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Case Report

Successful Therapy of a Patient with Prospected Autoimmune Encephalitis and Negative Laboratory Results-Confirmation of Diagnosis by Immunocytochemistry

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Abstract

Autoimmune encephalitis (AIE) presents with a broad spectrum of neurological and psychiatric symptoms and can cause persistent brain damage. However, the absence of pathognomonic findings in CT and MRI screening, and the fact that relevant antigens for laboratory detection of autoantibodies mostly are unknown, makes its diagnosis still a challenging task.

In the present report the latter problem is overcome by the immunocytochemical detection of autoantibodies using brain sections, which in fact present nearly all neuronal antigens. Furthermore, we used a highly optimized immunoperoxidase technique, which allowed morphological high-resolution analyses of the immunoproducts.

The 82-old female patient presented with unsteady gait pattern, ataxia, myoclonus, apraxia, as well as an unclear language. She suffered from panic attacks, cognitive and mnestic deterioration as well as delusions. She was alert but disoriented. Despite of normal MRI and EEG results and normal CSF laboratory data we prospectively diagnosed autoimmune encephalitis. After five days high dose corticosteroid therapy all symptoms disappeared completely. The autoimmune pathogenesis subsequently was verified unequivocally, when the patient´s CSF strongly stained neuronal and glial cell bodies in brain sections.

This report should be taken as proof of principle that immunocytochemistry with CSFs of neurological patients provides the possibility to recognize a considerable number morphological details at high resolution. This will provide the possibility to form groups of patients showing similar distributions of immunoreactivities. Such groups may represent distinct nosological entities and finally may allow for elaborating selective treatments for patients with distinct types of autoimmune inflammation of the CNS.

Keywords: Autoimmune encephalitis; Immunocytochemistry; CSF antibodies; High-resolution detection; Case report

Introduction

Autoimmune encephalitis (AIE) presents with a broad spectrum of neurological and psychiatric symptoms and can cause persistent brain damage [1]. Antibody-mediated encephalitides can be separated in two groups: group I presenting with antibodies against intracellular antigens, and group II with antibodies targeting antigens on the cell surface. This distinction is clinically important regarding treatment response, association with an underlying malignancy and long-term prognosis [2].

Several factors including antibody testing, yet unknown antibodies, nonspecific findings on MRI, EEG, and cerebrospinal fluid (CSF) analysis and numerous differential diagnoses make diagnosing autoimmune encephalitis (AE) complicated [3]. Quite recently an international team of experts developed a practical, syndrome-based approach including guidelines to diagnose autoimmune encephalitis [4]. The absence of pathognomonic findings in CT and MRI screening, and the fact that relevant antigens for laboratory detection of autoantibodies mostly are unknown, makes diagnosing antibody-mediated encephalitis still a challenging task.

The latter problem is overcome by using brain sections, which in fact present nearly all neuronal antigens, to immunocytochemically disclose any autoantibody in prospected autoimmune patients, while conventional workups will fail.

Case presentation

An 82-year-old female presented to our Emergency Department complaining of gait deterioration and dizziness for 4-5 months. Previously, she could walk unaided. She also reported headache for 6 months. Her family reported also a stutter and a word-finding difficulty. All these symptoms had been worsening in the last 2-3 weeks. Worth mentioning in the past history were type 2 Diabetes mellitus, arterial hypertension and coronary artery disease. An MRI examination of brain and cervical spinal cord carried out before the admission had shown no relevant abnormalities. The clinical neurological examination showed

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no focal deficits except an irregular swaying with undirected tendency to fall in Romberg's test. She was alert and oriented. She seemed afraid, insecure and spoke softly and stuttered.

She was admitted to the department of neurology. Brain CT and MRI did not show relevant abnormalities. Vital signs were within normal limits. Laboratory data showed no related disturbances. A cardiac workup including an ECG, 24-h long-term ECG and 24 h-long-term RR was normal. The electrophysiological studies including EEG, nerve conduction studies and electromyography also revealed no explanation of the gait disorder and dizziness. Suspected of having a depressive syndrome, she could be discharged with Mirtazapin after exclusion of a somatic pathology as well in the clinical, electrophysiological and laboratory studies as in the imaging of brain and cervical spinal cord.

Three weeks later she was admitted to the department of psychiatry because of panic attacks, as well as cognitive and mnestic deterioration. A day before, she did scarcely eat or drink due to fear of poisoning and scary feeling in the chest. It was not possible to take a personal history. She was alert and for person and situation oriented. For time she was partially oriented. She seemed helpless, haphazard, fearful with a flattened affectivity and depressed mood. There was no clue of delusions, hallucinations, self-disorders, suicide or aggression. During the inpatient stay there, she offered an unsteady gait pattern, apraxia as well as an unclear language. She progressively lost body control and became dependent on nursing support with supplying food intake and basic care. Due to the fast-advancing psychopathological and neurological findings, the patient was admitted to the department of neurology once again.

At arrival, myoclonus of the lower extremities and a tendency to fall backwards when sitting were noticeable in addition to the findings listed above. She was alert but disoriented. Generally, she did not follow any commands. The MRI of the brain did not provide new findings. EEG showed a mild encephalopathy with no focal lateralizing or epileptiform discharges. Laboratory data including thyroid autoantibodies remained normal. CSF analysis revealed a mild lymphocytic pleocytosis accompanied by massive disturbance of the blood-brain barrier, but no organisms on Gram stain. Assays for neurodegenerative biomarkers (NSE, S100, protein 14-3-3, phosphorylated and non-phosphorylated tau-protein, ß-Amyloid 1-42) and other commercially available antibodies against intracellular and cell surface antigens in CSF were negative. At this stage, an autoimmune pathogenesis of the patient´s status moved into the foreground (Table 1).

Therapy was started on a 5-day course of intravenous high dose corticosteroid pulse with methylprednisolone 1000 mg/day. By the end of the corticosteroid-course, the patient experienced a drastic improvement in her neurologic and psychopathological status. She was completely oriented for time, location, person, and situation. She was independent from nursing and fully able to control her body when sitting or walking, so that she was discharged home (Table 1). Retrospectively, the nonspecific symptoms during the first hospital admission probably represented a prodrome of her autoimmune encephalitis.

Table 1: Relevant Data from the Episode of Care.

6 Month ago:	Unspecific symptoms
5 Weeks ago:	First admission to neurological department
4 Weeks ago:	Discharged home
1 Week ago:	Admission to psychiatric department
Day 1:	Second admission to neurological department
Day 3:	Diagnosis of autoimmune encephalitis
Day 4:	Start of corticoid therapy encephalitis
Day 9:	End of corticoid therapy
Day 13:	Discharged home with minimal residual complaints.

To ascertain the autoimmune pathogenesis we aimed to unequivocally verify the presence of CNS-reactive antibodies in the CSF of our patient. To overcome the problem that relevant antigens for laboratory detection of autoantibodies mostly are unknown, we used brain sections, which naturally present nearly all neuronal antigens. This approach was strongly improved by the use of a highly sensitive immunoperoxidase technique. Consequently, our sections showed that the patient´s CSF strongly stained neuronal and glial cell bodies as well as the neuropil (Figure). In fact, our unconventional approach allowed us to support the diagnosis of antibody-mediated encephalitis, confirming the correctness of our treatment strategy.

Discussion

Our case supports the idea that antibody-mediated encephalitis should be considered in patients with rapidly progressing unclear CNS-symptoms (memory deficits, movement disorders, seizures, or psychiatric symptoms). This remains true even when other mimicking diseases can be excluded and when no supporting findings in classical CSF analysis and brain imaging can be obtained.

The immune process targeting intracellular antigens mostly is cell-mediated by T-cells resulting in progressive destruction of cells. This process often is associated with malignancy and poor response to treatment. In contrast, autoreactivity against cell surface antigens is directly antibody-mediated, associated less with malignancy and combined with better response to immunotherapy [5, 6]. Due to different properties, the detection methods of these antibodies are not uniform applicable to both groups. Antibodies targeting intracellular proteins (onconeuronal, GAD) are detectable with many techniques including western blot analysis, line assays, enzyme-linked immunosorbent assay (ELISA), fixed tissue- based assays (fixed TBA), cell-based assays (CBA) or radioimmunoassay (RIA). To identify of anti-neuronal surface antibodies, methods that recognize conformation dependent epitopes (e.g., CBAs and unfixed/postfixed TBAs) are required [7].

Limitations of the classical CSF study and commercially available antibody detection for autoimmune encephalitis should kept in mind. The absence of inflammatory signs in CSF or of conventionally detectable antibodies against brain structures does not exclude any immune-mediated pathogenesis. In contrast, positive tests may be obtained in healthy individuals or among

Table 2: Criteria for prospective autoimmune encephalitis when autoantibody-negative.

Diagnosis of autoimmune encephalitis can be made when all four of the following criteria have been met (Graus et al, 2016):

1) Rapid progression (less than 3 months) of working memory deficits (short-term memory loss), altered mental status, or psychiatric symptoms

2) Exclusion of well defined syndromes of autoimmune encephalitis (eg, typical limbic encephalitis, Bickerstaff's brainstem encephalitis, acute disseminated encephalomyelitis)

3) Absence of well characterised autoantibodies in serum and CSF, and at least two of the following criteria:

- MRI abnormalities suggestive of autoimmune encephalitis
- CSF pleocytosis, CSF-specific oligoclonal bands or elevated CSF IgG index, or both
- Brain biopsy showing inflammatory infiltrates and excluding other disorders (eg, tumor)

4) Reasonable exclusion of alternative causes.

people with various neurological diseases [8-11]. Yet unknown or rare antibodies will be overlooked by most currently available commercial tests, especially if only the serum is analyzed [12]. Bilateral temporomesial hyperintensities on T2/FLAIR MRI sequences may point to limbic encephalitis, but in most cases conventional imaging reveals only unspecific changes or a lack of abnormalities [13].

Another approach, the response to immunotherapy as a component of diagnosis process, may not be always helpful. Some patients do not respond to immunotherapy. Others could need prolonged therapies that are not always available unless a diagnosis has been confirmed. Conversely, patients with other disorders such as primary CNS lymphoma or Hashimoto-encephalopathy [4] may respond to immunotherapy.

In 2016 an international team of experts developed a practical, syndrome-based approach to diagnose autoimmune encephalitis. It is based on conventional clinical neurological assessment and standard diagnostic tests (MRI, EEG, and CSF studies). To avoid any delay in diagnosis and to initiate treatment in a timely fashion, the authors have suggested criteria (Table 2) to diagnose autoimmune encephalitis without evidence for autoreactive CSF antibodies [4].

Quite recently, immunocytochemistry has been used to overcome the problem that relevant antigens for laboratory detection of autoantibodies mostly are unknown [14]. Even when corresponding antigens remain unknown the importance of this approach in clinical practice cannot be overemphasized and surpasses the necessity of a brain biopsy [4].

Unfortunately, available attempts to use brain sections suffer from a number of restrictions.

The use of unfixed brains is possible but results in very poor morphological localization. Immunofluorescence suffers from its low sensitivity [15], often rendering high-resolution analysis

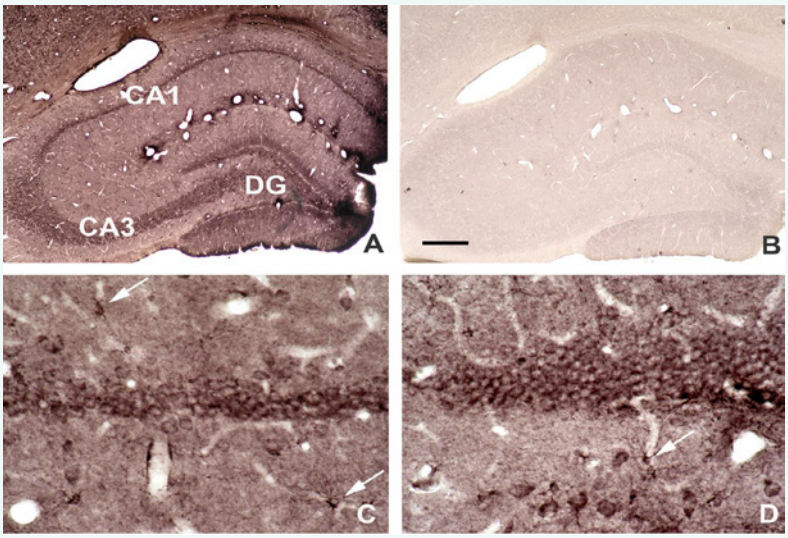

Figure 1 Immunocytochemical reactivities of the patient´s CSF(A) and that of a patient presenting with headache (control, B). CSFs were applied to mildly fixed coronal sections of a rat brain. Immunoreactivities were visualized using biotinylated secondary antibodies and an optimized ABC-procedure [15]. Note the strong staining in cortex (A, upper left corner) and hippocampus. Hippocampal areas CA1, CA3, and dentate gyrus (DG) are labelled accordingly. Higher magnification of the hippocampal CA1 region (C) discloses positive pyramidal neurons, interneurons, astrocytes (white arrows) and neuropil. Similarily, in (D) positive granule cells, hilar interneurons, astrocytes (white arrow), and neuropil also are detected in the area of the dentate gyrus (DG). Bar in (B) indicates 400 µm in (A) and (B) and 50 µm in (C) and (D).

impossible, which may be necessary to differentiate reactions with unconventionally distributed antigens from tissue artifacts. In our present report we have used an optimized avidin biotin-peroxidase technique, which allows us to demonstrate anti-neuronal activity against several types of neurons. Furthermore, we see that the patient´s CSF also reacts with astrocytes and the neuropil (Figure 1). Taking together, our procedure allows for detecting brain-reactive antibodies in the CSFs of so far undiagnosed neurological patients.

This report also should be taken as proof of principle that immunocytochemistry with CSFs of neurological patients provides the possibility to recognize a considerable number morphological details at high resolution. This will allow for building groups among patients showing similar distributions of immunoreactivities. Such groups may represent distinct nosological entities and finally may allow for elaborating selective treatments for patients with distinct types of autoimmune inflammation of the CNS.

Data availability

Data supporting the conclusions of this report are available by contacting the corresponding author.

Conflicts of interest

The authors declare that they have no conflicts of interest pertaining to this article.

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