Determination of Neuronal Antibodies in Suspected and Definite Creutzfeldt-Jakob Disease

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IMPORTANCE Creutzfeldt-Jakob disease (CJD) and autoimmune encephalitis with antibodies against neuronal surface antigens (NSA-abs) may present with similar clinical features. Establishing the correct diagnosis has practical implications in the management of care for these patients.

OBJECTIVE To determine the frequency of NSA-abs in the cerebrospinal fluid of patients with suspected CJD and in patients with pathologically confirmed (ie, definite) CJD.

DESIGN, SETTING, AND PARTICIPANTS A mixed prospective (suspected) and retrospective (definite) CJD cohort study was conducted in a reference center for detection of NSA-abs. The population included 346 patients with suspected CJD and 49 patients with definite CJD.

MAIN OUTCOMES AND MEASURES Analysis of NSA-abs in cerebrospinal fluid with brain immunohistochemistry optimized for cell-surface antigens was performed. Positive cases in the suspected CJD group were further studied for antigen specificity using cell-based assays. All definite CJD cases were comprehensively tested for NSA-abs, with cell-based assays used for leucine-rich glioma-inactivated 1 (LGII), contactin-associated protein-like 2 (CASPR2), N-methyl-D-aspartate (NMDA), and glycine (Gly) receptors.

RESULTS Neuronal surface antigens were detected in 6 of 346 patients (1.7%) with rapid neurologic deterioration suggestive of CJD. None of these 6 patients fulfilled the diagnostic criteria for probable or possible CJD. The target antigens included CASPR2, LGII, NMDAR, aquaporin 4, Tr (DNER [Δ/notch-like epidermal growth factor–related receptor]), and an unknown protein. Four of the patients developed rapidly progressive dementia, and the other 2 patients had cerebellar ataxia or seizures that were initially considered to be myoclonus without cognitive decline. The patient with Tr-abs had a positive 14-3-3 test result. Small cell lung carcinoma was diagnosed in the patient with antibodies against an unknown antigen. All patients improved or stabilized after appropriate treatment. None of the 49 patients with definite CJD had NSA-abs.

CONCLUSIONS AND RELEVANCE A low, but clinically relevant, number of patients with suspected CJD had potentially treatable disorders associated with NSA-abs. In contrast, none of 49 patients with definite CJD had NSA-abs, including NMDAR-abs, GlyR-abs, LGII-abs, or CASPR2-abs. These findings suggest that cerebrospinal fluid NSA-abs analysis should be included in the diagnostic workup of patients with rapidly progressive central nervous system syndromes, particularly when they do not fulfill the diagnostic criteria of probable or possible CJD.
Creutzfeldt-Jakob disease (CJD) and central nervous system (CNS) disorders associated with antibodies against neuronal surface antigens (NSA-abs) may present with similar clinical features. Unlike CJD, CNS disorders associated with NSA-abs are potentially treatable and often have a good outcome if diagnosed and treated early. The diagnosis of these immune-mediated disorders is based on the rapid presentation of symptoms and detection of antibodies in serum and cerebrospinal fluid (CSF); other tests, such as electroencephalography or magnetic resonance imaging, are less useful because they may show nonspecific findings, and magnetic resonance imaging is normal in 10% to 60% of patients depending on the antibody type. Creutzfeldt-Jakob disease usually presents with rapidly progressive cognitive and/or motor symptoms, and the diagnosis is supported by the presence of characteristic electroencephalographic and magnetic resonance imaging findings as well as increased levels of neuronal injury markers in the CSF, particularly the 14-3-3 protein. The diagnostic accuracy of each test varies and depends on the phase of the disease and the genotype on codon 129. The 14-3-3 test is negative in approximately 12% to 14% of definite sporadic CJD, and false-positive results have been found in several neurologic diseases characterized by acute and extensive neuronal damage, including paraneoplastic neurologic disorders. On the other hand, recent studies suggest that antibodies to the N-methyl-D-aspartate receptor (NMDAR-abs), glycine receptor (GlyR-abs), and voltage-gated potassium channel (VGKC-abs) complex may occur in the serum of patients with CJD, further complicating the diagnostic dilemma of these disorders. The aim of the present study was to systematically determine the frequency of NSA-abs in 346 suspected and 49 pathologically confirmed cases of CJD and to describe the cases with treatable immune-mediated disorders.

Methods

Patients

Our laboratory is a reference center for 14-3-3 and neuronal antibody testing in Spain and has collaborated in the CJD surveillance system in Catalonia, Spain, for CJD case detection and diagnosis since 1997. We receive approximately 400 CSF samples per year obtained from patients with suspected CJD for 14-3-3 protein determination. During 2012, we prospectively tested all CSF samples received for 14-3-3 determination for NSA-abs. In addition, CSF samples from 49 patients with pathologically confirmed CJD registered in the epidemiologic surveillance center of Catalonia and the Neurologic Tissue Bank of the Biobank of Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) were tested for NSA-abs. We examined the frequency of positive 14-3-3 test results in the CSF of 24 consecutive patients with severe anti-NMDAR encephalitis (modified Rankin scale score, 4-5 [full range of the scale, 0-6]) and 29 with anti-leucine-rich glioma-inactivated 1 (LGII) encephalitis (modified Rankin scale score not specified in the clinical records, but all patients had variable degrees of memory loss and confusion).

Results

During 2012, we received CSF samples of 346 patients to be tested for the 14-3-3 protein. Forty patients (11.6%) had a positive 14-3-3 test result, 10 patients (2.9%) had positive staining in rat brain immunohistochemistry, and 1 patient (0.3%) had both. In 6 of the patients (1.7%) with positive rat immunohistochemistry results, cell-based assays confirmed the presence of antibodies targeting the following antigens: CASPR2, LGII, NMDAR, AQP4, Tr (DNER [5-notch-like epidermal growth factor-related receptor]), and 1 unidentified NSA, which was confirmed on cultures of hippocampal neurons. The other 4 cases with positive rat immunohistochemistry findings did not react with NSAs in cultures of dissociated rat hippocampal neurons, and the unknown intracellular antigens were no longer studied.

The clinical features of the 6 patients with NSA-abs are summarized (Table), and a detailed clinical description is provided (Supplement eAppendix). Briefly, 4 patients developed rapidly progressive cognitive decline, 3 of them with associated psychiatric symptoms (CASPR2-abs, NMDAR-abs, and unknown-abs). In the other 2 patients (LGII-abs and Tr [DNER]-abs), only motor abnormalities without cognitive decline were reported. The patient with LGII-abs presented with myoclonus-
like movements involving the face and limbs (afterward described as faciobrachial dystonic seizures or tonic seizures),5,16 with alternate laterality. The patient with Tr (DNER)-abs developed a subacute cerebellar syndrome. The median time from symptom onset to lumbar puncture was 24 days (range, 9–180 days). Analysis of the CSF revealed mild pleocytosis in 2 patients (Tr [DNER]-abs and unknown-abs), high protein concentration in 2 patients (NMDAR-abs and AQP4-abs), and a positive 14-3-3 test result in 1 patient (Tr [DNER]-abs). Magnetic resonance imaging features of limbic encephalitis were observed in the patient with CASPR2-abs. Small cell lung carcinoma; Tr(DNER), δ/notch-like epidermal growth factor–related receptor; WBCs, white blood cells.

* Time from first symptom to first medical evaluation: acute (<1 wk), subacute (1 wk–3 mo), and insidious (>3 mo).

** Initially considered as myoclonus, retrospectively considered as faciobrachial dystonic seizures.

All 49 CSF samples of pathologically confirmed CJD were negative for NSA-abs. A nonspecific pattern of immunoreactivity in paraformaldehyde-fixed rat brain was observed in 5 of the 49 samples, but additional studies using neuronal cultures were negative, indicating that the immunoreactivity was not directed to surface antigens. Specific cell-based assays for LGI1-abs, CASPR2-abs, GlyR-abs, and NMDAR-abs were also negative in all 49 samples. None of the 24 patients with anti-NMDAR encephalitis tested positive for 14-3-3 protein, and 1 of 29 patients (3.4%) with anti-LGI1 encephalitis was positive for 14-3-3 protein.

**Discussion**

Our study indicates that a low, but clinically relevant, number of patients with suspected CJD have NSA-abs–associated neurologic disorders that are potentially responsive to immunotherapy. Moreover, our patients with definite CJD did not have CSF antibodies against NMDAR, GlyR, LGI1, or CASPR2 (the latter 2 included within the term VGKC complex antibodies). Conversely, none of the patients with anti-NMDAR en-
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Can be mistaken for CJD.21 Patients with encephalitis associated with LGI1-abs may present with myoclonus-like movements and other symptoms that almost 9% of the disorders had been initially diagnosed as CJD.22 The studies of NSA-abs in CJD-suspected cases is important for the initial assessment of patients with rapidly progressive dementia. However, negative determination does not rule out the possibility of a potentially treatable immune-mediated disorder. Therefore, it is important to consider both prion and immune-mediated diseases in the differential diagnosis of rapidly progressive neurologic deficits, with or without cognitive impairment, given the broad spectrum of symptoms of both disorders. Using reported criteria24 that include immunohistochemistry testing on brain tissue optimized for cell surface or synaptic proteins and recombinant cell-based assays, we did not find antibodies directed against LGI1, CASPR2, NMDAR, GlyR, or any other cell-surface antigen reported to date (excluding dopamine receptors, which were not investigated) in the CSF of 49 patients with definite CJD. These findings differ from those of previous studies that included few patients, suggesting that some patients with CJD may have NMDAR-abs, GlyR-abs, or VGKC complex-abs. The interpretation of those studies, however, has important limitations. First, a poor definition of the antigen was used.25 For example, the interpretation of VGKC complex-abs without clarifying whether they are directed against LGI1 or CASPR2 is complicated because VGKC complex-abs that differ from LGI1 and CASPR2 have limited syndrome specificity and are not reliable indicators of response to immunotherapy.25-26 Second, the NMDAR-abs and GlyR-abs were detected only in serum, casting doubts on the potential pathogenic relevance.25 In all, these findings emphasize the need to also determine antibodies in CSF to avoid potential pitfalls.

Overall, data from the present study and of previous reports suggest that patients suspected to have CJD, particularly those without supportive ancillary tests (typical magnetic resonance imaging or electroencephalographic findings) should be examined for the presence of NSA-abs. Detection of these antibodies indicates a potentially treatable disorder. However, negative determination does not rule out the possibility of a potentially treatable immune-mediated disorder. Patients in this series with definite CJD did not have antibodies directed against NMDAR, GlyR, LGI1, or CASPR2 in their CSF. Conversely, patients with anti-NMDAR encephalitis did not have 14-3-3 protein in their CSF, although this test may be positive in a small percentage of patients with anti-LGI1 encephalitis.
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