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ORIGINAL RESEARCH

Auto-Immune Glial Fibrillary Acidic Protein Astrocytopathy with Active Intrathecal Epstein-Barr Virus: A Single-Center Case Series Report

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Purpose: Auto-immune glial fibrillary acidic protein (GFAP) astrocytopathy is a disease with unclear mechanisms and no diagnostic and treatment guidelines. Epstein-Barr virus (EBV) infection is reportedly involved in glial activities. However, the relationship between GFAP astrocytopathy and EBV infection is not clear. This study reports a case series of auto-immune GFAP astrocytosis with positive cerebrospinal fluid (CSF) EBV DNA, describing its clinical manifestations and treatment experience.

Patients and Methods: In the serial case study, we reported six patients diagnosed with GFAP astrocytopathy having intrathecal EBV.

Results: The significant signs included headache, fever and urination disorder, ataxia, limb weakness, numbness, consciousness disorder, psychological disorder, and blindness, among others. CSF analysis showed increased pressure, white blood cell count, abnormal biochemical components, positive GFAP antibody, and EBV. The positive results of metagenomic next-generation sequencing (mNGS) and PCR in CSF indicated that there might be active replication of EBV in the CSF of patients. The results of EBV-associated antibodies in blood suggest no evidence of acute primary EBV infection in six patients. Initial single antivirus therapy did not show satisfactory effects, but all patients showed improvement in clinical features and laboratory analysis after immunotherapy.

Conclusion: This study indicated that intrathecal EBV activity was closely related to auto-immune GFAP astrocytopathy, of which the mechanism remains to be further studied.

Keywords: autoimmune GFAP astrocytopathy, Epstein-Barr (EB) virus, cerebrospinal fluid, glial fibrillary acidic protein, next-generation sequencing, MRI

Introduction

Auto-immune glial fibrillary acidic protein (GFAP) astrocytopathy is a recently defined disease of the central nervous system.[1,2] Because the pathology may involve multiple sites from the cortex to the spinal cord and from meninges to nerve roots, the primary clinical manifestations of auto-immune GFAP astrocytopathy are not specific and may include headache, ataxia, seizure, and urinary disorder.[1,3–7] In contrast to its considerable influence on patients, treatment for auto-immune GFAP astrocytopathy is not satisfactory, even with an immunosuppressive strategy, although the disease is caused by immune dysfunction.[3,8] Diagnosing auto-immune GFAP astrocytopathy depends on clinical manifestation, laboratory examination, characteristic imaging findings, and therapeutic effects.[4] As a signature of the pathological change of astrocytes in neurological diseases, GFAP provides unique values for

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diagnosing and therapeutic evaluation of auto-immune GFAP astrocytopathy.[2,4,9,10] However, it is still difficult to distinguish auto-immune GFAP astrocytopathy from other diseases, such as multiple sclerosis (MS) and lymphocytic inflammation.[11,12] In addition, auto-immune GFAP astrocytopathy has complex and unclear pathological mechanisms, which need further investigations.[5,13,14] Some studies implied that auto-immune GFAP astrocytopathy may be related to infection and tumors[9,15] and may demonstrate prodromal infection symptoms such as runny nose and sore throat, which may also occur after infection with herpes simplex virus, varicella-zoster virus, human immunodeficiency virus, etc.[9,15–17] The involvement of EBV in auto-immune GFAP astrocytopathy is rarely reported.[18,19]

EBV is a type of herpes virus in humans and primates. It can induce systemic auto-immune diseases, such as systemic lupus erythematosus syndrome and lymphocytosis, and diseases of the central nervous system, such as MS.[20,21] In addition, a link between EBV infection and Alzheimer's disease and Parkinson's disease is also reported.[22,23] Although EBV infection is usually considered benign, its lethal outcomes are also observed.[24] Therefore, it is worth understanding the mechanism of EBV infection. Recently, the involvement of glial cells in the pathological process of EBV infection has been observed.[25,26] However, very few cases of the involvement of the EBV in auto-immune GFAP astrocytopathy are reported.[18,19] In this study, we reported six patients who suffered from auto-immune GFAP astrocytopathy with complex active intrathecal EBV. These findings would contribute to understanding auto-immune GFAP astrocytopathy and aid in developing a clear diagnosis and therapeutic strategies for these patients. These results would further enrich the study of auto-immune GFAP astrocytopathy and be helpful for clear diagnosis and therapeutic strategies for these patients with the same disease.

Materials and Methods

Subjects and Inclusion Criteria

From November 5, 2021, to April 5, 2022, our hospital admitted ten cases of auto-immune GFAP astrocytopathy. Among them, six patients showed positive EBV by metagenomic next-generation sequencing (mNGS) and EBV DNA RNA testing of cerebrospinal fluid (CSF) and were included in the study, according to the inclusion criteria as below: 1) having clinical features of single or combination of encephalitis, meningitis, and myelitis; 2) positive GFAP-IgG in CSF; 3) CSF EBV DNA test positive; 4) diffuse abnormal T2 signals in the brain and/or spinal cord, linear enhancement in surrounding vessels of the ventricle, and enhancement in pia mater.[1]

The patients were evaluated for Modified Rankin Score (MRS), according to the following criteria: 0, no residual symptoms; 1) symptomatic but no significant dysfunction and able to carry out all daily responsibilities and activities; 2) slight disability and unable to carry out all pre-stroke activities but able to look after self without daily help; 3) moderate disability and requiring some external help but able to walk without the assistance; 4) moderately severe disability, unable to walk independently and to meet self-requirement without assistance; 5) severe disability, unable to walk independently and to meet self-requirement without assistance; 6) death. Response to immunotherapy was defined as at least 1 point change of MRS.

The study was approved by the Ethics Committee of the Second Hospital of Hebei Medical University. All subjects signed the informed consent form. This study was performed in line with the principles of the Declaration of Helsinki.

Data Collection

The patient underwent lumbar puncture within 2 days of admission, and 5–6 mL of CSF was collected to assess routine biochemistry, cytology, mNGS, and demyelination antibody-related examinations. The patients with positive EBV by mNGS were further verified with PCR for EBV DNA and EBV-related antibodies in serum.

The GFAP antibody detection of all patients was based on the cell-based assay (CBA). Among them, two patients were found positive by tissue-based assay (TBA) and then confirmed by CBA using the IgAGM detection kit (EUROIMMUNAG, Germany).

Image Screening

The six patients received plain and contrast-enhanced magnetic resonance imaging (MRI) scanning for the brain and spinal cord with a 1.5T/3.0T MRI scanner (GE optima, 360 1.5/3.0T, MRI, USA) with contrast gadolinium (0.1mmol/kg, i.v). The sequential MRI images of the brain and spinal cord were analyzed, including axial T1, axial T2, fluid-attenuated inversion recovery, sagittal T2, and enhanced axial T1. Three patients received additional examination with positron emission tomography-computed tomography (PET-CT) (Philips Vereos PET-CT, Netherlands). Patients #1 and #6 were subjected to PET-CT scanning by a local hospital before transferring to our hospital. Patient #2 received a PET-CT examination to exclude neoplastic diseases because the headache was not significantly improved during antiviral treatment, and significant back pain developed. Patient #4 strongly requested PET-CT scanning to exclude inflammation in the spinal cord because of dissatisfactory improvement of lower limb weakness. Other patients were screened because of fever or to exclude cancer.

EEG

Video electroencephalogram (EEG) monitoring was performed with 64-channel EEG (EEG-1200C, Nihon Kohden, Japan). The electrodes were placed according to the international 10–20 system, plus bilateral surface pterygoid electrode recording, electrocardiography monitoring, and bilateral electromyographic monitoring of the deltoid muscle. The recording leads were set to earlobe reference leads, and the recording parameters were set as a low-pass filter of 0.53Hz, high-pass filter of 70Hz, speed of 3cm/s, and sensitivity of 10μV/mm for 2 h. Eye-open and eye-close evoked tests were performed with simultaneous video monitoring to record the EEG of patients at the wake and sleep stages. All patients completed EEG within three days of admission, and most patients were discharged without repeated EEG.

Treatment and Follow-Up

All patients received antivirus treatment after the initial diagnosis. The first-line immunotherapy was administered after confirmation of positive GFAP antibody in CSF. One patient received immediate antivirus treatment and immunotherapy after initial diagnosis because of a severe condition before detecting the GFAP antibody.

The patients were followed up for 1 year by phone call or hospital visit.

Results

General Information

The patients included one female (16.67%) and five males (83.33%) with a median age of 38.5 years (36–47 years) at the onset. The onset was the first time for all patients. The demography and clinical features are listed in Table 1. The initial signs were headache, dysuria, and fever, which appeared in all six patients. Other clinical signs at the acute stage included ataxia (4/6, 66.67%), limb weakness (4/6, 66.67%), numbness (3/6, 50%), consciousness disorder (1/6, 16.67%), psychological disorder (1/6, 16.67%), blind (1/6, 16.67%), dizziness (1/6, 16.67%), vomiting (1/6, 16.67%), radiculalgia (1/6, 16.67%), respiratory failure (1/6, 16.67%), weight loss (1/6, 16.67%), and nuchal rigidity (2/6, 33.3%). The patients had MRS of 3–5 before treatment.

Results of Laboratory Examination

The patients received lumbar punctures to collect CSF for laboratory examination (Table 1). Intracranial hypertension (>200 mmH_2O) occurred in all patients, with a median of 247 mmH_2O (200–280 mmH_2O). The protein concentration was increased in all patients, with a median of 1.67 g/L (0.85–5.64 g/L). Four patients demonstrated low glucose, and five demonstrated low chloride in CSF. The WBC in CSF increased in all patients, with a median of 166 /L (74–472 /L), and lymphocytes were predominant (84–97%). After immunotherapy, the CSF was improved in five patients who received re-examination (Table 1).

GFAP antibody in CSF was detected in all patients at the acute stage, with a titer of 1:10–1:32. In addition, three patients showed positive GFAP antibody in serum, with a titer of 1:100. One patient (#6) had positive GAD antibody in serum and CSF, as well as headache, fever, limb tremor, and blindness, but no GAD antibody-related

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Table 1 Clinical Features of the Six Patients

#	Gender/ Age (Years)	Clinical Features	Change of MRS by Therapy				MRI/PET-CT	OB and IgG Synthesis Rate (mg/24h)		CSF Examination: pre-/post-Immunotherapy							GFAP AB pre-/ post-Immunotherapy	
			Pre-	Post-(Days in Hospital at Evaluation)	At Follow-Up (Days)	1-year Prognosis				Pressure (mmH_2O)	WBC (/L)	Lymphocytes (%)	Protein (g/L)	Glucose (mM)	$[Cl^-]$ (mM)	Other Auto-AB	Serum	CSF
1	M/47	Headache, fever, weight loss, unstable walking, dysuria, ataxia, hand tremors, fingertip numbness	3	1 (20)	0 (180)	0	Radial enhancement of vessels surrounding lateral ventricle in brain MRI; diffuse hypermetabolism from medulla to the lumbar spinal cord in PET-CT	Positive OB and IgG increase in CSF	11.45	225/nc	175/nc	96/nc	0.85/nc	2.82/nc	125.4/ nc	Anti SC1-70 AB (LIA)/nc	(-)/nc	1:10/nc
2	M/37	Headache, fever, dizziness, vomiting, radiculalgia, dysuria, ataxia, nuch rigidity	3	2 (22)	0 (156)	0	Multiple supratentorial and subtentorial enhancements of pia mater in brain MRI; Strip-like low T1 and high T2 signals in bilateral posterior spinal cord area at C4-5 level in spinal MRI; Linear high signal in spinal pia mater at the cervicothoracic segment in contrast-enhanced MRI; diffuse hypermetabolism in the spinal cord by PET-CT	-	nc	200/130	175/28	95/nc	1.32/0.71	2.34/2.81	118.4/ 126	Anti-nuclear AB (ANA +, 1:100); Anti-RNP AB(+, 24)/ nc	(-)/nc	1:10/(-)
3	M/40	Headache, fever, psychological disorder, limb weakness, dysuria, non-fluent language	5	2(28)	1(158)	0	Diffuse membrane thickness in contrast-enhanced brain MRI.	Positive OB and IgG increase in CSF	23.25	260/190	190/37	84/98	1.34/0.96	2.41/3.32	117.1/ 123.4	nc/nc	1:100/ nc	1:32/nc

4	F/44	Headache, fever, dysuria, lower limbs weakness, hands and feet numbness	5	2 (29)	0 (146)	0	Few enhancements in pia mater, thickness and enhanced T2/FLAIR in the bilateral insular cortex, a high signal of T2 and FLAIR in brain MRI; multiple, long and sparse linear enhancement in spinal cord and membranes in spinal MRI. PET-CT showed negative at the recovery stage.	No OB in blood or CSF, IgG increase	36.26	235/150	472/45	8793	1.99/0.76	3.42/4.87	113.2/125.1	nc/nc	1:100/nc	1:32/(-)
5	M/36	Headache, fever, dysuria, weakness of muscle strength, tinnitus, ataxia, consciousness impairment, respiratory failure, neck resistant	5	2 (44)	1 (129)	0	Flaky low signal in bilateral corona radiata, basal ganglia, and thalamus by brain-enhanced MRI; no signal enhancement in the thoracic and lumbar spinal cord by enhanced MRI.	Positive OB and IgG increase in CSF	−24.21	28075	83/54	97/75	1.84/0.46	1.77/2.45	115.2/125	ANA AB (+, 1:100), AntiSSA60 AB (+), AntiSSA52 AB (++)/nc	1:100/nc	1:32/(-)
6	M/37	Headache, fever, dysuria, blind in left eye, ataxia, four limbs tremor	3	1 (27)	1 (138)	1	Slightly increase of enhancement in brain membrane by enhanced MRI; obvious enhancement in left cerebella tentorium and postcentral gyrus by tentorium enhanced CUBE MRI; a large area of T2WI high signal in the cervical spinal cord by plain MRI; diffuse heterogeneous hyper metabolism in the whole spinal cord by PET-CT, no intraspinal canal occupation or abnormal density.	Positive OB and IgG increase in CSF	13.86	270/150	74	94/nc	56.4/65.6	44.81/43.02	116/121	GAD (+)	(-)/nc	1:10/nc

Abbreviations: AB, antibody; CSF, cerebrospinal fluid; MRS, Modified Ranking Score; MRI, magnetic resonance imaging; nc, not check; OB, oligoclonal band; PET-CT, positron emission tomography–computed tomography; WBC, white blood cell; ANA, anti-nuclear antibody.

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clinical signs such as diabetes, cerebellar ataxia, auto-immune epilepsy, limbic encephalitis, progressive encephalomyelitis with atonia and myoclonus, and eye movement disorder. Other auto-immune encephalitis-related antibodies, such as the AQP4 and Mog antibodies, were undetected in all other patients. Five patients underwent an oligoclonal band (OB) examination; four showed positive OB in CSF and increased IgG (Table 1). Five patients were examined for autoantibody, and three patients were positive, including one patient (#1) showing positive anti-SCI-70 (LIA) and one patient (#2) showing positive anti-nuclear antibody (ANA) and anti-RNP antibody. One patient (#6) was positive for ANA, anti-SSA60, and anti-SSA52 antibodies. After immunotherapy, CSF was re-examined in three patients (#2, #4, #5) for GFAP antibody and EBV, and all showed negative results (Tables 1 and 2).

Influenza virus and Mycoplasma pneumonia were weak positives for IgM for the pathogens producing respiratory tract infections. Tuberculosis mycobacterium, TORCH [toxoplasmosis, other (syphilis, varicella-zoster, and parvovirus B19), rubella, cytomegalovirus, and herpes infections], Borrelia burgdorferi, Bunyamwera virus DNA, and B-encephalitis virus IgM were all negative by acid-resistant staining, India ink preparation, and bacterial culture.

The results of mNGS indicated that the EBV in CSF was positive in all six patients. The DNA of the EBV in the blood of all six patients was negative, as determined by the PCR test. Patients #2 and #4 had their blood tested for EBV antibodies, displaying anti-EBV-CA IgM AB (-), anti-EBV-CA IgG AB (++, 1:3.2), anti-EBV-CA IgG high affinity, anti-EBV-NA IgG AB (++, 1:100), and anti-EBV-EA IgG (-) (Table 2).

In addition, five patients were screened for tumors, and all of them were negative.

After immunotherapy, only patient #4 among the six patients had GFAP antibody rechecked several times. On February 10, 2022, the CSF GFAP (++) was 1:32, and the patient's CSF antibody was rechecked on both days 16 and 46 after immunotherapy, both of which were (+) 1:10. At this time, the patient's lower limb weakness symptoms were relieved. The patient was rechecked four months later, and the result was negative.

Imaging Data

Plain and contrast-enhanced MRI scanning was performed, and abnormality was found in all six patients. Two patients (#1, #2) showed linear perivascular radial enhancement patterns extending outward from the lateral ventricle (Figures 1 and 2), and three patients (#2, #4, #6) showed enhancement of signal in pia mater (Figures 2 and 3), in brain MRI. Three patients (#2, #4, #6) with spinal MRI showed multiple enhanced signals in the cervicothoracic/cervical pia mater or intraspinal cord (Figures 2 and 3). PET-CT was performed in three patients (#1, #2, #6), and

Table 2 Examination of EB Virus in Blood and CSF of Six Patients with Auto-Immune GFAP Astrocytopathy

#	NGS of EBV in CSF, Sequence Number	EBV Nucleic Acid in CSF/(Post-Immunotherapy)	EBV Nucleic Acid in Blood	EBV Antibody in Serum
1	+, 151	+/(nc)	-	n/a
2	+, 6	+/(-)	-	Anti-EBV-CA IgM AB (-), anti-EBV-CA IgG AB (++, 1:3.2), anti-EBV-CA IgG high affinity anti-EBV-NA IgG AB (++, 1:100) anti-EBV-EA IgG (-)
3	+, 47	+/(nc)	na	n/a
4	+, 1	+/(-)	na	Anti-EBV-CA IgM AB (-), anti-EBV-CA IgG AB (++, 1:3.2), anti-EBV-CA IgG high affinity, anti-EBV-NA IgG AB (++, 1:100) anti-EBV-EA IgG (-)
5	+, 1	+/(-)	na	n/a
6	+, 47	+/(nc)	-	n/a

Abbreviations: AB, antibody; CA, capsid antigen; CNS, cerebrospinal fluid; EBV, Epstein-Barr virus; GFAP, glial fibrillary acidic protein; n/a, not applicable; nc, not checked; NGS, next-generation sequencing.

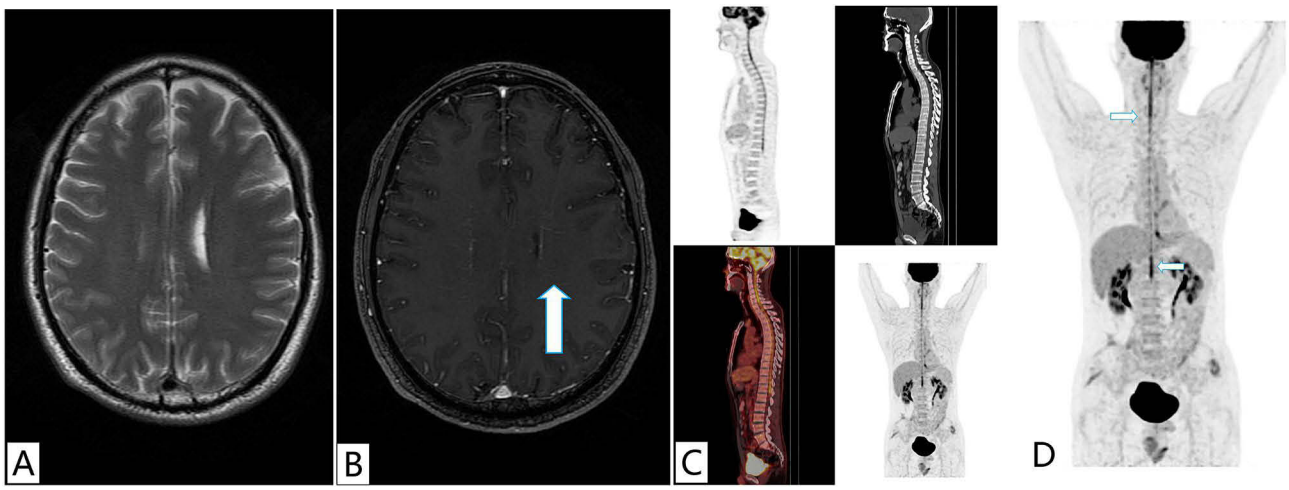

Figure 1 MRI images of patient #1, a male, 47 years old, with headache, fever, weight loss, unsteady walking, dysuria, ataxia, tremors in hands, and numbness of the fingertips of both hands. (**A**) Plain MRI scanning of the brain showing normal signal; (**B**) Contrast-enhanced MRI scanning of the brain showing linear enhancement (arrow) beside ventricle; (**C** and **D**) PET-CT showing diffuse hypermetabolism from medulla to lumbar spinal cord (arrow).

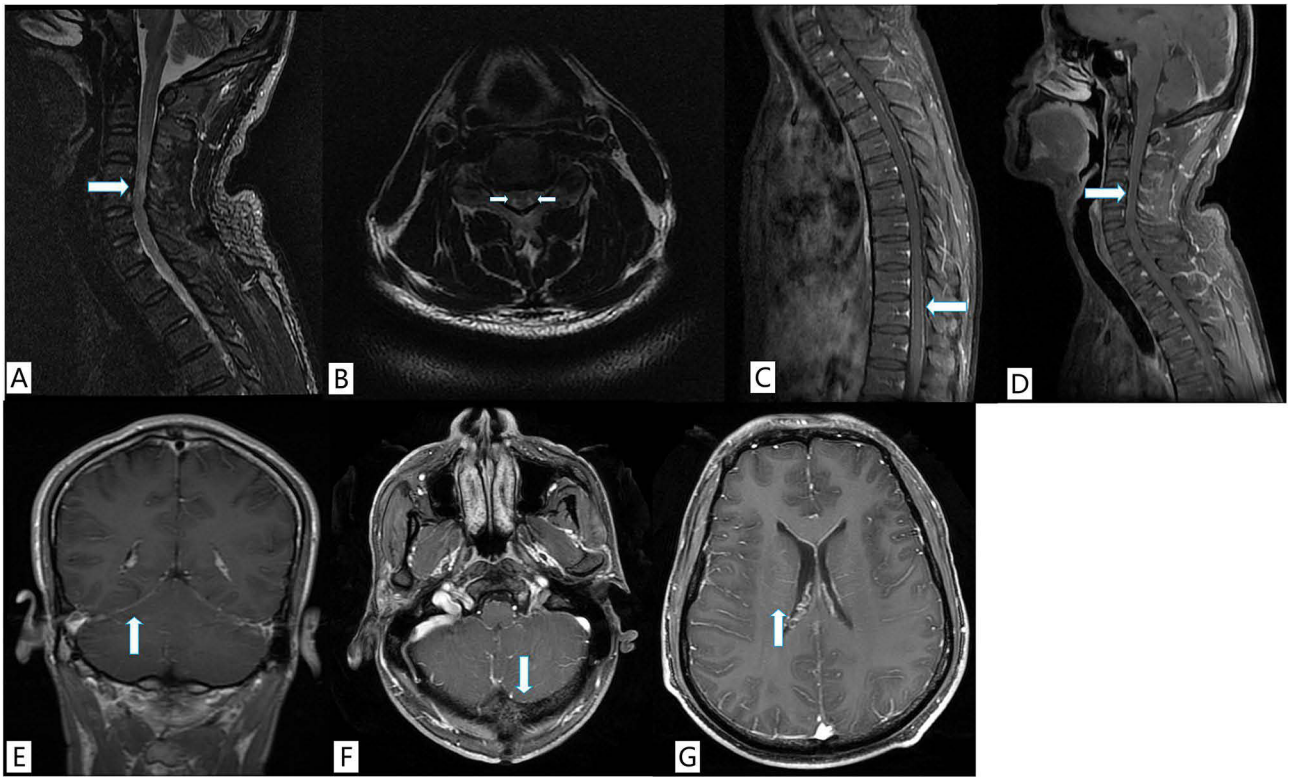

Figure 2 MRI images of the brain and spinal cord of patient #2, a male, 37 years old, with headache, fever, dizziness, vomiting, radicular pain, dysuria, and ataxia. (**A** and **B**) Plain MRI images at C4-5 level showing symmetrical strip-like T2 enhancement in the medial posterior region of the spinal cord (arrow); (**C** and **D**) Cervical contrast-enhanced MRI images showing abnormal enhancement of signal in pia mater of cervical/thoracic spinal cord (arrow); (**E** and **F**) Brain contrast-enhanced MRI images showing multiple enhancement of signal at supratentorial and subtentorial pia mater (arrow); (**G**) Contrast-enhanced MRI scanning of the brain showing linear enhancement (arrow) beside ventricle (arrow).

diffuse heterogeneous hypermetabolism was observed (Figure 1). One patient (#4) received PET-CT screening after immunotherapy, and no abnormality was detected. Three patients (#1, #3, #5) received spinal plain and contrast-enhanced MRI; no enhancement was detected.

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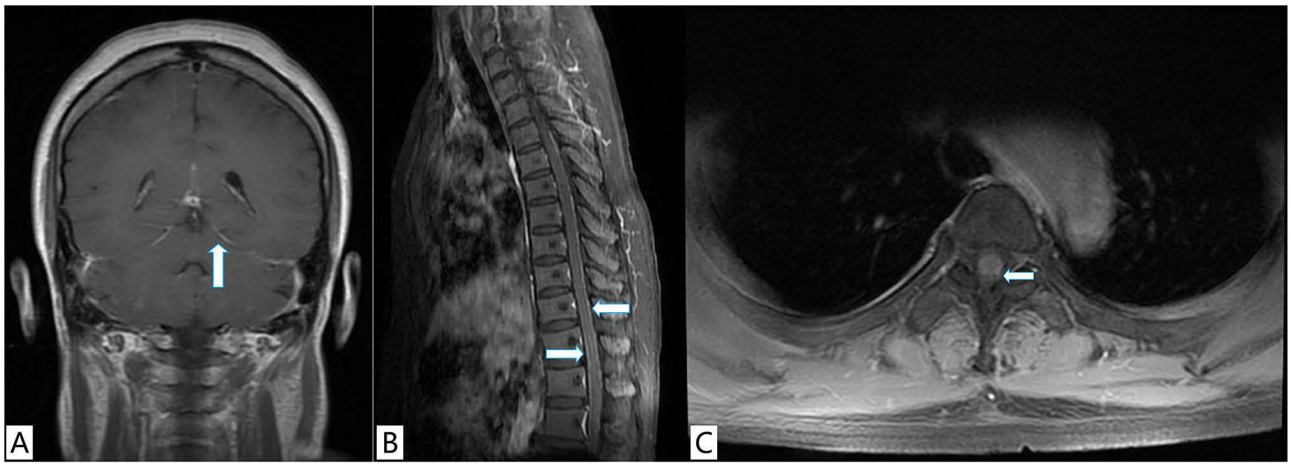

Figure 3 Contrast-enhanced MRI images of patient #4, a female, 44 years old, with headache, fever, dysuria, bilateral weakness, and numbness in four limbs. (**A**) Brain MRI image showing the enhancement of signal at pia mater (arrow); (**B**) Sagittal spinal MRI image showing strip-like enhancement of signal at thoracic pia mater (bold arrow) and intramedullary piece of enhancement of signal (arrow); (**C**) Axial thoracic contrast-enhanced MRI showing enhancement of signal at thoracic pia mater (arrow).

EEG Examination

EEG examination was performed in six patients within two days of admission, and four patients (#1, #2, #3, #5) showed a mild-moderate increase of slow wave. One patient (#6) demonstrated boundary EEG oscillation, and one patient (#4) showed normal EEG. There were no significant specific findings in EEG, and no repeated EEG examination was performed on these patients who were discharged from the hospital since there was no seizure or obvious cognitive dysfunction.

Response to Treatment and Prognosis

All patients received antivirus treatment after initial diagnosis (Ganciclovir, 0.25 g, Bid, i.v., for 14–21 days) but without obvious alleviation. Two patients (#1 and #6) only showed alleviation of headache but displayed numbness in the fingertips of both hands (patient #1) and vision impairment (patient #6) during antivirus treatment alone. After confirmation of GFAP antibody in CSF, five patients (#2, #3, #4, #5, and #6) received first-line immunotherapy with consecutively intravenous injections of methylprednisolone plus intravenous immunoglobulin (IVIg). One patient (#5) immediately received Ganciclovir +Methylprednisolone +IVIg (i.v). after initial diagnosis without confirmation of GFAP in CSF because of the quick disease progression. All patients showed significant alleviation of all signs after the first-line immunotherapy. Five patients (#2, #3, #4, #5, and #6) were repeated for CSF examination after alleviation and demonstrated improvement in CSF (Table 1). After being discharged from the hospital, all patients were given oral prednisolone, and the dose was slowly reduced (5 mg per week) for long-term preventive therapy. All patients demonstrated improvement in neural functions and MRS after antivirus therapy and immunotherapy, and there was no death. Follow-up was performed for 1 year, and no patient showed recurrence. The MRS scores decreased to 0 in three patients and 1 in the other three patients.

Discussion

The present study reported six patients (five males and one female) with positive EBV in CSF. All patients were diagnosed with auto-immune GFAP astrocytopathy with GFAP antibodies in CSF and serum. The major clinical features included headache, fever, and urinary disorder. Intracranial hypertension, monocytic pleocytosis, increases in WBC and protein, and decreases in glucose and chloride in CSF were detected. The MRI and PET-CT showed multiple enhanced signals and hypermetabolism in the brain and spinal cord. All patients responded well to immunotherapy after unsatisfactory antivirus treatment and demonstrated an excellent prognosis during follow-ups of one year. Few studies have reported auto-immune GFAP astrocytopathy combined with EBV infection.[18,19] The patients reported in this article all had auto-immune GFAP astrocytopathy with positive CSF EBV DNA. The clinical data include mNGS of CSF EBV,

PCR verification of EBV, blood EBV antibodies, and detailed clinical data such as diagnosis, treatment process, and prognosis, which can accumulate clinical data for future research.

As a human herpes virus, EBV can attack the immune system, produce autoantibodies, and thus induce auto-immune diseases such as MS.[20,21] The present study indicated that EBV was detected in all six patients by mNGS, which was confirmed by PCR for nuclear antigen, suggesting that there may be active replication of EBV in the CSF of patients. However, positive anti-EBV-VCA IgM or anti-EBV-VCA IgG with low affinity in blood was not detected in any of the six patients. According to previous studies,[27,28] the body produced anti-EBV-VCA IgG or IgM at the acute infection stage, and anti-early antigen antibody appeared at the late stage of acute infection or recurrent infection, whereas the anti-EBV-NA antibody was produced at the late stage of recovery. The body would first produce antibodies with low affinity at re-infection and, subsequently, high-affinity antibodies following persistent infection. The above results of EBV-associated antibodies suggest no evidence of acute primary EBV infection in our six patients. In addition, the six patients did not show significant alleviation after antivirus therapy, although the EBV was confirmed in CSF in all patients. In contrast, all patients responded well to immunotherapy after a confirmed diagnosis of auto-immune GFAP astrocytopathy. These results suggest that the direct cause of the patient's symptoms is GFAP astrocytosis. The presence of the EBV in the CSF of the patients in this case series is an interesting phenomenon. According to existing reports, EBV is associated with inflammatory diseases of the nervous system, such as MS and acute disseminated encephalomyelitis.[23,29,30]

EBV can induce central nervous system inflammation through specific potential mechanisms, such as molecular mimicry.[31–35] This may be why GFAP antibodies were detected, and patients eventually achieved significant relief after immunosuppressive treatment. However, the relationship between active intrathecal EBV duplication and auto-immune GFAP astrocytopathy, ie, whether auto-immune GFAP astrocytopathy reactivates EBV or EBV active replication promotes auto-immune GFAP astrocytopathy, needs to be studied further, reminding clinicians to be cautious when making diagnoses.

Auto-immune GFAP astrocytopathy is a newly defined disease in the central nervous system and has several similar manifestations of meningoencephalitis and flu. Among the reported symptoms in previous studies,[1,3–7] headache, fever, ataxia, and dysuria were observed in all patients in the present study. Other symptoms in individuals included nuchal rigidity, limb numbness or fatigue, or sensory disorder. These results suggest that when the signs of meningoencephalitis were presented, diagnosis of auto-immune GFAP astrocytopathy should be considered with other supporting evidence, particularly when antiviral, anti-tuberculous, and other empirical treatments failed. However, because the clinical features of auto-immune GFAP astrocytopathy are not specific, other diseases should be considered for differential diagnosis with caution, including MS, radiculoneuritis, tuberculosis, and encephalomyelitis with unresponsiveness to immunotherapy.[3,11,12,36,37] Among these diseases, easily to be confused with auto-immune GFAP astrocytopathy, tuberculous meningitis (TBM) is worthy of more attention because both diseases could demonstrate fever, headache, meningeal irritation signs, an increase of total white blood cells, hypochlorhydria, hypoglycemia, and an increase of protein in CSF. However, there are still some differences between TBM and auto-immune GFAP astrocytopathy. For example, TBM usually demonstrates mixed cytologic reaction with increased neutrophils and lymphocytes in CSF at the beginning and a gradual decrease of the proportion of neutrophils following anti-tuberculosis treatment; in contrast, auto-immune GFAP astrocytopathy consistently demonstrates lymphocytic inflammation without neutrophils in CSF and fails to anti-tuberculosis. The response to therapy is also helpful for correct diagnosis. In this study, most patients did not show clear alleviation to antivirus or anti-tuberculosis but responded well to immunotherapy after confirmation of auto-immune GFAP astrocytopathy by GFAP in CSF.

In addition to clinical signs, other laboratory examinations can provide valuable support for the correct and timely diagnosis of auto-immune GFAP astrocytopathy. CSF examination is considered an important test for correctly diagnosing auto-immune GFAP astrocytopathy.[4,9,38,39] In the present study, all six patients demonstrated positive GFAP antibodies in CSF, and three had positive GFAP in serum. The result was consistent with previous studies[4,9] and suggested that positive GFAP in CSF is a vital marker for auto-immune GFAP astrocytopathy and can be used for diagnosis.[5] Other changes in CSF are also worthy of attention, for example, changes in protein, chloride, and WBC percentages. Consistent with the present study, previous studies also reported an auto-immune GFAP astrocytopathy

patient with lymphocytic pleocytosis in CSF.[6,38] Imaging examination is another method to support the diagnosis. In this study, all patients demonstrated abnormal signals in enhanced MRI and/or PET-CT scanning, consistent with previous studies.[13,40,41]

The present study had some limitations. First, the study did not enroll control participants, including healthy volunteers or patients with negative EBV and/or GFAP. Enrolling such patients may help determine the correlation between the EBV and auto-immune GFAP astrocytopathy. Second, the data on GFAP and EBV-associated antibodies in serum were not collected in three patients after being discharged from the hospital. This was partially due to the financial burden placed on the patients' families, making it difficult to reach a clear conclusion about the relationship between EBV and therapy. Third, the failure mechanism of antiviral therapy after the initial diagnosis was unclear and remained to be further studied.

Conclusion

Six patients showed positive GFAP antibodies and EBV DNA in CSF. We recorded the laboratory tests, imaging data, and treatment experience of this type of patient, which provided us with valuable clinical data for further exploration of the relationship between EBV and auto-immune GFAP astrocytopathy in the future. The patients responded well to first-line immunotherapy but not to antivirus therapy. The active EBV in auto-immune GFAP astrocytopathy should be given enough attention for diagnosis, and the mechanism needs further studies.

Consent for Publication

The patients have provided written informed consent to have the case details and accompanying images published.

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Disclosure

The authors report no conflicts of interest in this work.

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