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SHORT COMMUNICATION

Seroprevalence and specificity of NMO-IgG (anti-aquaporin 4 antibodies) in patients with neuropsychiatric systemic lupus erythematosus

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Abstract Neuropsychiatric manifestations are present in 30–40% of patients with systemic lupus erythematosus (SLE). Recently, antibodies to aquaporin-4 (termed AQP4-Ab, or NMO-IgG), a water channel protein, were reported to be present in a subset of patients with SLE and neurological involvement. To evaluate the syndrome specificity and prevalence of serum NMO-IgG/anti-AQP4 antibodies in patients with neuropsychiatric systemic lupus erythematosus (NPSLE). Sera of 76 patients with SLE and neurological symptoms, 50 of whom met the ACR case definitions of NPSLE, were tested for AQP4-Ab in an indirect immunofluorescence assay employing HEK293 cells transfected with recombinant human AQP4. Only one of the examined sera was positive for NMO-IgG/AQP4-Ab. This patient suffered from TM, ranging over two vertebral segments on spinal MRI. None of the 75 NPSLE without TM was found to be seropositive for NMO-IgG/AQP4-Ab. NMO-IgG/AQP4-Ab in NPSLE were present only in a patient with TM and were not detectable in NPSLE patients with other neurological manifestations. Testing for NMO-IgG/AQP4-Ab positivity should be considered in patients presenting with SLE and TM. Non-longitudinally extensive lesions do no not exclude NMO-IgG/AQP4-Ab in patients presenting with SLE and TM.

Keywords Neuropsychiatric systemic lupus erythematosus · Neuromyelitis optica · Optic neuritis · Longitudinally extensive transverse myelitis · Aquaporin4 · Anti-aquaporin 4 antibody

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Introduction

Neuropsychiatric manifestations are present in 30–40% of patients with systemic lupus erythematosus (SLE) [1]. Transverse myelitis (TM) and optic neuritis (ON), or the combination of both (termed neuromyelitis optica, NMO), are rare but serious complication of SLE, and have been described in approximately 1% of SLE patients [2–4]. Recently, pathogenic autoantibodies against the aquaporin-4 (AQP4) water channel (termed anti-AQP4-Ab or NMO-IgG) have been reported in a subset of patients with TM and ON [5, 6]. The prevalence and specificity of these antibodies has been examined also in small cohorts of patients with suspected SLE [7]. However, not all patients included in those studies met the ACR criteria and not all of them had definite neuropsychiatric SLE (NPSLE) according to the ACR case definitions [8]. In this study, we evaluated the seroprevalence and syndrome specificity of NMO-IgG/AQP4-Ab in a large cohort of patients with unequivocally diagnosed NPSLE.

Patients

Consecutive patients enrolled between January 2002 and January 2009 into the Lupus Registry of the Institute of Rheumatology in Prague were studied. The Institute of Rheumatology receives referrals from primary care physicians, general internists, rheumatologists, and other subspecialties from the whole Czech Republic. The registry currently contains data and sera from 471 SLE patients. All patients in the registry met the revised ACR classification criteria for SLE [9] and signed the informed consent. Patients were first evaluated upon enrollment into the registry and the serum samples from the enrollment visit of selected patients were used for the current study. For the purpose of selection of true NPSLE cases, data both from the Lupus Registry and from the institutional clinical database were used. As *possible* NPSLE cases were designated all SLE patients that were referred to neuroimaging by MRI and/or passed examination by a neurologist specialized in NPSLE. As *definite* cases of NPSLE were designated those patients that in addition unequivocally fulfilled the ACR case definitions for neuropsychiatric lupus syndromes [8]. The study protocol was approved by the institutional research ethics committee.

Methods

Samples were analyzed by means of a recombinant immunofluorescence assay (rIFA) using AQP-4 transfected HEK293 cells. In direct comparison with the original procedure for the detection of NMO-IgG/anti-AQP4 Ab as described by Lennon et al. [5], the rIFA exhibited an increase in sensitivity of 12.5% (overall sensitivity 78.1%; specificity 100%) in a study based on 183 samples from NMO patients and relevant neurologic disease controls, including MS [10]. Serum samples were classified as being positive or negative for NMO-IgG/anti-AQP4 Ab by two independent investigators who were unaware of the clinical data.

Results

We have identified overall 76 SLE cases with neuropsychiatric involvement. Of these, 50 were classified as definite NPSLE cases by using the above-mentioned criteria. The average age of patients with definite NPSLE was 43 years (±16) and 90% were women.

Most of the 50 NPSLE patients fulfilled more than one ACR case definitions (average 2), with cognitive disorder being the most common (25/50), followed by mood disorder (14/50), cerebrovascular disease (13/50), headache (13/50), seizure disorder (11/50), and psychosis (8/50). Other case definitions were less frequently represented (aseptic meningitis 2/50, movement disorder 1/50, myelopathy 1/50, acute confusional state 2/50, anxiety disorder 3/50, cranial neuropathy 3/50, and polyneuropathy 4/50)—see Table 1. Only one of the 50 NPSLE patients was diagnosed with myelitis, while optic neuritis, another condition frequently associated with NMO-IgG/AQP4-Ab, was not documented in this cohort; 47/50 NPSLE patients were examined by MRI and 39/47 had white matter changes suggestive of NPSLE. These changes were mostly small punctiform MRI lesions located predominantly in the periventricular and subcortical white matter. All 76 patients with possible/definite NPSLE were ANA positive. Of the 50 patients with definite NPSLE 21 were positive for anti-dsDNA, 25 for anti-Ro, 14 for anti-La, 13 for anti-RNP, and 13 for antiphospholipid antibodies (defined as anticardiolipin/anti-GPB2 ELISA and/or lupus anticoagulants). Only the patient who was diagnosed with myelitis was found to be NMO-IgG/anti-AQP4 Ab positive, all other patients with possible or definite NPSLE were NMO-IgG/anti-AQP4 Ab negative. The positive patient was a 48 years old women who developed myelitis 12 years after being diagnosed with SLE (based on the following ACR criteria: ANA+, anti-dsDNA+, leucopenia, pericarditis, arthritis). She also fulfilled European American Consensual Group criteria for both primary and secondary Sjögren's syndrome (ocular symptoms, oral symptoms, objective evidence of dry eyes, and presence of anti-Ro and anti-La antibodies). Her autoantibody profile included positivity for ANA, anti-dsDNA, anti-Ro 52, anti-Ro 60, anti-La, and anti-nucleosomes. She manifested predominantly by sensitive disturbances (quadriparesthesia, Lhermitte sign), and mild paraparesis without sphincter disturbances. In T2-weighted MRI scans, she had a lesion extending over two cervical vertebral segments (C2–C3) of the spinal cord (see Fig. 1), and NMO-IgG/anti-AQP4 Ab was detected at a titer of 1:1,000. She was treated by pulses of methylprednisolone and cyclophosphamide with a good response. She developed two further clinical relapses of sensitive disturbances in the following 3 years.

Discussion

In this large cross-sectional cohort study, we evaluated the seroprevalence of NMO-IgG/anti-AQP4 Ab in patients with established NPSLE. NMO-IgG/AQP4-Ab was present in the only patients with TM in our cohort, but was negative in 75 NPSLE patients with neurological signs or symptoms other than TM. Our findings demonstrate that NMO-IgG/AQP4-Ab is highly specific for TM also in NPSLE, a disease associated with a broad spectrum of

Table 1 Neuropsychiatric SLE (NPSLE) syndromes and seroprevalence of autoantibodies

NPSLE syndrome	Number of patients with syndrome *N*	ANA *n* (%)	Anti-dsDNA *n* (%)	Anti-Ro *n* (%)	Anti-La *n* (%)	APLA *n* (%)	Anti-ribosomal P *n* (%)	Anti-AQP4 *n* (%)
Aseptic meningitis	2	2 (100%)	1 (50%)	1 (50%)	0 (0%)	1 (50%)	2 (100%)	0 (0%)
Cerebrovascular disease	13	13 (100%)	3 (23%)	7 (54%)	0 (0%)	4 (31%)	6 (46%)	0 (0%)
Demyelinating syndrome	0							
Headache	13	13 (100%)	8 (62%)	5 (38%)	2 (15%)	6 (46%)	5 (38%)	0 (0%)
Movement disorder	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Myelopathy	1	1 (100%)	1 (100%)	1 (100%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)
Seizure disorder	11	11 (100%)	7 (64%)	6 (55%)	5 (45%)	2 (18%)	2 (18%)	0 (0%)
Acute confusional state	2	2 (100%)	0 (0%)	2 (100%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)
Anxiety disorder	3	3 (100%)	2 (67%)	3 (100%)	1 (33%)	0 (0%)	3 (100%)	0 (0%)
Cognitive dysfunction	25	25 (100%)	10 (40%)	12 (48%)	7 (28%)	7 (28%)	4 (16%)	0 (0%)
Mood disorder	14	14 (100%)	7 (50%)	7 (50%)	5 (36%)	1 (7%)	5 (36%)	0 (0%)
Psychosis	8	8 (100%)	1 (13%)	3 (38%)	3 (38%)	0 (0%)	3 (38%)	0 (0%)
Movement disorder	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Guillain–Barre syndrome	0							
Autonomic neuropathy	0							
Mononeuropathy	2	2 (100%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	0 (0%)
Myasthenia gravis	0							
Cranial neuropathy	3	3 (100%)	1 (33%)	1 (33%)	0 (0%)	2 (67%)	1 (33%)	0 (0%)
Plexopathy	0							
Polyneuropathy	4	4 (100%)	0 (0%)	3 (75%)	1 (25%)	2 (50%)	1 (25%)	0 (0%)

The seroprevalence of autoantibodies in altogether 103 neuropsychiatric syndromes described in the 50 patients with SLE. Most of the patients have thus fulfilled more than one ACR case definition for neuropsychiatric syndrome in SLE. Anti-AQP4 antibodies were only present in the patient with myelopathy/myelitis

autoantibodies, many of which are not disease-specific. Importantly, all patients included in the present cohort met the strict ACR criteria for SLE. In addition, 50 of our patients fulfilled the ACR case definition for neuropsychiatric syndromes.

Our data are in good accordance with findings from some previous studies. In a study of 153 patients with neuromyelitis optica spectrum disorders, Pittock et al. [7] found 3 NMO-IgG/AQP4-Ab positive SLE patients (2%). By contrast, none out of 49 control patients with either SLE or Sjögren's syndrome but no neuropsychiatric symptoms was positive for NMO-IgG/AQP4 Ab. In another study [11], 8 SLE patients with either longitudinally extensive TM or ON were found NMO-IgG/AQP4-Ab seropositive. None of the 40 control patients with SLE, of which 20 had NPSLE and 1 of these had TM involving less than 3 vertebral segments, was positive. The high specificity of NMO-IgG/AQP4-Ab for TM both in patients without SLE and patients with SLE found in our study and previous ones suggests that AQP4-Ab positive TM and SLE are independent conditions, which may co-exist in a similar manner as, e.g., autoimmune thyroiditis.

Our cohort consisted of patients that were included in the lupus register and referred to a neurological exam or brain MRI imaging. Thus, we could not capture patients referred exclusively to ophthalmology. Therefore, no patients with optic neuritis (ON) were included in the present cohort. However, previous studies [12] suggest that NMO-IgG/AQP4-Ab is also present in a subset of patients with ON, and testing for this antibody in patients with SLE and ON seems thus highly justifiable.

It is of special note that the spinal cord lesion was limited to two vertebral segments in our patients, and thus was not "longitudinally extensive" as reported in most previous NMO-IgG/AQP4-Ab positive patients. This underscores that NMO-IgG/AQP4-Ab in patients with TM should thus be assessed independently of lesion length as detected on MRI, which is subject to timing issues [13].

Due to the retrospective design of this study, blood testing was mostly not done at the onset of neurological symptoms in this study and in previously published ones. However, it is known from patients with long-term follow-up that AQP-4 antibodies remain detectable in the serum also during remission over many years, in part at high

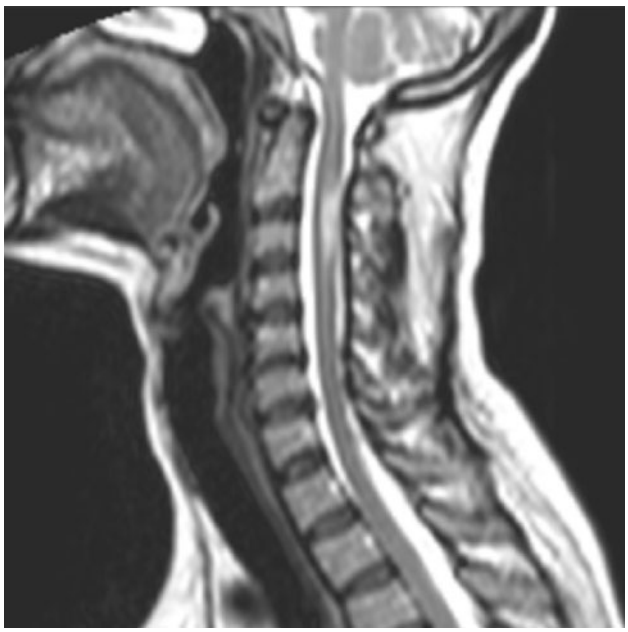

Fig. 1 Sagittal image of the cervical spinal cord illustrating signal hyperintensity in the C2–C3 level

titers, suggesting that AQP-4 antibody testing can be of diagnostic relevance independently of disease activity [14].

TM have been reported to evolve in about 1% of SLE patients. While part of these cases may be truly and only SLE-related events (perhaps caused more often by arterial thrombosis or vasculitis than by demyelinization), significant percentage may in fact prove to be NMO-IgG/AQP4-Ab-mediated. In NMO-IgG/AQP4-Ab positive patients, however, repeated relapses of TM are common [15], and plasmapheresis and immunosuppressive (e.g., azathioprin or rituximab) are required in many cases. The pathogenicity of AQP4-antibodies is strongly supported by passive transfer experiments in mice or rat [16, 17]. AQP4-antibodies are detectable in serum during relapse as well as during remission, but there is growing evidence that the levels of anti-AQP4 antibodies correlate with disease activity. Furthermore, an increase in AQP4 titer often precedes each clinical relapse in NMO [18]. Higher AQP4 titers have been also associated with the severity of NMO manifestations (complete blindness and more extensive spinal cord and brain involvement) [19]. NMO-IgG/AQP4-Ab testing is therefore of high importance to assist diagnostic, prognostic, and therapeutic decisions in patients presenting with SLE and TM [20].

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Conflict of interest KPW is an employee of Euroimmun, Lübeck. Euroimmun had no influence on study design, interpretation of the results, or the decision of publish the manuscript. All other authors have no conflict of interest.

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