Two cases of paraneoplastic limbic encephalitis associated with small cell lung cancer and a literature review

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Abstract. In the present study, two cases of paraneoplastic limbic encephalitis (PLE) associated with small cell lung cancer were reported. Using avidin-biotin immunoperoxidase methods, purified recombinant HuD western blotting and Euroline Neuronal Antigens Profile 2 immunoglobulin G western blotting, it was found that the well-characterized anti-Hu and anti-amphiphysin onconeural antibodies were present in the serum/cerebrospinal fluid of the patients. With a review of the literature, it was found that patients with PLE of Chinese Han nationality had two types of clinical manifestations, simple and complex, and that the lesions could also be divided into focal and scalable lesions. Furthermore, the clinical manifestations and lesion scopes were associated with certain types of cancer and antibodies. In addition, it was found that the prognosis for patients with PLE with autoantibodies targeting membrane antigens is improved compared with that for patients with PLE with autoantibodies targeting intracellular antigens, due to an increased sensitivity to immunomodulatory treatments and anti-cancer therapy.

Introduction

Limbic encephalitis is a rare neurological syndrome that selectively affects the structures of the limbic system, including the hippocampus, amygdala and hypothalamus. Since the initial cases of this disease were often accompanied by cancer, such as small cell lung cancer (SCLC), the disease was subsequently referred to as paraneoplastic limbic encephalitis (PLE) (1-6). The main clinical manifestations of PLE are seizures associated with progressive short-term memory loss, which may develop into dementia. In addition, there may be different degrees of involvement in such extra-limbic-system tissues as the cerebellum, brainstem and thalamus. Electroencephalography (EEG) typically exhibits epileptic activity in the unilateral or bilateral temporal lobes, with focal or global slow waves; magnetic resonance imaging (MRI) T2 or flair images show high-signal, abnormal lesions in the interior sides of the unilateral or bilateral temporal lobes. In the majority of cases, temporal lobe atrophy develops. Cerebrospinal fluid examination typically exhibits inflammatory changes, with mildly to moderately increased lymphocytes, as well as increased protein levels; glucose levels would be normal and the immunoglobulin G (IgG) index would most likely be increased. Furthermore, oligoclonal bands would be apparent (7-9).

A number of studies have revealed that the pathogenesis of PLE is an immune-mediated response, primarily effected by cytotoxic T cells and antibodies that act on neuronal antigens, such as anti-Hu (10,11), anti-Ma2 (12), anti-amphiphysin (13) and anti-Yo (14) antibodies. Treatments for PLE include anti-cancer therapy and immunotherapy; the effects of the former are more marked, but the overall prognosis is typically poor (8,15). Research into PLE has made progress over the past decade (16), and certain clinical manifestations and imaging appearances have been found to be consistent with PLE. Furthermore, since the generation of antibodies targets neuronal cell membrane antigens, the development of specific immunotherapies could lead to improvements in prognosis for patients with PLE (15,17). To date, few studies have focused on PLE in patients of Chinese Han nationality; therefore, the present study described two cases of PLE in patients of Chinese Han nationality and summarized four cases in the literature.

Materials and methods

Clinical data. Two male patients with PLE associated with SCLC were hospitalized in the Department of Neurology, the First Affiliated Hospital of Bengbu Medical College (Bengbu, China) between October 1999 and July 2013. The patients were aged 69 and 83 years, respectively, and exhibited serious memory impairment, which prevented the patients from recalling the five designated objects presented on admission 5 min later. One of the patients complained of headache. One patient suffered from generalized tonic-clonic seizures (GTCSs), while the other suffered from complex partial seizures.
seizures. The serum sodium levels of the two patients were as low as 115 and 130 mmol/l, and one patient had intractable hyponatremia. Cranial MRI was performed prior to treatment in both cases. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of Bengbu Medical College. Written informed consent was obtained from all participants.

Wechsler Adult Intelligence Scale (WAIS) determination. A psychiatric doctor performed the test on the two patients prior to treatment (18). The language assessment included six aspects (knowledge test, comprehension, arithmetic, similarities, digit span and vocabulary) and the operation test consisted of five parts (number signs, picture filling, block design, picture arrangement and object assembly). The scores were obtained from the coarse score scale, and added to obtain the language IQ score, the operation IQ score and the total IQ points.

Immunohistochemistry. The cerebral cortex and cerebellum were obtained from a neurologically normal individual within 6 h after mortality (the brain tissue was provided by Professor Zhou Jiangning from the School of Life Sciences, University of Sciences and Technology of China, Hefei, China). Consent was provided by the family of the deceased. Pieces were embedded in optimal cutting temperature compound and snap-frozen in isopentane cooled by liquid nitrogen, prior to being stored at -80°C. Tissue sections measuring 6 µm were sequentially incubated with 0.3% hydrogen peroxide (to block endogenous peroxidase activity) for 10 min and 10% normal goat serum (Organon Teknika-Cappel, West Chester, PA, USA) was then added as the blocking serum, prior to incubation for 15 min. The sera of the patients were serially diluted overnight at 4°C and then incubated with biotinylated goat anti-human IgG (Vector Laboratories, Burlingame, CA, USA) for 1 h and the Vectastain® avidin-biotin complex (Vector Laboratories) for 30 min at room temperature. The substrate staining was developed with 0.05% diaminobenzidine tetrahydrochloride (Sigma), 0.5% Triton X-100 and 0.01% hydrogen peroxide in phosphate-buffered saline (PBS).

Western blotting. Western blotting was performed using the Euroline Neuronal Antigens Profile 2 IgG kit (DL1111-1601-2 G; Euroimmun AG, Lübeck, Germany). The film strip was removed and placed in the incubation tank. Sample buffer (1.5 ml) was added and incubated in a shaker (Euroimmun AG, Lübeck, Germany) at room temperature for 5 min, prior to the absorption of the liquid into the tank. Diluted serum sample (1.5 ml) was then added into the incubation vessel and agitated at room temperature (18-25°C) for 30 min incubation. Following incubation, the samples were washed four times for 15 min in the aforementioned buffer and incubated with biotinylated goat anti-human IgG (Vector Laboratories) for 1 h and the Vectastain avidin-biotin complex (Vector Labs) for 30 min at room temperature. The substrate staining was developed with 0.05% diaminobenzidine tetrahydrochloride (Sigma), 0.5% Triton X-100 and 0.01% hydrogen peroxide in PBS.

Results

WAIS determination. Prior to the treatment, the language IQ scores of the two patients were 45 and 57 points, the operation IQ scores were 42 and 43 points and the total IQ scores were 40 and 48 points. The patients scored poorly in the arithmetic, picture filling, block design, picture arrangement and object assembly.

EEG. EEG revealed abnormalities in both cases, including focal or sharp slow waves in the bilateral frontotemporal lobes (Table I).

Cranial MRI. One case had atrophy in the bilateral temporal lobe and hippocampal area and one case had high signal intensity on the flair and T2-weighted images in the bilateral amygdala and hippocampal area (Fig. 1).

Immunohistochemistry. The 69-year-old patient with PLE and SCLC was found to have anti-Hu antibodies in the serum. Following incubation of a section of the frontal cortex for 60 min with the patient’s serum [serum dilution, 1:1,000-1:16,000; cerebrospinal fluid (CSF) dilution, 1:100-1:800], positive staining of the neuronal nuclei was observed in a homogeneous pattern. No staining of the nucleoli was observed, and negative results were obtained for anti-Yo and -Ri antibodies (Fig. 2). The 83-year-old patient was found to have anti-amphiphysin antibodies in the serum. Anti-amphiphysin antibodies belong to the antibodies targeting the synaptic vesicle. Immunohistochemical tests are unable to show positive staining even in the presence of anti-amphiphysin antibodies.
| Patient no. | Gender, age in years | Tumor type/location | Symptoms and signs | EEG | CSF protein (mg/day) | Brain MRI and \(^{18}\)F-FDG/PET-CT | Neuronal antibodies | Prognosis |
|------------|----------------------|---------------------|-------------------|-----|---------------------|-------------------------------------|---------------------|----------|
| 1 (present study) | M, 69 | SCLC | Disorientation and GTCS for 90 days, Na\(^+\) 115 mmol/l | Bilateral frontal slow wave, right temporal lobe focal sharp-wave | Normal | Brain MRI: Atrophy in the bilateral temporal lobe and hippocampal area | Anti-Hu* | Mortality |
| 2 (present study) | M, 83 | SCLC | Progressive short-term memory loss, partial complex seizure Na\(^+\) 130 mmol/l | Bilateral frontal, right temporal lobe slow wave | Normal | MRI: High signal intensity on the flair and T\(_2\)-weighted image in the bilateral amygdala and hippocampal area | Anti-amphiphysin* | Mortality |
| 3 | M, 49 | Pancreatic cancer | Clumsy, apathy, echoing speech, memory loss, partial complex seizure; sucking, groping and grasping reflexes and a diffuse, brisk, deep tendon reflex; disorganization | Bilateral frontal, right temporal lobe, focal slow wave | Protein: 900 mg/l | MRI: Bilateral frontal lobe, temporal lobe, left parietal lobe, occipital lobe, cerebellar hemisphere, right parietal lobe. PET-CT: Bilateral frontal temporal lobe, right parietal lobe, occipital lobe, decreased metabolism | Negative | Mortality |
| 4 | F, 22 | Ovarian teratoma | Apathy, babbling, conscious disturbance, GTCS status, Na\(^+\) 130 mmol/l | Bilateral diffuse slow wave | WBC: 16x10\(^6\)/l Protein: 50 mg/l | Brain MRI: Normal | Negative | Recovery |
| 5 | F, 17 | Ovarian teratoma | Short-term memory loss, emotional disturbance, GTCS status | Bilateral diffuse height amplitude \(\delta\) waves | Intracranial hypertension, 220 mm H\(_2\)O; WBC: 16x10\(^6\)/l, Protein: 580 mg/l | Brain MRI: Normal | NMDAR* | Recovery |
| 6 | M, 52 | SCLC | Short-term memory loss, partial complex seizure, Lambert-Eaton syndrome | - | - | MRI: Brain atrophy | Negative | Mortality |

Ref., reference number; EEG, electroencephalography; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; \(^{18}\)F-FDG/PET-CT, \(^{18}\)F-fluorodeoxyglucose/positron emission tomography-computed tomography; SCLC, small cell lung cancer; GTCS, generalized tonic-clonic seizure; WBC, white blood cells; NMDAR, N-methyl-D-aspartate receptor.
Western blotting. The paraneoplastic neuronal antibody spectrum examination included six well-characterized onconeural antibodies: Anti-Hu, anti-Yo, anti-Ri, anti-CV2, anti-paraneoplastic antigen Ma2 (PNMA2) and anti-amphiphysin. One of the patients with PLE and SCLC was positive for anti-amphiphysin antibodies (Fig. 3).

Purified recombinant HuD. Immunoblots of purified recombinant HuD reacted with the serum of one of the patients with PLE and SCLC. The serum of the patient was positive for anti-Hu antibody (dilation, 1:50-1:200) (Fig. 4).

Discussion

PLE is considered a rare manifestation that is characterized by the development of the neuropsychiatric symptoms of a paraneoplastic neurological disorder, but additionally associated with cancer in the absence of invasion of the nervous system by tumor cells. The tumor most commonly found in association with PLE is SCLC. The disease can affect individuals aged 10-85 years, with the females being less susceptible than males (5,20). Rarer malignancies associated with PLE are thymoma (21), ovarian teratoma (22,23), esophageal squamous cell carcinoma (24), adenocarcinoma of the colon (14), prostate cancer (25), testicular neoplasm (26,27), Hodgkin’s lymphoma (20), non-Hodgkin’s lymphoma (28), leukemia, lymphoma (29) and acute myeloid leukemia (17). A literature search identified four studies containing data on cancer-related PLE in patients of Chinese Han nationality (30-33). Through the clinical manifestations, psychology, WAIS determination, CSF analysis, electrophysiology, imaging, immunological
anti-N-methyl-D-aspartate receptor (NMDAR) (35) and bodies, such as anti-Hu (34,1), anti-PNMA2 (12), anti-Yo (14), the identification of a number of specific circulating autoantibodies targeting membrane in neuromuscular transmission (Table I). The lesions in the left cerebellar hemisphere (31). PET-CT confirmed that the metabolism in the bilateral frontal, temporal, right parietal and occipital lobe was reduced. The patient had sucking, groping and grasping reflexes and a diffuse, brisk, deep tendon reflex (31,32). One case with PLE and SCLC had Lambert-Eaton myasthenic syndrome, and the lesion involved voltage-sensitive calcium channels of the presynaptic membrane in neuromuscular transmission (Table I).

The clinical diagnosis of PLE is problematic; however, the identification of a number of specific circulating autoantibodies, such as anti-Hu (34,1), anti-PNMA2 (12), anti-Yo (14), anti-N-methyl-D-aspartate receptor (NMDAR) (35) and anti-voltage-gated potassium channel (17) antibodies, in these patients has revolutionized the diagnosis and understanding of these syndromes and demonstrated a role for the immune system in such neurological disorders. We have speculated that the clinical manifestations and lesions scopes are associated with certain types of tumors and antibodies. In cases of autoantibodies targeting intracellular antigens (anti-Hu, anti-PNMA2, anti-Yo and anti-amphiphysin), an associated malignancy can nearly always be observed. These neurological disorders are predominantly associated with neuronal death, and patients are rarely sensitive to immunomodulatory treatments; cellular immunity appears to play a major role in this lack of sensitivity. By contrast, patients with autoantibodies targeting membrane antigens (receptors, channels or receptors associated with proteins) almost always have ovarian teratoma (21,35,33,30), and the neurological disorders are associated with a reversible neuronal dysfunction. These patients are mostly sensitive to immunomodulatory treatments, and it appears that humoral immunity and autoantibodies play a major role.

Using avidin-biotin immunoperoxidase methods, it was found that the patients had two types of clinical manifestations, simple and complex, and that the lesions could also be divided into focal and scalable types. Of the four patients identified in the literature and the present two cases, three patients had PLE with SCLC (32), one case had PLE with pancreatic cancer (31) and two patients had PLE with ovarian teratoma (30,33). Four of the cases presented as an isolated neurological syndrome (progressive short-term memory loss, GTCSs) (32,33). One of the patients MRI showed the involvement of the bilateral frontal, right temporal and occipital lobes, as well as the left cerebellar hemisphere (31). PET-CT confirmed that the metabolism in the bilateral frontal, temporal, right parietal and occipital lobe was reduced. The patient had sucking, groping and grasping reflexes and a diffuse, brisk, deep tendon reflex (31,32). One case with PLE and SCLC had Lambert-Eaton myasthenic syndrome, and the lesion involved voltage-sensitive calcium channels of the presynaptic membrane in neuromuscular transmission (Table I).

The clinical manifestations and lesion scopes were associated with certain types of tumors and antibodies. Compared with patients with PLE with autoantibodies targeting intracellular antigens, the prognosis for patients with PLE with autoantibodies targeting membrane antigens is improved as a result of immunomodulatory treatments and anti-cancer therapy.
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