brazil) and positivity to the antibody was correlated with demographic data, prognosis, and association with LETM.

Patients and methods

The medical records were reviewed for consecutive patients with inflammatory ATM receiving care at the neurological department of the Hospital da Lagoa, Rio de Janeiro, Brazil from 2000 to 2009. The primary author personally followed patients attended in the last two years.

Definition of cases

ATM was identified by the combination of varying degrees of motor, sensory, and/or autonomic dysfunction with acute onset (from 4 hours to 21 days). The inflammatory etiology was confirmed by spinal cord MRI T2 lesion with or without gadolinium uptake and CSF features as pleocytosis or high-IgG index. In patients who had only one acute spinal cord event, the course of the disease was considered monophasic, whereas patients who had additional episodes of ATM with no clinical indications that the disease had affected any other site in the CNS were considered to have the recurrent form.

Classification

Acute myelitis was classified as APTM or ACTM according to Scott *et al.*[3]

Inclusion criteria

Patients with idiopathic ATM with recurrent clinical course, tested for anti-AQP4 were selected for the study.

Exclusion criteria

Based on medical history, clinical examination, and supplementary tests, patients with the following conditions were excluded from the study: previous or concomitant systemic autoimmune diseases; infections (human immunodeficiency virus, human T lymphotropic virus type 1, syphilis, neuro-parasitoses, herpes simplex, cytomegalovirus, toxoplasmosis, and herpes zoster) and other idiopathic inflammatory demyelinating disorders as MS and NMO.

Data collection

The following data were collected directly from the medical charts: gender (male/female), age (years), race (white/of African descent), age at onset of the disease (years), total number of ATM per patient, time between the first and the second episodes of myelitis and description of motor, sensory, and autonomic dysfunction at the last follow-up.

Supplementary tests

Spinal cord MRI was analyzed with respect to the extent of inflammatory lesions and then classified as: LETM (lesions extending over three or more vertebral segments) and non-LETM (small lesion, less than three vertebral segments). Brain MRI was analyzed and classified as suggestive or not of MS. The anti-AQP4 antibody was tested by IIF assay.

Long-term impairment

Dysfunction and disability were staged according to Kurtzke's Functional Systems (FS)/Expanded Disability Status Scale (EDSS) scale at the last neurological evaluation. Severe spinal cord dysfunction was scored by motor, sensorial and bowel, and bladder FS ≥ 4 and severe disability by EDSS ≥ 6.

Statistical analysis

The data were saved and analyzed using the SPSS statistical software program, version 14; the missing values were excluded from analysis. Categorical variables were expressed as percentages together with their respective 95% confidence intervals. Continuous variables were presented as medians and ranges. Comparison of subgroups was done using the χ^2 and Mann-Whitney U test. *P*-values <0.05 were considered statistically significant.

Ethical aspects

The study was approved by the Internal Review Board of the Federal University of the State of Rio de Janeiro (UNIRIO).

Results

Forty five cases of idiopathic ATM were identified in the period 2000 through 2009, after exclusion of other inflammatory syndromes associated with secondary etiologies as infectious or systemic autoimmune diseases ($N = 8$) and cases that convert to other IIDD (idiopathic inflammatory demyelinating diseases) ($N = 23$). Fifteen patients remained monophasic and thirty developed further relapses. Among the recurrent cases, 26 were tested for anti-AQP4 and enrolled in this study.

Clinical findings

Of the 26 patients with idiopathic ATM who had recurrent clinical course, 21 (80.8%) were female and five (19.2%) male; 17 (65.4%) were white and nine (34.6%) of African descent. Onset of the disease occurred at a mean age of 38.04 ± 12.71 years (12–60 years). The first myelitis was partial in 61.5% (16 of 26) and complete in 38.5% (10 of 26). During the course of the disease six patients with APTM at onset developed ACTM. The median time between the first and the second episode ATM was eight months (1–150 months). The number of myelitis episodes ranged from two to seven per patient. At the last neurological evaluation, after a median time of 40.5 months (range 12 from 192 months) the median FS scores were pyramidal 2 (0–5), sensory 2.5 (0–5), bowel and bladder 1 (0–5), and the majority of the patients presented mild disability with EDSS score three (0–9). Four patients had no disability (15.4%); 14 mild disability (53.8%), one moderate (3.8%), and seven severe disability (26.9%). In the latter group, two patients required aids for walking, three used a wheelchair, and two were bedridden. One of these patients died following a severe cervical lesion that resulted in respiratory failure; this occurred during the fourth episode of ATM.

Supplementary tests

Serial vertebral MRI studies identified LETM in 17 patients (65.4%); the remaining patients presented with small lesions and were classified as no_LETM. The anti-AQP4 antibody was positive in seven cases (26.9%), all of them with LETM. Cranial MRI was normal or not suggestive of MS in all cases.

LETM versus no-LETM

Comparing ATM subgroups classified by spinal cord extension as LETM and no-LETM according to the extension of the spinal cord lesion ($\geq$ or $<$ than three vertebral segments) we found significant clinical and laboratorial differences as shown at Table 1. LETM had later onset, severe clinical course and higher seropositivity for anti-AQP4 (41.2%).

AQP4 antibody and long-term prognosis

The influence of the positivity of anti-AQP4 in the severity of LETM was analyzed at Table 2. No statistical difference was found concerning the severity of the spinal cord dysfunction and disability at last evaluation when we compared anti-AQP4 positive and negative subgroups.

Discussion

Idiopathic ATM with relapsing–remitting clinical course is a rare chronic inflammatory condition that affects mainly women. In the present study, we found an anti-AQP4 seropositivity rate of 26.9% but the frequency rose to 41.2% in a subgroup of patients with LETM. In the studies that have been carried out in Western countries the positivity for anti-AQP4 in ATM associated with LETM ranged from 15.4[14] to 100%.[11] Some factors, such as the laboratory assay used to identify the autoantibody, the criteria for selecting patients and ethnicity, may explain this variation. The method applied in the present study to detect the antibody was the IIF on rat cerebellum. This method was described by Lennon *et al.*[6] and applied firstly in patients from Mayo Clinic with NMO syndromes and until now is the most widely used for IgG-NMO detection. After that, other methods have been developed to improve the sensitivity, based on the hypothesis that the use of nerve tissue from rats and not human material as substrate for IIF could constitute a limiting factor. A recent study published by Fazio *et al.*[14] assessed sensitivity and specificity of five different assays for testing anti-AQP4 antibodies in NMO, syndromes with high risk to develop NMO (hrNMO), MS, APTM, and health controls. Two assays were able to detect perivascular IgG reactivity on brain tissue by

Table 1 Demographic, clinical, and laboratorial data of patients with LETM versus no-LETM

	LETM *N* = 17	no-LETM *N*= 9	*P*
Gender (*n*/%)			
Female	14 (82.4%)	07 (77.8%)	0.78
Male	03 (17.6%)	02 (22.2%)	
Race (*n*/%)			
White	12 (70.6%)	05 (55.6%)	0.43
Afrobrazilian	05 (29.4%)	04 (44.4%)	
Age at onset (years)	43 (19–60)	31 (12–47)	0.023
Median (min–max)			
Time of disease (months)			0.483
Median (min–max)	36 (17–168)	46 (12–192)	
First myelitis (*n*/%)			
APTM	08 (47.1%)	07 (77.8%)	0.216
ACTM	09 (52.9%)	02 (22.2%)	
Clinical presentation (*n*%)			
APTM only	05 (29.4%)	04 (44.4%)	0.443
ACTM in some attack	12 (70.6%)	05 (55.6%)	
Anti-AQP4 antibody (*n*/%)			
Positive	07 (41.2%)	0 (0%)	0.024
Negative	10 (58.8%)	09 (100%)	
Severity of disease (*n*%) (FS ≥ 4; EDSS ≥ 6)			
Motor FS	6 (35.3%)	0	0.042
Sensory FS	5 (29.4%)	0	0.07
Bladder and bowel FS	3 (17.6%)	1 (11.1%)	0.66
EDSS	7 (41.2%)	0	0.024

LETM, longitudinally extensive transverse myelitis; APTM, partial transverse myelitis; ACTM, complete transverse myelitis; FS.EDSS, functional system/expanded disability status score; anti-AQP4, antibody anti-aquaporin4.

immunofluorescence (NMO-IgG and three other assays have been set to detect anti-AQP4 antibodies: immunofluorescence and flow cytometry on AQP4-expressing cells, and a radioimmunoprecipitation assay). The presence of serum NMO-IgG and anti-AQP4 reactivity was almost exclusively restricted to patients with NMO and hrNMO. Seroprevalence and sensitivity ranged from 30 to 47%, depending on the assay. Specificity ranged from 95 to 100%. Comparing results obtained in the five assays, lack of concordance occurred in some samples. The authors concluded that the current lack of a gold standard to detect anti-AQP4 antibodies implies the necessity to standardize the detection of these antibodies.

A large number of trials aimed to establish the frequency of the positivity for anti-AQP4 in high-risk syndromes. In patients with acute LETM evaluated in the study conducted by Lennon *et al.*[6], the antibody was present in 52% of patients, a frequency that was confirmed in later studies using the same method: 50[9,12] and 53.8%.[13] Only one study reported positivity of 80%; however, in this case, sample size was small (5 patients).[10] Using immunoprecipitaion fluorescence, the seropositivity rate found was 55%,[12] whereas with radioimmunoprecipitation this rate was 100%,[11] however, the sample consisted of only six patients. In the present study the blood samples of Brazilian patients were tested by the original assay and the frequency of anti-AQP4 in LETM was 41.2%, very similar to 37.9% in patients in the USA tested with the same method.[7]

On the contrary, in patients with myelitis with small lesions (no-LETM), presence of the antibody is

Table 2 Morbidity in patients with LETM anti-AQP4 (+) versus anti-AQP4 (–)

Features	Anti-AQP-4 (+) *N* = 7	Anti-AQP-4 (–) *N* = 10	*P*
Gender female:male	6:1	8:2	0.761
Race white:Afrobrazilian	5:2	7:3	0.949
Severity of disease at last evaluation (*n*%) (FS ≥ 4; EDSS ≥ 6)			
Motor FS	3/7 (42.8%)	3/10 (30.0%)	0.585
Sensory FS	2/7 (28.5%)	3/10 (30.0%)	0.949
Bladder and bowel FS	2/7 (28.5%)	1/10 (10.0%)	0.323
EDSS	3/7 (42.8%)	4/10 (40.0%)	0.906

practically non-existent, reinforcing the strong correlation between the antibody and the extent of the spinal lesion. In a study carried out by Scott *et al.*[8] only one of the twenty-two patients with ATM and small spinal cord lesions tested positive for anti-AQP4. This patient went on to develop an extensive spinal lesion. Jarius *et al.*[10] detected the antibody in 80% of cases of acute LETM; however, all 11 patients no-LETM tested were negative. Likewise, Marigner *et al.*[13] detected the antibody in seven of the 21 patients with ATM, LETM being found in all the seropositive patients and in none of the seronegative individuals. In our study, we confirm these previous reports: all Brazilian patients with no-LETM were negative for anti-AQP4.

Conclusion

The influence of the anti-AQP4 in long-term prognosis of recurrent ATM with LETM was analyzed here. We found no significant difference in severity of spinal cord dysfunction and long-term disability in subgroups positive and negative to AQP4.

Although the presence of the anti-AQP4 in NMO, OS–MS and in high-risk syndromes for NMO has been confirmed, its role in the pathogenesis of these conditions has yet to be established. What has yet to be established is the participation of this antibody in the inflammatory process: cause or consequence?

References

1 Wingerchuk DM, Weinshenker BG. Unusual presentations and variants of central nervous system idiopathic demyelinating diseases. In: Raine CS, McFarland HF, Hohlfeld R. (eds.) Multiple sclerosis. A comprehensive text. Saunders Elsevier: Philadelphia, PA, USA, 2008. p. 24–42.

2 Transverse Myelitis Consortium Working Group. Proposed diagnostic criteria and nosology of acute transverse myelitis. Neurology 2002;59(4):499–505.

3 Scott TF, Kassab SL, Singh S. Acute partial transverse myelitis with normal cerebral magnetic resonance imaging: transition rate to clinically definite multiple sclerosis. Mult Scler 2005;11(4): 373–7.

4 Ford B, Tampieri D, Francis G. Long-term follow-up of acute partial transverse myelopathy. Neurology 1992;42(1):250–2.

5 Wingerchuk DM, Lennon VA, Lucchinetti CF, Pittock SJ, Weinshenker BG. The spectrum of neuromyelitis optica. Lancet Neurol 2007;6(9):805–15.

6 Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Luchinetti CF, Fujihara CF, *et al.* A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. Lancet 2004; 364(9451):2106–12.

7 Weinshenker BG, Wingerchuk DM, Vukusic S, Linbo L, Pittock SJ, Luchinetti CF, *et al.* Neuromyelitis optica IgG predicts relapse after longitudinally extensive transverse myelitis. Ann Neurol 2006;59(3):566–9.

8 Scott TF, Kassab SL, Pittock SJ. Neuromyelitis optica IgG status in acute partial transverse myelitis. Arch Neurol 2006;63(10): 1398–400.

9 Saiz A, Zuliani L, Blanco Y, Tavolato B, Giometto B, Graus F, *et al.* Revised diagnostic criteria for neuromyelitis optica (NMO). Application in a series of suspected patients. J Neurol 2007; 254(9):1233–7.

10 Jarius S, Franciotta D, Bergamaschi R, Wright H, Littleton E, Palace J, *et al.* NMO-IgG in the diagnosis of neuromyelitis optica. Neurology 2007;68(13):1076–7.

11 Paul F, Jarius S, Aktas O, Bluthner M, Bauer O, Appelhans H, *et al.* Antibody to aquaporin 4 in the diagnosis of neuromyelitis optica. PLoS Med 2007;4(4):e133.

12 Waters P, Jarius S, Littleton E, Leite MI, Jacob S, Gray B, *et al.* Aquaporin-4 antibodies in neuromyelitis optica and longitudinally extensive transverse myelitis. Arch Neurol 2008;65(7):913–9.

13 Marigner R, De Seze J, Vukusic S, Durand-Dubief F, Zéphir H, Vermersch P, *et al.* NMO-IgG and Devic's neuromyelitis optica: a French experience. Mult Scler 2008;14(4):440–5.

14 Fazio R, Malosio V, Lampasona D, De Feo D, Privitera F, Marnetto D, *et al.* Antiacquaporin 4 antibodies detection by different techniques in neuromyelitis optica patients. Mult Scler 2009; 15(10):1153–63.

15 Alvarenga MP, Thuler LC, NETO SP, Vasconcelos CC, Camargo SG, Alvarenga MP, *et al.* The clinical course of idiopathic acute transverse myelitis in patients from Rio de Janeiro. J Neurol 2010;257(6):992–8.

16 Nakashima I, Fujihara K, Miyazawa I, Misu T, Narikawa K, Nakamura M, *et al.* Clinical and MRI features of Japanese patients with multiple sclerosis positive for NMO-IgG. J Neurol Neurosurg Psychiatry 2006;77(9):1073–5.

17 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(Pt 5):1206–23.

18 Kim SH, Kim SM, Vincent A, Ahn SW, Hong YH, Park KS, *et al.* Clinical characteristics, prognosis, and seropositivity to the anti-aquaporin-4 antibody in Korean patients with longitudinally extensive transverse myelitis. J Neurol 2010;257:920–5.