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Glial Fibrillary Acidic Protein Astrocytopathy Based on a Two-Center Chinese Cohort Study

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ABSTRACT

Objective: Glial fibrillary acidic protein astrocytopathy (GFAP-A) is a recently defined nosological form belonging to the class of autoimmune inflammatory disorders affecting the central nervous system (CNS). Here, we report the clinical and MRI characteristics, treatment, and prognosis of a GFAP-A cohort from two centers in China.

Methods: We retrospectively analyzed the data from 38 adult patients with positive GFAP antibodies and diagnosed as GFAP-A between June 2019 and September 2024. Clinical features, semiquantitative antibody test results, MRI features, treatment approaches, and prognosis were collected.

Results: Among the 38 patients, 24 were male, and the median age at disease onset was 49.5 years. The clinical phenotype included encephalomyelitis (28.9%), myelitis (23.7%), encephalitis (18.4%), meningoencephalomyelitis (18.4%), meningitis/spinal meningitis (7.9%), and peripheral neuropathy (2.6%). In enhanced MRI images, 4 (10.5%) of the patients showed enhancement of the cerebral meninges, 2 (5.3%) had enhancement of the ependyma, and 5 (13.2%) had enhancement of the spinal cord pia mater. 77.1% of the patients responded to the glucocorticoid treatment, while 65.8% had a monophasic course. Spearman correlation analysis showed that CSF-specific oligoclonal bands were significantly correlated with 1-year relapse (CI = 0.527, $p = 0.003$).

Interpretation: The clinical manifestations of GFAP-A are highly diverse, encompassing encephalitis, myelitis, and meningitis, including spinal meningitis. The enhancement of the spinal pia mater and ependyma on MRI was confirmed. Most patients exhibit a positive response to glucocorticoid therapy. The presence of CSF-specific oligoclonal bands could potentially serve as an indicator for predicting recurrence.

1 | Introduction

Primary astrocytopathies contribute to the pathogenesis of several neurological diseases [1]. Glial fibrillary acidic protein astrocytopathy (GFAP-A) is an autoimmune disorder of the central nervous system described in 2016 [2]. Glial fibrillary acidic protein (GFAP), the main intermediate filament in astrocytes [3], acts as an autoantigen. There are at least ten

isoforms of GFAP, which exhibit variable expression patterns in astrocytes and peripheral glial cells. GFAPα is the predominant isoform in astrocytes of the central nervous system and is also expressed in the peripheral nervous system [4]. Multiple regions of the nervous system may be affected in GFAP-A resulting in encephalitis, meningitis, myelitis, and spinal meningitis. Clinical manifestations of GFAP-A are diverse and may include pre-infection symptoms such as fever and headache, movement disturbances, paresthesia, visual disturbance, brainstem syndrome, and autonomic dysfunction [5–7]. The etiology and pathogenesis of GFAP-A remain elusive, although infections may serve as a trigger. Genetic susceptibility was also identified showing association with human leucocyte antigens HLA-A*3303 and HLA-DPB1*0501 [8]. Pathological examinations of autopsy and biopsy specimens have revealed a perivascular inflammatory reaction characterized by predominant CD8+ T-cell infiltration [9, 10]. Antibodies against GFAP in serum and cerebrospinal fluid are crucial for the diagnosis of GFAP-A [2, 4, 6]. Cell-based assays (CBA) utilizing the GFAPα isoform exhibit a comparatively elevated level of sensitivity [11]. MRI findings typically demonstrate linear enhancement perpendicular to the lateral ventricles, extensive segmental myelitis, as well as meningeal and spinal pia enhancement. High-dose corticosteroid therapy usually yields a positive response; however, relapses can occur and several cases may exhibit a poor prognosis [12, 13].

The aim of this study was to present findings from a two-center Chinese cohort of GFAP-A, thereby providing a comprehensive and detailed analysis of its clinical characteristics, semi-quantitative antibody test results, imaging features, treatment approaches, and prognosis.

2 | Methods

2.1 | Study Design and Participants

We retrospectively retrieved a total of 46 adult patients with positive GFAP antibodies among inpatients in the neurology department of Tianjin Medical University General Hospital and Beijing Tiantan Hospital, between June 2019 and September 2024. According to the following inclusion and exclusion criteria, 38 patients were finally included. Inclusion criteria were: (1) clinically diagnosed as GFAP-A; (2) GFAP IgG test positive; (3) absence of other inflammatory diseases of the CNS; (4) availability of follow-up information. Patients diagnosed with other conditions or with incomplete information were excluded from the study. The detailed information of the cohort inclusion can be found in Figure S1.

Collected data included demographics, initial clinical manifestations, accompanying diseases, immune factors, MRI images, and treatment approaches during both the acute and remission stages. Patients' clinical features were summarized by clinical symptoms and imaging findings as encephalitis, myelitis, encephalomyelitis, and others. Additionally, initial clinical manifestations were categorized as motor, sensory, autonomic symptoms, blurred vision, brainstem symptoms, cortical symptoms, ataxia, epilepsy, headache, and fever. MRI images of the patients' brains and spinal cords were collected. During follow-up, the Modified Rankin Scale (mRS) score, Activities of Daily Living (ADL) score, and maintenance treatment information were collected via telephone or in face-to-face conversations. The study was approved by the Ethics Committees of both centers, and informed written consent was obtained from patients or their representatives. Anonymized data from this study are available upon reasonable request.

2.2 | Antibody Assay

Both centers employed a fixed cell-based assay for testing serum or cerebrospinal fluid. In the fixed CBA for detecting GFAP antibodies, plasmids containing GFAPα (clone NM_002055.5; Akriva, Wuxi, China) and GFP were transfected into 293 T cells cultured in 96-well plates. Forty hours post-transfection, the cells were fixed with 4% polyformaldehyde. After incubating the fixed cells with patients' sera (or CSF), GFAP antibodies from patients would bind to the GFAP antigen expressed on 293 T cells, forming an antigen–antibody complex that can be detected using immunofluorescence-labeled anti-human IgG secondary antibodies under an immunofluorescence microscope. The serum of enrolled patients was diluted in ratios of 1:10, 1:32, 1:100, 1:320, and 1:1000 (CSF: 1:1, 1:3.2, 1:10, 1:32, 1:100, and 1:320) for fixed CBA detection in this study. There were six and seven titers (serum: < 1:10, 1:10, 1:32, 1:100, 1:320, and 1:1000; CSF: < 1:1, 1:1, 1:3.2, 1:10, 1:32, 1:100, and 1:320) used to estimate the relative intensity of GFAP antibodies for each sample. Antibody negativity in the fixed CBA is defined as a titer < 1:10 if no positive signal is detected when the serum is diluted to 1:10. GFAP antibody positivity is assigned upon a positive reaction for GFAP IgG at a titer of 1:10 in serum. For cerebrospinal fluid test results, the demarcation value is determined to be 1:1. In cases of inconsistent interpretation results, samples will be re-tested and re-interpreted to ensure alignment of interpretation results between the two readers.

2.3 | Statistical Analysis

Data were statistically analyzed using SPSS Version 24.0. Continuous variables were expressed as median (ranges), and categorical variables were presented in proportional form. The relapse tendency over time was illustrated using a line chart. Spearman correlative analysis was used to investigate the relative factors influencing one-year relapse, the mRS score during attack and recovery conditions. A $p < 0.05$ was considered indicative of statistical significance.

3 | Results

3.1 | Demography and Clinical Characteristics

From June 2019 to September 2024, we collected data from 38 patients diagnosed with GFAP-A and treated in two centers, with 21 cases from Tianjin General Hospital and another 17 cases from Beijing Tiantan Hospital. The demographics and clinical characteristics of these 38 patients are summarized in Table 1. Of the total patients, 24 (63.2%) were male. The median age at disease onset was 49.5 years (range: 19–75 years).

TABLE 1 | Demographics and clinical characteristics of GFAP-A patients.

Demographics	
Sex, male, percent (*n*)	63.2% (24)
Age onset (years), median (range)	49.5 (19–75)
BMI, median (range)	23.9 (16.8–31.1)
Clinical phenotype, percent (*n*)	
Myelitis	23.7% (9)
Encephalomyelitis	28.9% (11)
Encephalitis	18.4% (7)
Meningoencephalomyelitis	18.4% (7)
Meningitis/spinal meningitis	7.9% (3)
Peripheral neuropathy	2.6% (1)
Clinical manifestation, percent (*n*)	
Motor	57.9% (22)
Sensory	63.2% (24)
Autonomic symptom	28.3% (10)
Blurred vision	7.9% (3)
Brainstem symptom	18.4% (7)
Cognitive, memory, and psychiatric symptoms	23.7% (9)
Ataxia	34.2% (13)
Epilepsy	5.3% (2)
Headache	31.6% (12)
Fever	31.6% (12)
Accompanied disease, percent (*n*)	
Autoimmune disease	5.3% (2)
Endocrine disease	26.3% (10)
Infection within 3 months prior to onset	10.5% (4)

The most common clinical phenotype was encephalomyelitis, accounting for 28.9% (11). Nine patients (23.7%) presented with myelitis. Both encephalitis and meningoencephalomyelitis accounted for 18.4% (7). In terms of clinical manifestations, the most common signs and symptoms included sensory abnormalities (63.2%, 24) and motor dysfunctions (57.9%, 22), followed by ataxia (34.2%, 13), fever (31.6%, 12), and headache (31.6%, 12). Out of the 38 patients, 10(26.3%) had comorbidities related to endocrine or metabolic disorders, including 6 with diabetes, 3 with thyroid disorders, and 1 with gout. Two cases (5.4%) presented with accompanying autoimmune diseases: one had immune thrombocytopenia, and another connective tissue disease. The chest-enhanced CT scan of one patient revealed space-occupying lung lesions highly suggestive of a tumor; however, due to the lack of biopsy confirmation, this information was not included in the analysis.

TABLE 2 | Serum and CSF findings of GFAP-A patients.

Factors in serum and CSF	
Coexisting autoantibody, percent (*n*)	50% (19)
CSF pressure (mmH_2O), median (range)	150 (60–310)
CSF WBC count (per μL), median (range)	17 (0–249)
CSF protein before therapy (g/L), median (range)	0.67 (0.20–2.42)
Oligoclonal bands type, percent (*n*)	
I	45.2% (14/31)
II	45.2% (14/31)
III	9.7% (3/31)
IV	0
V	0
GFAP-antibody	
Serum, positive, percent (*n*)	62.2% (23/37)
CSF, positive, percent (No.)	83.8% (31/37)
Both in serum and CSF, positive, percent (*n*)	44.4% (16/36)
Serum antibody titer[a], median (range)	1:32 (1:10–1:320)
CSF antibody titer[a], median (range)	1:10 (1:1–1:320)

Abbreviations: CSF = cerebral spinal fluid, WBC = white blood cell.
[a]The analysis only included patients who tested positive for the GFAP antibody.

3.2 | Serum and Cerebrospinal Fluid Findings

The test results of the patients' serum and cerebrospinal fluid are summarized in Table 2. Among the 38 patients, 19 (50%) tested positive for coexisting autoantibodies. These included 8 cases of positive anti-nuclear antibodies (ANA), 1 case of positive anti-GD2 antibodies, 1 case of positive aquaporin-4 antibodies (serum titer 1:32), and 1 case of positive myelin oligodendrocyte glycoprotein antibodies (CSF titer 1:100). Additionally, there was 1 case with positive anti-mitochondrial M2 (AMA-M2) and Jo-1 antibodies. Two patients exhibited positivity for SSA and Ro-52 antibodies, as well as AMA-M2 antibodies. Furthermore, one patient tested positive for rheumatoid factor, one for thyroglobulin antibody, one for anti-streptolysin O (ASO) antibody, and one for thyroid peroxidase antibody.

The majority of patients exhibited a slight elevation in the white blood cell count and protein levels in cerebrospinal fluid. A total of 31 patients had available data for oligoclonal bands (OCB) testing. The statistical results for different types of OCB [14] indicate that Type I constitutes 45.2% (14), while Types II and III collectively account for 54.8% (17). Among all patients, 54.8% (23) tested positive for serum GFAP antibodies, while 73.8% (31) had GFAP antibodies in cerebrospinal fluid. Of these, 38.1% (16) exhibited positivity in both serum and CSF. In patients with positive GFAP antibody tests, the median serum titer was 1:32 (range: 1:10–1:320), and the

median CSF titer was 1:10 (range: 1:1–1:320). Notably, CSF and serum GFAP antibodies were both negative for four patients.

3.3 | Imaging Features

The MRI features of the brain and spinal cord are summarized in Table 3. The location and morphology of lesions within the brain are highly variable, potentially affecting multiple regions, including the cerebral white matter, cerebellum, diencephalon, and brainstem (Figure 1). In the brain MRI, 50% (19) of the patients exhibited white matter T2 hyperintense lesions; 28.9% (11) had brainstem lesions. Typical linear enhancement perpendicular to the lateral ventricles was observed in 6 patients. Ependymal and leptomeningeal enhancement was evident in some patients (Figure 1C.a–C.b). Diffusion-weighted imaging revealed a hyperintense ring signal in certain lesions, which exhibited dynamic changes throughout the course of the disease (Figure 1E.a–E.h).

More than half of the patients exhibited involvement of spinal parenchyma. Of the 38 patients, 65.8% (25) exhibited spinal lesions on imaging, with 73.9% (17/23) of those showing longitudinally extensive T2 hyperintensities. Spinal cord enhancement was typically characterized by spot-like or scattered enhancement, and thickened pia mater with continuous enhancement was observed (Figure 2). In all 36 cases for which post-contrast MRI data were available, 19 (52.8%) of the patients exhibited lesion enhancement during the acute phase.

TABLE 3 | Imaging features of GFAP-A patients.

T2 lesion on brain MRI, percent (*n*)	
Cerebral cortex	2.6% (1)
Cerebral white matter	50% (19)
Diencephalon	13.2% (5)
Brainstem	28.9% (11)
Optic nerve	2.6% (1)
Cerebellum	5.3% (2)
T2 lesion on spinal MRI, percent (*n*)	
Spinal cord parenchyma	65.8% (25)
Longitudinally extensive	73.9% (17/23)
Enhanced MRI, percent (*n*), $n = 36$[a]	
Enhanced lesion in acute stage	52.8% (19)
Thread enhanced perpendicular to the lateral ventricle	16.7% (6)
Cerebral meninges	10.5% (4)
Ependyma	5.3% (2)
Spinal cord pia mater	13.2% (5)

[a]Thirty-six cases with available enhanced MRI.

3.4 | Treatment and Prognosis

The treatment and prognosis outcomes are shown in Table 4. The median follow-up time was 29 months (range: 9–132 months). During the disease attack period, 76.3% (29) of patients received intravenous methylprednisolone (IVMP) treatment alone, while 13.2% (5) were treated with a combination of IVMP and intravenous immunoglobulin (IVIG). One patient received a combination of IVMP and efgartigimod; another patient was treated solely with IVIG, and two patients received symptomatic treatment. Among the patients receiving glucocorticoid therapy, 77.1% (27/35) responded positively. Thirty-one of the thirty-eight patients continue to receive maintenance therapy at the last follow-up. Two patients had previously been on low-dose oral glucocorticoids but discontinued treatment after achieving disease stability. Three patients did not receive any immunomodulatory therapy. One patient was switched to mycophenolate mofetil (MMF) after discontinuing ofatumumab due to a pulmonary infection.

The majority of patients (65.8%) presented with a monophasic course. The median time to relapse was 7 months, with a range of 2 to 98 months. The median mRS score during the disease attack period was 3 (1–5), and the median ADL score was 75 (0–100). At the final follow-up, the median mRS score was 1 (0–5), while the median ADL score was 100 (0–100). The trend of relapse over time is illustrated in Figure 3: the relapse percentage at 3 months was 2.6% (1/38), at 6 months it was 13.2% (5/38), at 1 year it reached 25% (9/36), and at 2 years, the relapse rate was 42.3% (11/26).

Spearman correlation analysis was employed to evaluate factors associated with relapse at 1 year, disease severity, and recovery condition for cases positive for GFAP antibodies in serum and/or cerebrospinal fluid. The results are presented in Tables S1 and S2. The presence of CSF-specific oligoclonal bands (CI = 0.527, $p = 0.003$) showed a positive correlation with one-year relapse. Age at onset was negatively associated with one-year relapse (CI = -0.035, $p = 0.037$). Additionally, in the 37 cases with complete mRS score data, serum antibody titer (CI = 0.539, $p = 0.008$) showed a positive correlation with mRS score during the attack. CSF WBC count (CI = 0.502, $p = 0.002$), CSF protein (CI = 0.378, $p = 0.028$), GFAP-antibody positive in both sample (CI = 0.370, $p = 0.029$), and thread enhanced perpendicular to the lateral ventricle (CI = 0.490, $p = 0.003$) were significantly associated with ΔmRS score, defined as mRS score on attack minus follow-up mRS score.

4 | Discussion

This study analyzed 38 patients diagnosed with GFAP-A from two centers. With a median follow-up time of 29 months, we presented demographic characteristics, clinical manifestations, imaging features, treatment, and prognosis of the patients, providing a comprehensive overview of GFAP-A patients within the Chinese population.

The pathogenesis of GFAP-A remains poorly understood. Unlike other well-characterized, clinically relevant glial cell autoantigens, such as aquaporin-4 (AQP4) and myelin oligodendrocyte glycoprotein (MOG), which are plasma membrane targets, GFAP is an intracellular antigen [15]. Consequently, the direct pathogenicity of GFAP-specific IgG is considered

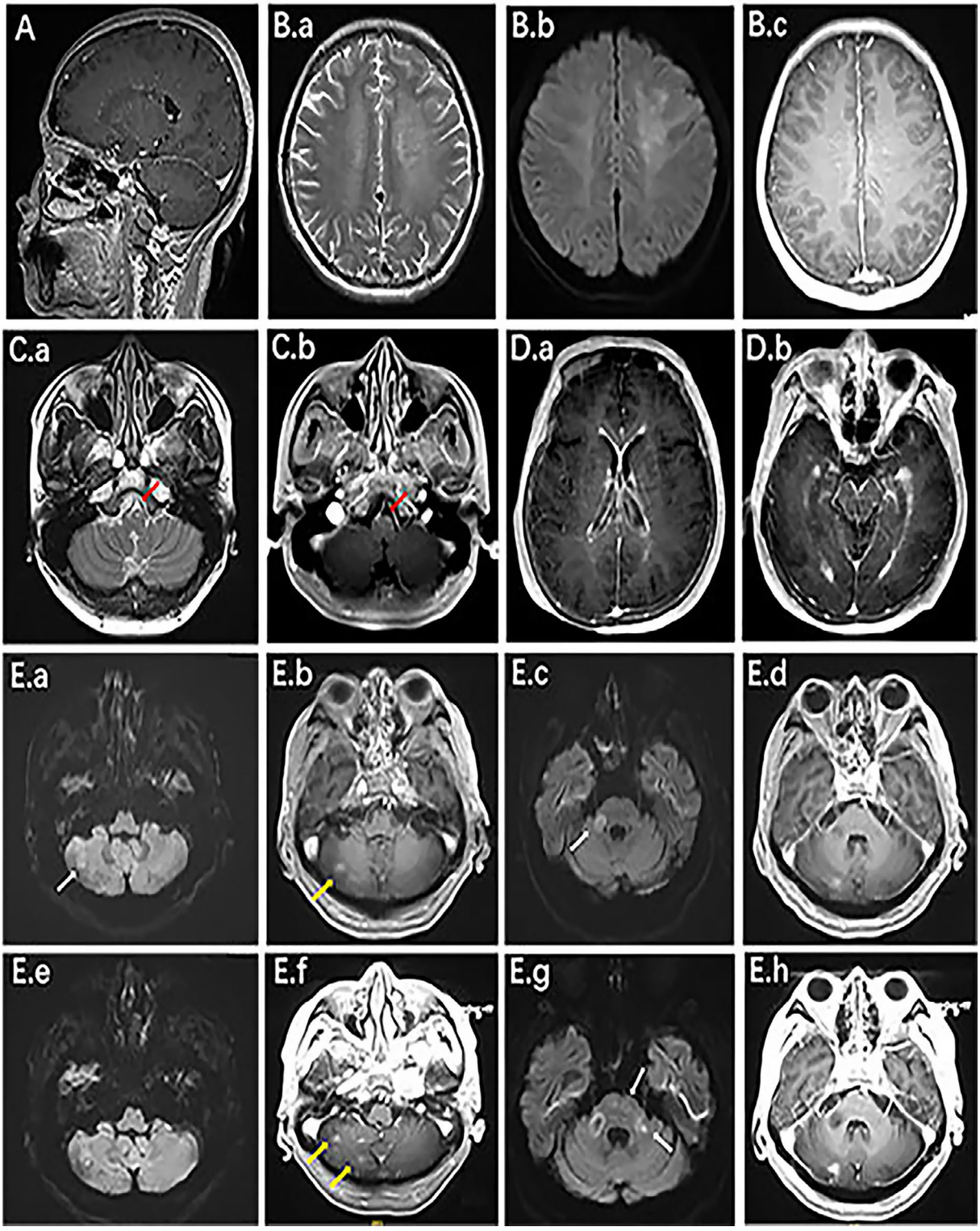

FIGURE 1 | Legend on next page.

relatively low. Meningeal biopsy and histopathological analyses have demonstrated the infiltration of various lymphocytes, including CD4+, CD8+ T cells, and B cells, in inflamed tissues, with a predominance of $CD8^+$ *T* cells [9, 11]. An earlier mouse model of GFAP-related neurogenic autoimmunity confirmed the pathogenic role of cytotoxic T cells specific to GFAP peptides [16]. With respect to the mechanisms underlying cellular activation, given that patients with GFAP-A frequently exhibit infection-like intracranial symptoms, viral infections may serve as potential triggers. Furthermore, data on patients with GFAP-A complicated with tumors have been frequently reported. GFAP expression in tumors, particularly astrocytomas, may also act as a trigger for inducing immune responses [6]. A study by Shu et al. revealed an association between the HLA-A*3303 allele and increased susceptibility to GFAP-A [8]. Animal model research has also identified two distinct disease

FIGURE 1 | Brain MRI features in GFAP-A patients. A sagittal T1-weighted post-contrast image reveals radial perivascular enhancement extending from the ventricles (A). A middle-aged patient reported dizziness and hand tremors persisting for one week (B.a–B.c). Axial T2-weighted imaging demonstrated bilateral fronto-parietal white matter hyperintensities (B.a), with diffuse hyperintensity visible on diffusion-weighted imaging (B.b), and mild post-contrast enhancement (B.c). A patient experienced lower limb numbness, with axial T2-weighted imaging showing abnormal signals in the medulla oblongata (C.a, C.b). Another patient presented with limb pain, weakness, and urinary retention. Enhanced MRI imaging revealed linear and nodular enhancement throughout the ependymal region (D.a), and leptomeningeal enhancement in the brainstem (D.b). Lesions in the right cerebellum and pontine arm were observed in an elderly patient presenting with fever and drowsiness. Diffusion-weighted imaging showed high signal intensity (E.a, E.c white arrow), and enhanced MRI indicated cerebellar lesion enhancement (E.b, yellow arrow), with no enhancement in the pontine arm (E.d). After 12 days, the patient's consciousness disturbances worsen. Diffusion-weighted imaging revealed that the lesions in the cerebellum and right pontine arm were more pronounced (E.e, E.g), with new lesions developing in the left pontine arm and pontine region (E.g, white arrow). The cerebellar lesions exhibited enhancement (E.f, yellow arrow), while the pontine lesions showed no enhancement (E.h).

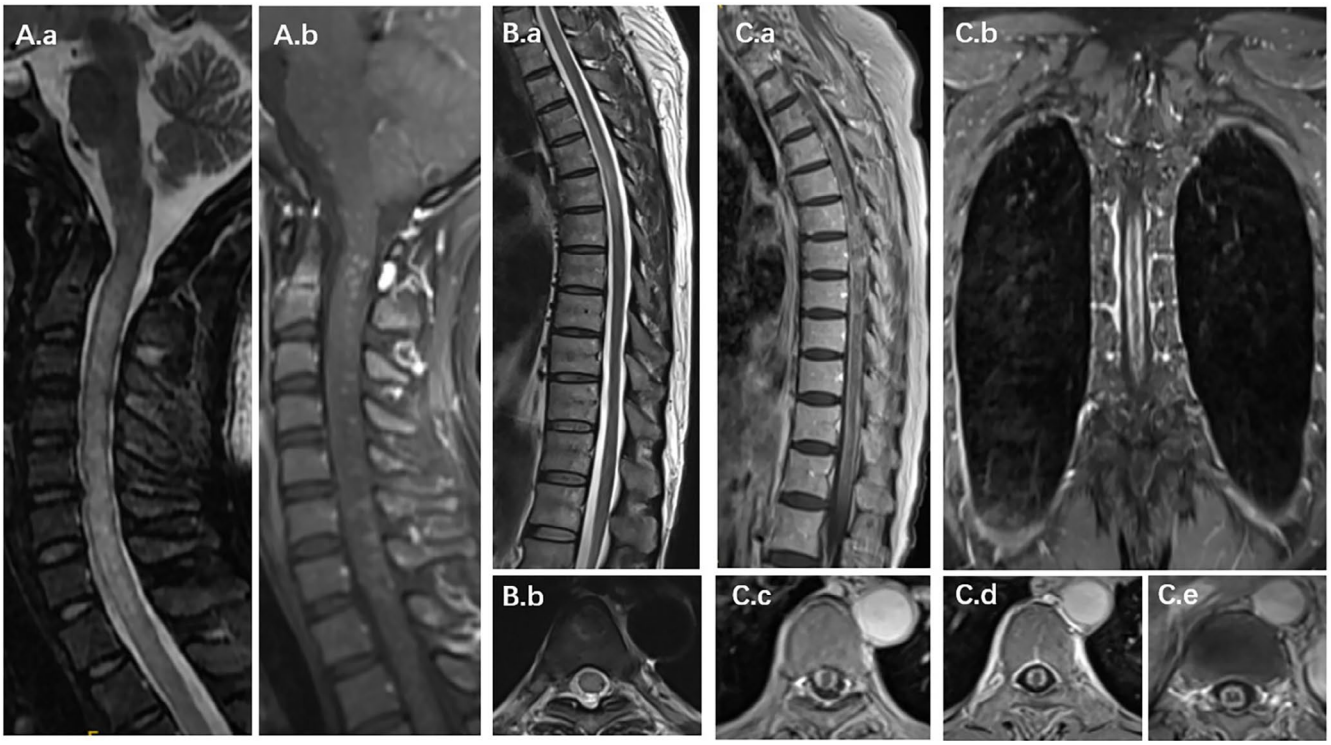

FIGURE 2 | Spinal cord MRI features in GFAP-A patients. A patient presented with numbness in both lower limbs. Cervical MRI demonstrated a long segmental T2 hyperintensity with spinal cord swelling (A.a), and the contrast-enhanced image revealed scattered enhancement (A.b). Another patient reported numbness in both lower extremities and urinary retention. Thoracic MRI showed an abnormal T2 signal in the long thoracic medullary segments (B.a) and an axial transverse spinal cord lesion (B.b). A separate patient presented with bilateral lower limb pain and weakness, as well as urinary and fecal retention (C.a–C.e). Abnormal enhancement of the pia mater was observed from the thoracic segment to the conus medullaris (C.a). Diffuse thickening and enhancement of the pia mater were noted in the coronal (C.b) and axial (C.c–C.e) views.

course phenotypes: acute onset and slower relapse-remission patterns, potentially reflecting different triggering mechanisms and levels of B-cell involvement [16]. To date, only two autopsies of GFAP-A cases have been reported, identifying two distinct pathological phenotypes: lymphocytic and granulomatous [9]. Whether these phenotypic differences represent different stages of the disease process or are linked to specific genetic risk factors remains unclear. Further basic research and long-term follow-up studies are warranted to elucidate these mechanisms and their clinical implications.

Demographically, our cohort is similar to previously reported cohort [17, 18], with a median onset at middle age and a slight male predominance. The clinical picture of GFAP-A is highly heterogeneous, with pathological changes in the brain, spinal cord, meninges, pia mater, and peripheral nerves. GFAP is the key type III intermediate filament expressed in astrocytes in the CNS, and is also expressed in non-myelinating Schwann cells and subepithelial glial cells of the enteric nervous system [4]. The presence of GFAP in the paraventricular, spinal cord, nerve root ganglia, and peripheral nerves has been confirmed through indirect immunofluorescence staining of cerebrospinal fluid from positive patients and rat tissue in previous studies [2, 19]. This finding partially explains why patients exhibit both central and peripheral nervous system symptoms. The electrophysiological examination of the patient in our cohort with peripheral neuropathy revealed injuries to motor axons consistent with a previously reported data [20].

TABLE 4 | Treatment and prognosis of GFAP-A patients.

Follow-up time (months), median (range)	29 (9–132)
Attack treatment, percent (*n*)	
IVMP[a]	76.3% (29)
IVMP[a] + IVIG	13.2% (5)
IVMP[a] + Efgartigimod	2.6% (1)
IVIG	2.6% (1)
Symptomatic treatment	5.3% (2)
Response to Glucocorticoid[b]	77.1% (27/35)
Maintenance treatment, percent (*n*), *n* = 31[c]	
Glucocorticoid[d]	51.6% (16)
Rituximab	29% (9)
MMF	6.5% (2)
Ofatumumab	6.5% (2)
CTX	3.2% (1)
Inebilizumab	3.2% (1)
Prognosis	
Monophasic course, percent (*n*)	65.8% (25)
Time to relapse (months), median (range)	7 (2–98)
mRS score (during attack), median (range)	3 (1–5)
mRS score (follow-up), median (range)	1 (0–5)
ADL score (during attack), median (range)	75 (0–100)
ADL score (follow-up), median (range)	100 (0–100)

Abbreviations: ADL = Activities of Daily Living, CTX = cyclophosphamide, IVIG = intravenous immunoglobulin, IVMP = intravenous methylprednisolone, MMF = mycophenolate mofetil, mRS = Modified Rankin Scale.
[a]High-dose intravenous methylprednisolone.
[b]Only included 35 patients who received glucocorticoid therapy.
[c]Thirty-one patients were still undergoing maintenance therapy at the time of follow-up.
[d]Low-dose oral glucocorticoids.

Visual symptoms observed in three patients from our cohort were characterized by blurry vision, with or without reduced visual acuity. Their lumbar puncture pressure was within the normal range, while OCT or fundus examinations revealed papilledema. Previous studies reported that GFAP-A patients with optic disc edema often exhibit normal or slightly elevated lumbar puncture pressure [21, 22]. A prominent venular leakage on fluorescein angiography in GFAP autoantibody–positive meningoencephalitis was also reported [23], suggesting that optic disc edema may result from inflammatory vasculopathy rather than elevated intracranial pressure. At the same time, more than 60% of patients with GFAP antibody– positive papilledema were asymptomatic [24]. Therefore, it is crucial to conduct fundus and OCT examinations for all individuals with suspected GFAP-A to avoid underestimating the pathology.

The reported prevalence of tumors in patients with GFAP-A ranged from 16.1% to 46.7% [2, 5, 6, 11, 25–27]. However, only one patient in our cohort exhibited a strongly suspected lung tumor; the prevalence of tumors in other Chinese studies [18, 28] was similarly low, consistent with our findings. Medical records from both centers indicated that all patients underwent tumor screening, including blood tests and imaging examinations. This discrepancy in tumor prevalence may be attributed to the exclusion of patients with other diagnoses, including paraneoplastic syndrome, at the time of enrollment. Further studies involving larger populations are necessary to determine whether the incidence of GFAP-A related tumors has been overestimated.

More than half of the patients (17/31) in this study were tested positive for OCB in CSF, with no individual seropositive cases identified. The observed high rate of OCB positivity aligns with previous findings from France (77%) and the United States (46%) [5, 25], although contradictory results have been reported in other studies (11.1% and 31.8%) [18, 29]. These data are lower than the commonly recognized OCB positivity rate in multiple sclerosis but higher than the positivity rate in neuromyelitis optica spectrum disorder (NMOSD). The meta-analysis, which included 681 patients, demonstrated a higher positivity rate of GFAP IgG in CSF compared to serum [17]. Our findings further support this observation with a CSF positivity rate of 83.8%. Biopsies revealed that antibody-secreting CD138+ cells, distributed around blood vessels, may account for intrathecal antibody synthesis [7], which may thus explain the high OCB and antibody positivity rates in CSF.

Coexisting antibodies are frequently observed in GFAP-A [12, 17]. However, the most commonly reported antibody against N-methyl-D-aspartate type of ionotropic glutamate receptors (NMDAR) was not detected in our cohort, likely due to the exclusion of other central nervous system inflammatory diseases during enrollment. The second most commonly reported were antibodies against aquaporin 4 (AQP4) and myelin oligodendrocyte glycoprotein (MOG). In our study, the initial AQP4 antibody titers in serum and cerebrospinal fluid were 1:32 and 1:10, respectively, while the GFAP antibody titers were 1:320 and 1:10, respectively, at the onset of myelitis in a patient whose spinal cord lesion did not exceed three vertebral body lengths. After 8 months, this patient tested negative for serum AQP4 antibodies but retained a GFAP antibody titer of 1:10, thus supporting GFAP-A diagnosis. Another patient exhibited a cerebrospinal fluid MOG antibody titer of 1:100, along with serum GFAP antibody titers of 1:10 and CSF GFAP antibody titers of 1:100. The serum MOG antibody of this patient was negative, and the MRI revealed point-linear enhanced lesions in the brainstem and cervical spinal cord. Consequently, a diagnosis of GFAP-A was considered more likely. After undergoing B-cell depletion treatment for three months, both antibodies became negative. Given the prevalence of coexisting antibodies in GFAP-A, diagnoses should be made with caution.

The hallmark MRI feature of GFAP-A is linear enhancement perpendicular to the lateral ventricle [2], a finding also observed in our patients (Figure 1A). The brain MRI lesions appeared as punctate or linear T2 hyperintensities. Approximately 52.8% of patients in the acute phase exhibited enhancement lesions, primarily presenting as perivascular punctate or linear enhancement, consistent with previous reports [26, 27, 30]. Radial linear enhancement was also noted in the cerebellum and brainstem

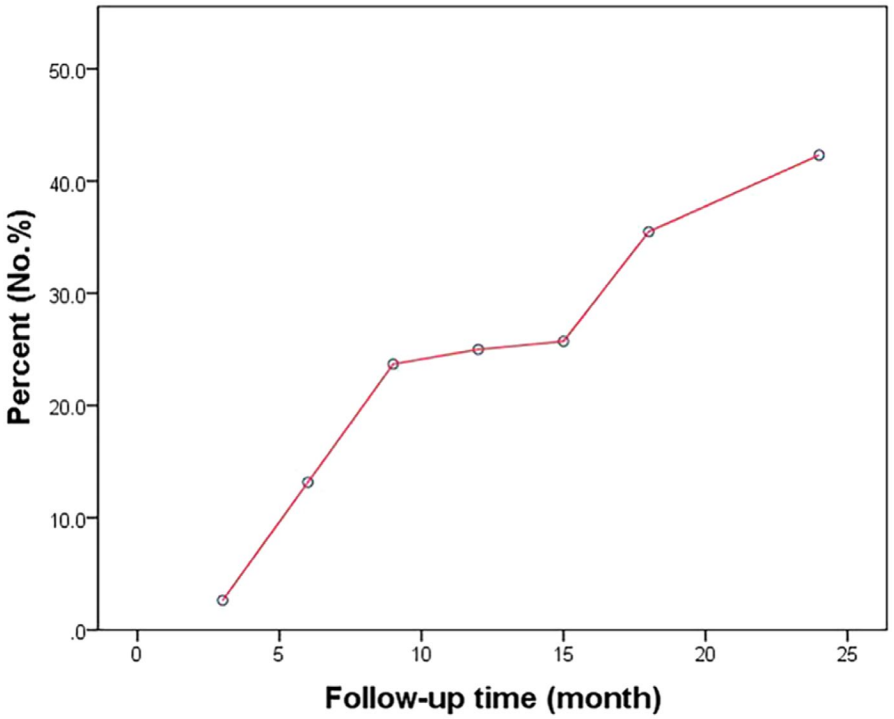

FIGURE 3 | Relapse tendency over time (months).

outside the lateral ventricles [6, 28, 31], while no ring enhancement was detected [25]. Other reported sites of brain involvement include the area postrema, basal ganglia, and corpus callosum [5, 32]. Leptomeningeal involvement was evident in our patients. Previous imaging reports mentioned pial or subependymal enhancements [33], but we are the first to demonstrate significant whole-ependymal enhancement (Figure 1D.a). This patient also exhibited substantial enhancement throughout the spinal cord pia mater (Figure 2C.a–C.e), similar to a published report [34], but with more pronounced thickening of the pia mater. The observed MRI lesions are in accordance with the pathological findings. The linear enhancement around the lateral ventricles corresponds to rat tissue-based immunofluorescence staining results [2, 28]. Biopsies of the pia further confirmed the inflammatory reaction and revealed an accumulation of CD8+ T cells, along with associated inflammatory factors [11]. Pia mater involvement, or speckled enhancement of parenchymal lesions, may be more characteristic of GFAP-A than other inflammatory demyelinating diseases, such as multiple sclerosis (MS), neuromyelitis optica spectrum disorder (NMOSD) and myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) [17].

Data on the treatment of GFAP-A remain limited, particularly from large multi-center studies. The majority of patients in our study received intravenous methylprednisolone (IVMP) during the acute phase of the disease. 77.1% of patients in this study responded to high-dose IVMP therapy with partial or complete remission of symptoms, a response rate similar to the 83%, reported in a previous meta-analysis [17]. We report, for the first time, the use of neonatal Fc receptor (FcRn) blocker Efgartigimod, during the acute phase of GFAP-A. This case involves an elderly male patient with encephalomyelitis who failed to respond to glucocorticoid therapy. However, his symptoms still did not improve significantly after the application of FcRn blocker, and his prognosis was poor. These outcomes may be attributed to the prolonged duration (1.5 months) between initial pulmonary infection and the administration of glucocorticoid and Efgartigimod, resulting in severe and irreversible nerve damage. Regarding maintenance therapy, in addition to previously reported conventional immunosuppressants (MMF, CTX, AZA) and intravenous rituximab [5, 25], two of our patients received ofatumumab and one received inebilizumab. None of the three patients receiving targeted B-cell therapy experienced relapse during follow-up.

The prognosis of our cohort was relatively favorable, with a median mRS score of 1 and an ADL score of 100 at the final follow-up. The relapse rate has been reported in other cohorts to range from 20%–50% [6, 7, 11, 27, 35]. In our study, approximately 35% of patients experienced relapse. Further statistical analysis of patients followed for one year ($N = 36$) showed that CSF-specific oligoclonal bands were positively correlated with a 1-year relapse (CI = 0.527, $p = 0.003$). CSF level of OCB is widely acknowledged as an independent predictor of the risk of a second attack in MS [36]. Given that this is a retrospective data analysis, further investigation, considering demographic and therapeutic factors, is needed to determine whether CSF-specific OCB holds the same predictive value in GFAP-A. We also identified positive associations between CSF WBC count, CSF protein, GFAP-antibody positive in both sample, partial imaging findings, and ΔmRS. This finding may be attributed to the fact that patients with higher white blood cell counts and protein levels presented with more severe acute symptoms, reflected by higher acute mRS scores, which subsequently resulted in a greater reduction in mRS scores during follow-up. It is crucial to conduct longer follow-up periods and larger population studies in order to enhance the confidence level of these results.

Our study has several limitations. The sample, derived from two centers in China, may introduce selection bias. Additionally, the limited follow-up duration may restrict a comprehensive evaluation of disease recurrence. Moreover, GFAP-A is a relatively recent disease entity, with a limited number of cases. While we conducted a retrospective analysis of available patient data, larger sample sizes and prospective studies are needed to provide more valuable insights.

Author Contributions

Conception and design of the study: T.W., F.-D.S. Acquisition, analysis, and interpretation of data: T.W., H.Z., C.G., Q.Y., M.F., L.-J.Z., H.C., Y.W., F.-D.S., T.S. Drafting the manuscript: T.W., H.Z., F.-D.S., T.S. Statistical analysis: T.W., H.Z., H-P.Z., T.S. Review and editing the manuscript: F.-D.S., A.V., F.P., C.B., T.W., T.S. All authors read and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data available on request from the authors.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.