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A Comparative Study of 141 Glial Fibrillary Acidic Protein Immunoglobulin G Positive Cases

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Keywords: anti-GFAP antibody | autoimmune diseases | autoimmune encephalitis | glial fibrillary acidic protein | viral encephalitis

ABSTRACT

Background: Glial fibrillary acidic protein-immunoglobulin G (GFAP-IgG) positivity is associated with autoimmune GFAP astrocytopathy (GFAP-A), but also with other autoimmune encephalitides and viral infections. We attempted to elucidate the characteristics of GFAP-A in relation to other GFAP-IgG-positive encephalitides and constructed a differential diagnosis model.

Methods: 141 GFAP-IgG-positive cases were identified, including 52 astrocytopathy (GFAP-A group), 48 autoimmune encephalitis (AE-G), and 41 viral encephalitis (VE-G). Multivariate logistic regression was employed to create a diagnostic model, with validation using an external cohort.

Result: Compared to the AE-G group, the GFAP-A patients showed more onset age ≥ 50 years, headache, fever, consciousness disturbance, MRI radial vascular enhancement, cerebrospinal fluid (CSF) antibody titer grade ≥ 4, and CSF proteins ≥ 700 mg/L, but less female sex, limb numbness, visual disturbances, and CSF chloride ≤ 120 mmol/L. Among these, CSF antibody titer grade ≥ 4, CSF protein ≥ 700 mg/L, and absence of visual disturbances were independent risk factors for GFAP-A diagnosis. Compared

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to the VE-G group, the GFAP-A patients showed more course ≥14 days, onset age ≥50 years, limb weakness, serum potassium ≤3.9 mmol/L, CSF antibody titer grade ≥4, CSF leukocytes ≤46*10, MRI radial vascular enhancement, MRI involvement of brainstem, and MRI involvement of spinal cord, but less headache, fever, nausea, and vomiting. Among these, serum potassium ≤3.9 mmol/L, MRI spinal cord involvement, and absence of nausea and vomiting were independent risk factors for GFAP-A diagnosis.

Conclusions: Based on critical clinical indicators identified, we constructed a differential diagnosis model for GFAP-A.

1 | Introduction

Autoimmune glial fibrillary acidic protein astrocytopathy (GFAP-A) is a type of autoimmune encephalitis (AE) first defined by the Mayo Clinic in 2016 [1–4]. The presence of glial fibrillary acidic protein immunoglobulin G (GFAP-IgG) in the cerebrospinal fluid (CSF) is an essential diagnostic criterion for GFAP-A. However, the specificity of GFAP-IgG relative to GFAP-A remains to be investigated. Viral encephalitis (VE), anti-N-methyl-d-aspartate receptor (NMDAR) encephalitis, and neuromyelitis optica (NMO) spectrum disorder can be associated with positive GFAP-IgG [5–8], and GFAP-A can be accompanied by the presence of other antibodies, such as aquaporin-4 (AQP-4) IgG, NMDAR-IgG, and myelin oligodendrocyte glycoprotein (MOG)IgG [4, 9, 10], making the diagnosis of GFAP-A challenging.

In this study, we enrolled 406 GFAP-IgG-positive cases across multiple centers. After screening, 141 encephalitis cases were included for observation, which included 41 cases of VE with GFAP-IgG, 48 cases of AE with GFAP-IgG, and 52 cases of GFAP-A. We attempted to elucidate the characteristics of GFAP-A in relation to other GFAP-IgG-positive encephalitis and constructed a differential diagnosis model.

2 | Methods

This study retrospectively identified 406 GFAP-IgG-positive cases from multiple centers until December 31, 2022: 55 cases with only positive serum GFAP-IgG were excluded, 63 with incomplete data were excluded; 45 cases without encephalitis, meningoencephalitis, myelitis, or meningoencephalomyelitis symptoms were excluded, which included symptoms of dizziness ($n=24$), fatigue($n=6$), tinnitus ($n=4$), abdominal pain with distension ($n=3$), trigeminal neuralgia ($n=2$), skin itchiness ($n=2$), transient speech impairment ($n=1$), chest tightness ($n=1$), eye pain ($n=1$) and sore throat ($n=1$); lastly, 62 cases with definitive diagnoses of nonencephalitic diseases, including vascular diseases ($n=21$), neurodegenerative disorders ($n=14$), neoplastic diseases ($n=9$), myasthenia gravis ($n=8$), motor neuron diseases ($n=4$), metabolic encephalopathies ($n=4$) and subacute combined degeneration ($n=2$), were excluded. Based on metagenomics next-generation sequencing results, the remaining 181 encephalitis cases were classified into 51 cases of infectious encephalitis and 130 of AE. Among the 51 cases of infectious encephalitis, VE with multiple antibodies ($n=4$), bacterial/fungal infection ($n=5$) and syphilis infection ($n=1$) were excluded, leaving 41 cases diagnosed as VE with GFAP-IgG (VE-G group). Of the 130 AE cases, 29 cases with rare or non-specific antibodies, including endothelial cell antibody ($n=4$), ANA antibody ($n=4$), SSA/Ro-52/AMA-M2 antibody ($n=3$), neuronal antibody ($n=3$), GM antibody ($n=3$), sulfatide antibody ($n=2$), non-specific intermediate filament antibody ($n=2$), GAD65 antibody ($n=1$), GABAR antibody ($n=1$), neutrophil antibody ($n=1$), Purkinje cell antibody ($n=1$), Musk antibody ($n=1$), GD1a antibody ($n=1$), DPPX antibody ($n=1$), ribosomal P protein antibody ($n=1$), and amphiphysin antibody ($n=1$) were excluded. One case with only serum NMDAR-IgG positivity was excluded [11]. The remaining 100 cases were divided into 52 cases in the GFAP-A group (without other antibodies) and 48 cases in the AE with GFAP-IgG (AE-G) group [including AQP4-IgG ($n=31$), NMDAR-IgG ($n=7$), and MOG-IgG ($n=10$)]. A total of 141 cases were included for analysis, comprising 52 cases of GFAP-A, 48 cases of AE-G, and 41 cases of VE-G (Figure 1).

To validate the efficacy of the diagnostic model, we collected GFAP-IgG-positive cases admitted to the Guangzhou Medical University Affiliated Second Hospital and the 999 Brain Hospital after January 2023 and simultaneously collected an external independent case series from Beijing Xuanwu Hospital for a total of 212 GFAP-IgG-positive cases. We excluded 52 patients with only serum GFAP-IgG positivity, 21 with clear diagnoses, and one with non-specific antibodies. Ultimately, 51, 60, and 27 patients were considered for GFAP-A, AE-G, and VE-G, respectively (Figure 1).

2.1 | Antibody Screening

GFAP-IgG detection using tissue basic assay (TBA) and cell-based assay (CBA) was positive in all cases included in this study. For TBA, antibodies were detected using standard rat or monkey hippocampal and cerebellar tissues, and CSF (1:1) or serum (1:150) diluted in PBS was reacted with tissue sections on glass slides for 2 h. The slides were rinsed twice with PBS, incubated with fluorescein-conjugated IgG for 1 h, rinsed again with PBS, and examined under a microscope. For CBA, GFAPα genes (Supporting Information—S1) were amplified from a human gene cDNA library by polymerase chain reaction and transfected into HEK-293 T cells. Control cells were transfected with an empty vector. Serum and CSF samples were prepared, and CBA was performed using TBA. All cases underwent comprehensive screening for various AE antibodies by CBA; details are in the Supporting Information—S2. The antibody titer grades are classified based on the antibody titers detected in the CSF using the CBA method: Grade 4: titer ≥1:32, Grade 3: 1:32 > titer ≥1:10, Grade 2: 1:10 > titer ≥1:3.2, Grade 1: titer <1:3.2.

The diagnostic criteria for AE are as follows: subacute onset (<3 months) of memory deficits, altered mental status, or

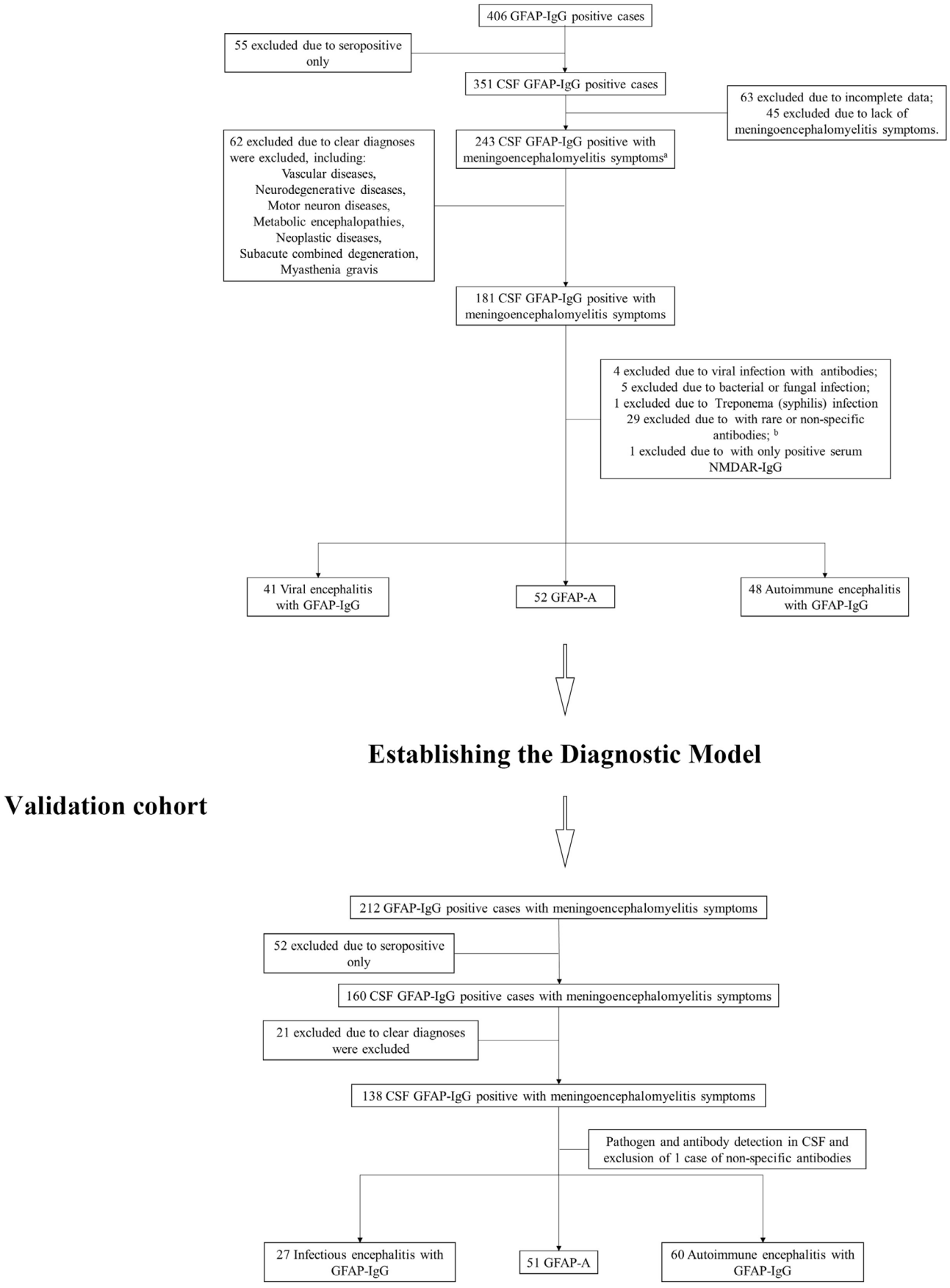

FIGURE 1 | Legend on next page.

FIGURE 1 | Flowsheet of patient identification, inclusion and exclusion. Key: GFAP-IgG, glial fibrillary acidic protein immunoglobulin G; CSF, cerebrospinal fluid; NMO, neuromyelitis optica; NMDAR, N-methyl-d-aspartate receptor; MOG, myelin oligodendrocyte glycoprotein. [a]The diagnostic criteria for autoimmune encephalitis are as follows: First, subacute onset (< 3 months) of memory deficits, altered mental status, or psychiatric symptoms; second, one or more of new focal CNS deficits, unexplained seizures, CSF pleocytosis, or MRI findings suggestive of encephalitis; third, reasonable exclusion of alternative disorders. [b]29 cases with rare or non-specific antibodies:4 endothelial cell antibodies, 2 non-specific intermediate filament antibodies, 1 GAD65 antibody, 1 GABAR antibody, 3 SSA/Ro-52/AMA-M2 antibodies, 1 neutrophil antibody, 1 Purkinje cell antibody, 3 neuronal antibodies, 2 GM antibodies, 2 sulfatide antibodies, 4 ANA antibodies, 1 Musk antibody, 1 GD1a antibody, 1 DPPX antibody, 1 ribosomal P protein antibody, 1 Amphiphysin antibody.

psychiatric symptoms; one or more new focal central nervous system deficits, unexplained seizures, CSF pleocytosis, or magnetic resonance imaging (MRI) findings suggestive of encephalitis; reasonable exclusion of alternative disorders [11]. This study was approved by the Ethics Committee of the Second Affiliated Hospital of Guangzhou Medical University (approval nos. 2019-hs-11 and 2020-hs-54).

2.2 | Statistical Analysis

Continuous variables were expressed as means ± standard deviation or median (interquartile range). Categorical variables were expressed as numbers (%). Comparisons were performed using Student's *t*-test and the Mann–Whitney *U*-test for continuous variables, and the chi-square test or Fisher's exact test for categorical variables. All statistical tests were two-sided. Statistical significance was set at $p < 0.05$. All variables with statistical significance were considered candidates for multivariate logistic regression analyses to develop a diagnostic model, and a regression equation (diagnostic model) was obtained. The regression coefficients of the model were regarded as weights for the respective variables, and the scores for each patient were calculated. The performances of the diagnostic models were evaluated using receiver operating characteristic (ROC) curve analysis. The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio were calculated. Data were analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

3 | Results

3.1 | Comparison and Characterization of the Three Groups of Cases

This study included 141 cases across three groups (Figure 1). Among the pathogens in the VE-G group, Epstein–Barr Virus (EBV) had the highest proportion (51%), followed by herpes simplex virus (HSV)-1 (20%), HSV-4 (12%), HSV-7 (2%), varicella zoster virus (12%), and cytomegalovirus (3%; Figure 2A). In the AE-G group, the highest proportion of combined GFAP-IgG was observed in the case of NMO (64.6%), followed by MOG-IgG-associated encephalitis (20.8%) and anti-NMDAR encephalitis (14.6%; Figure 2B).

Comparing the GFAP-A with the AE-G and VE-G groups, the statistically significant differences in baseline data were female sex (34.62% vs. 68.75%, $p = 0.001$), onset age (49.73 ± 16.28 vs. 39.69 ± 13.84 years, $p = 0.001$) between GFAP-A and AE-G, and onset age (49.73 ± 16.28 vs. 42.05 ± 18.2 years, $p = 0.035$) between GFAP-A and VE-G. The three groups did not differ significantly in terms of preceding infection, modified Rankin Scale score at admission, or death at admission (Table 1).

In terms of clinical symptoms, there were statistically significant differences between the GFAP-A and AE-G groups in the occurrence of headache (53.85% vs. 31.25%, $p = 0.023$), fever (63.46% vs. 27.08%, $p < 0.001$), disturbance of consciousness (26.92% vs. 8.33%, $p = 0.016$), limb numbness (17.31% vs. 39.58%, $p = 0.013$), and visual disturbance (11.54% vs. 33.33%, $p = 0.009$). The GFAP-A and VE-G groups were different in the occurrence of headache (53.85% vs. 85.37%, $p = 0.001$), fever (63.46% vs. 82.93%, $p = 0.038$), nausea and vomiting (15.38% vs. 39.02%, p = 0.01), and limb weakness (53.85% vs. 19.51%, $p = 0.001$; Table 1).

In terms of laboratory tests, the statistically significant differences between GFAP-A and AE-G were: CSF antibody titer grade(4.0 vs. 3.0, median, $p = 0.002$), CSF white blood cell (WBC) (39.0 vs. 10.0×10^6 cells/L, median, $p = 0.015$), CSF protein (870.0 vs. 462.5 mg/L, median, $p < 0.001$), CSF chloride (Cl^-) (120.4 vs. 122.8 mmol/L, median, $p = 0.025$), and MRI with radial vascular enhancement (32.0% vs. 7.89%, $p = 0.006$). Significant differences between GFAP-A and VE-G were: CSF antibody titer grade (4.0 vs. 2.0, median, $p = 0.007$), lumbar puncture (LP) pressure (160.0 vs. 180.0 mmH_2O, median, $p = 0.048$), CSF WBC (39.0 vs. 88.0 × 10 [6], median, $p = 0.008$), serum potassium (K^+) (3.68 vs. 4.03 mmol/L, median, $p = 0.011$), hypokalemia (29.55% vs. 8.11%, $p = 0.016$), involvement of brainstem (26.0% vs. 8.82%, $p = 0.049$), involvement of spinal cord (67.50% vs. 21.43%, $p < 0.001$), encephalomyelitis (50.00% vs. 17.86%, $p = 0.007$), and radial vascular enhancement (32.0% vs. 5.88%, $p = 0.004$; Table 1).

3.2 | Performance of Continuous Indicators for Differentiating GFAP-A From AE-G and VE-G

To screen for indicators that aid in differentiating the diagnosis of GFAP-A, ROC curve analysis was conducted to evaluate the discriminative performance of continuous indicators that showed statistical differences. In the comparison between GFAP-A and AE-G, onset age had an AUC of 0.687 (95% CI, 0.583–0.792), with a cutoff value of 50, showing a sensitivity of 57.7% and specificity of 77.1% (Figure 2C and Table 2).

The CSF antibody titer grade had an AUC of 0.699 (95% CI, 0.576–0.821), with a cutoff value of 4, sensitivity of 64.1%, and specificity of 80.0%. CSF WBC had an AUC of 0.659 (95% CI, 0.539–0.779), with a cutoff value of 6, sensitivity of 70.5%, and specificity of 50.0%. The CSF protein had an AUC of 0.740 (95% CI, 0.639–0.841), with a cutoff value of 700, sensitivity of 62.7%, and specificity of 81.0%. The CSF Cl^- level had an AUC of 0.637

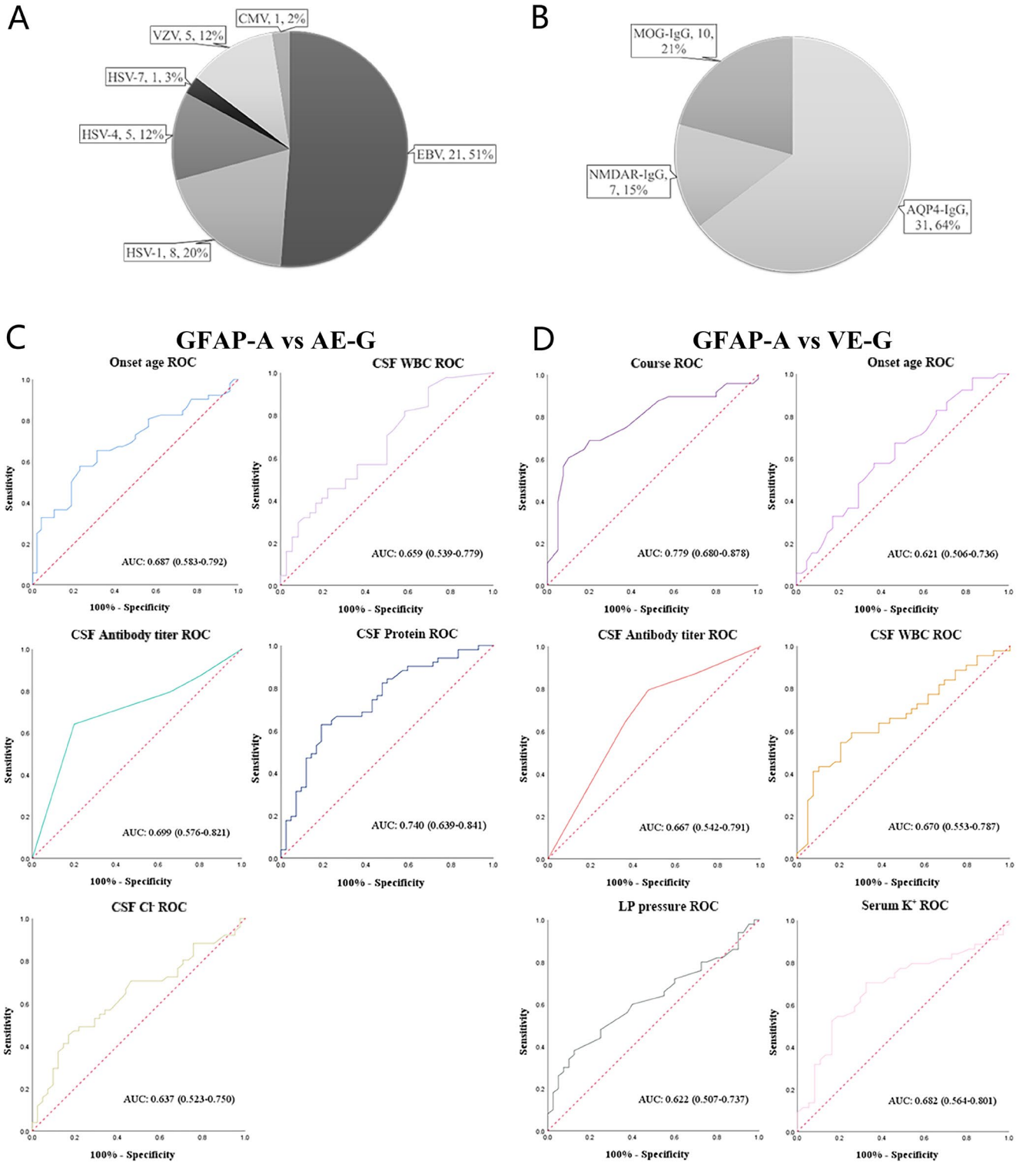

FIGURE 2 | Composition in encephalitis with GFAP-IgG and ROC of continuous indicators. AE-G, autoimmune encephalitis with GFAP-IgG; AQP-4, aquaporin-4; AUC, area under the ROC curve; Cl–, chloride; CMV, Cytomegalovirus; CSF, cerebrospinal fluid; EBV, Epstein–Barr Virus; GFAP-A, autoimmune glial fibrillary acidic protein astrocytopathy; GFAP-IgG, glial fibrillary acidic protein immunoglobulin G; HSV, herpes simplex virus; K+, potassium; LP, Lumbar puncture; MOG, myelin oligodendrocyte glycoprotein; NMDAR, N-methyl-d-aspartate receptor; VE-G, viral encephalitis with GFAP-IgG; VZV, varicella-zoster virus; WBC, white blood cell. (A) Composition in autoimmune encephalitis with GFAP-IgG; (B) Composition in viral encephalitis with GFAP-IgG; (C) ROC curve analysis showing the performance of continuous indicators in distinguishing GFAP-A from AE-G; (D) ROC curve analysis showing the performance of continuous indicators in distinguishing GFAP-A from VE-G.

TABLE 1 | Comparison and characterization of the three groups of cases.

	GFAP-A ($n=52$)	AE-G ($n=48$)	VE-G($n=41$)	P1[a]	P2[b]	P3[c]
Course, days, median (IQR)	30.0 (10.5–60.0)	30.0 (10.0–90.0)	10.0 (7.0–12.0)	**<0.001**	0.624	**<0.001**
Female sex, *N*, %	18.0/52.0, 34.62%	33.0/48.0, 68.75%	12.0/41.0, 29.27%	**<0.001**	**0.001**	0.584
Onset age, years, average ± SD	49.73 ± 16.28	39.69 ± 13.84	42.05 ± 18.2	**0.005**	**0.001**	**0.035**
Preceding infection, *N*, %	16.0/52.0, 30.77%	9.0/48.0, 18.75%	8.0/41.0, 19.51%	0.311	0.624	0.218
Admission mRs median (IQR)	4.0 (3.0–5.0)	3.0 (2.0–5.0)	4.0 (3.0–5.0)	0.102	0.067	0.854
Admission death, *N*, %	5.0/52.0, 9.62%	2.0/48.0, 4.17%	1.0/41.0, 2.44%	0.318	0.5	0.33
Symptom, *N* (%)						
Headache	28.0/52.0, 53.85%	15.0/48.0, 31.25%	35.0/41.0, 85.37%	**<0.001**	**0.023**	**0.001**
Fever	33.0/52.0, 63.46%	13.0/48.0, 27.08%	34.0/41.0, 82.93%	**<0.001**	**<0.001**	**0.038**
Dysarthria	4.0/52.0, 7.69%	5.0/48.0, 10.42%	5.0/41.0, 12.2%	0.778	0.9	0.707
Hiccups	1.0/52.0, 1.92%	3.0/48.0, 6.25%	1.0/41.0, 2.44%	0.526	0.554	1
Nausea and vomiting	8.0/52.0, 15.38%	9.0/48.0, 18.75%	16.0/41.0, 39.02%	**0.02**	0.654	**0.01**
Dysphagia, aspiration	3.0/52.0, 5.77%	2.0/48.0, 4.17%	0.0/41.0, 0.0%	0.44	1	0.331
Seizures	6.0/52.0, 11.54%	9.0/48.0, 18.75%	3.0/41.0, 7.32%	0.285	0.313	0.741
Neuropsychiatric abnormalities	15.0/52.0, 28.85%	11.0/48.0, 22.92%	9.0/41.0, 21.95%	0.734	0.499	0.451
Conscious disturbance	14.0/52.0, 26.92%	4.0/48.0, 8.33%	10.0/41.0, 24.39%	**0.045**	**0.016**	0.782
Involuntary movements	4.0/52.0, 7.69%	6.0/48.0, 12.5%	5.0/41.0, 12.2%	0.7	0.64	0.707
Cognitive impairment	5.0/52.0, 9.62%	2.0/48.0, 4.17%	2.0/41.0, 4.88%	0.523	0.5	0.643
Limb numbness	9.0/52.0, 17.31%	19.0/48.0, 39.58%	1.0/41.0, 2.44%	**<0.001**	**0.013**	0.05
Limb weakness	28.0/52.0, 53.85%	22.0/48.0, 45.83%	8.0/41.0, 19.51%	**0.003**	0.423	**0.001**
Ataxia	5.0/52.0, 9.62%	5.0/48.0, 10.42%	3.0/41.0, 7.32%	0.876	1	0.984
Facial paralysis	1.0/52.0, 1.92%	0.0/48.0, 0.0%	0.0/41.0, 0.0%	1	1	1
Sensory disturbances	8.0/52.0, 15.38%	13.0/48.0, 27.08%	2.0/41.0, 4.88%	**0.017**	0.151	0.198
Sleep disturbances	2.0/52.0, 3.85%	1.0/48.0, 2.08%	0.0/41.0, 0.0%	0.688	1	0.502
Visual disturbances	6.0/52.0, 11.54%	16.0/48.0, 33.33%	2.0/41.0, 4.88%	**0.001**	**0.009**	0.444
Urinary and bowel dysfunction	17.0/52.0, 32.69%	13.0/48.0, 27.08%	13.0/41.0, 31.71%	0.826	0.541	0.92
CSF and blood analysis, median (IQR)						
CSF antibody titer grade[d]	4.0 (3.0–4.0)	3.0 (2.0–3.0)	2.0 (1.0–4.0)	**0.004**	**0.002**	**0.007**
LP pressure, mmH_2O	160.0 (120.0–200.0)	150.0 (120.0–180.0)	180.0 (155.0–217.5)	**0.023**	0.521	**0.048**
CSF WBC, $*10^6$	39.0 (5.0–131.5)	10.0 (1.0–49.0)	88.0 (54.0–220.5)	**<0.001**	**0.015**	**0.008**
CSF protein, mg/L	870.0 (516.5–1531.3)	462.5 (347.0–670.0)	1121.4 (896.26–1464.3)	**<0.001**	**<0.001**	0.082
CSF Cl^-, mmol/L	120.4 (115.15–124.45)	122.8 (119.8–125.1)	119.85 (115.75–124.15)	**0.042**	**0.025**	0.57
CSF glucose, mmol/L	3.0 (2.4–4.1)	3.27 (2.98–3.88)	2.93 (2.4–3.29)	**0.026**	0.143	0.336
Hemoglobin, g/L	131.0 (118.0–138.0)	132.0 (124.5–143.0)	130.0 (116.0–136.0)	0.268	0.183	0.873

(Continues)

TABLE 1 | (Continued)

	GFAP-A ($n=52$)	AE-G ($n=48$)	VE-G($n=41$)	P1[a]	P2[b]	P3[c]
Blood glucose, mmol/L	4.89 (4.36–7.06)	5.15 (4.52–6.33)	5.6 (5.07–6.48)	0.214	0.474	0.083
Serum Na^+, mmol/L	138.0 (134.0–140.8)	139.0 (136.9–142.4)	136.4 (133.0–139.6)	**0.035**	0.101	0.271
Serum K^+, mmol/L	3.68 (3.48–4.0)	3.88 (3.65–4.16)	4.03 (3.71–4.19)	**0.015**	0.126	**0.011**
Hyponatremia[e], *N*, %	13.0/44.0, 29.55%	7.0/43.0, 16.28%	15.0/37.0, 40.54%	0.057	0.141	0.3
Hypokalemia[f], *N*, %	13.0/44.0, 29.55%	8.0/42.0, 19.05%	3.0/37.0, 8.11%	0.05	0.257	**0.016**
C-reactive protein, mg/L	2.05 (1.02–10.15)	1.22 (0.4–4.9)	2.39 (1.26–3.72)	0.229	0.116	0.93
Abnormal MRI, *N*, %						
Involvement of brain parenchyma	35.0/50.0, 70.00%	27.0/38.0, 71.05%	23.0/34.0, 67.65%	0.969	0.915	0.819
Involvement of brainstem	13.0/50.0, 26.00%	11.0/38.0, 28.95%	3.0/34.0, 8.82%	0.088	0.758	**0.049**
Involvement of meninges	12.0/49.0, 24.49%	5.0/38.0, 13.16%	14.0/34.0, 41.18%	**0.026**	0.202	0.107
Involvement of spinal cord	27.0/40.0, 67.50%	22.0/29.0, 75.86%	6.0/28.0, 21.43%	**<0.001**	0.45	**<0.001**
Encephalomyelitis	20.0/40.0, 50.00%	14.0/29.0, 48.28%	5.0/28.0, 17.86%	**0.018**	0.888	**0.007**
Radial vascular enhancement	16.0/50.0, 32.00%	3.0/38.0，7.89%	2.0/34.0，5.88%	**0.001**	**0.006**	**0.004**

Note: The bolded numbers in the table indicate a *p*-value less than 0.05.

Abbreviations: AE-G, autoimmune encephalitis with GFAP-IgG; Cl^-, chloride; CSF, cerebrospinal fluid; GFAP-A, autoimmune glial fibrillary acidic protein astrocytopathy; GFAP-IgG, glial fibrillary acidic protein immunoglobulin G; IQR, interquartile range; K^+, potassium; LP, Lumbar puncture; MRI, magnetic resonance imaging; mRS, Modified Rankin Scale; Na^+, sodium; SD, standard deviation; VE-G, viral encephalitis with GFAP-IgG; WBC, white blood cell.

[a]P1: *p*-value for comparison of the three groups.

[b]P2: *p*-value for comparison of GFAP-A and AE-G.

[c]P3: *p*-value for comparison of GFAP-A and VE-G.

[d]Antibody titer grading: Grade 4: titer ≥1:32, Grade 3: 1:32> titer ≥1:10, Grade 2: 1:10> titer ≥1:3.2, Grade 1: titer <1:3.2.

[e]Hyponatraemia is defined as serum sodium <136 mmol/L.

[f]Hypokalemia is defined as serum potassium <3.5 mmol/L.

(95% CI, 0.523–0.750), with a cutoff value of 120, sensitivity of 52.9%, and specificity of 70.7% (Figure 2C and Table 2).

In the differentiation between GFAP-A and VE-G, course had an AUC of 0.779 (95% CI, 0.680–0.878), with a cutoff value of 14, sensitivity of 68.8% and specificity of 80.0%. Onset age had an AUC of 0.621 (95% CI, 0.506–0.736) with a cutoff value of 50, sensitivity of 57.7% and specificity of 63.4%. The CSF antibody titer grade had an AUC of 0.667 (95% CI, 0.542–0.791), with a cutoff value of 4, sensitivity of 64.1% and specificity of 63.9%. LP pressure had an AUC of 0.622 (95% CI, 0.507–0.737), with a cutoff value of 167, sensitivity of 60.0% and specificity of 60.0%. The CSF WBC count had an AUC of 0.670 (95% CI, 0.553–0.787), with a cutoff value of 46, sensitivity of 54.5% and specificity of 79.5%. Serum K^+ levels had an AUC of 0.682 (95% CI, 0.564–0.801), with a cutoff value of 3.9, sensitivity of 70.5% and specificity of 67.6% (Figure 2D and Table 2).

After grouping according to the cutoff values, the indicators with statistical differences between GFAP-A and AE-G were: onset age ≥50 years, CSF antibody titer grade ≥4, CSF WBC ≥6×10 ^ 6[6] cells/L, CSF protein ≥700 mg/L, and CSF Cl^- ≤120 mmol/L. Statistical differences in the comparison between GFAP-A and VE-G were: Course ≥14 days, onset age ≥50 years, CSF antibody titer grade ≥4, CSF WBC ≤46×10 [6], and serum K^+ ≤3.9 mmol/L (Table 3).

3.3 | Establishing the Diagnostic Model in Differentiating GFAP-A From AE-G and VE-G

Further logistic regression analysis of the differential indicators showed that in the comparison between GFAP-A and AE-G, the independent risk factors for GFAP-A were CSF antibody titer grade ≥4, CSF protein ≥700 mg/L, and absence of visual disturbances. Comparison of GFAP-A and VE-G indicated that independent risk factors for GFAP-A were serum K^+ ≤3.9 mmol/L, involvement of the spinal cord, and absence of nausea and vomiting. The regression coefficients (*B* values) of these independent risk factors were used as weights to construct differential diagnostic models (Table 4). For the differentiation between GFAP-A and AE-G, the GFAP-A diagnosis model = (CSF antibody titer grade ≥4×2) + (CSF protein ≥700 mg/L×3)—(visual disturbances ×2). ROC curve analysis demonstrated that the model had an AUC of 0.879 (95% CI, 0.799–0.959), with a cutoff value of 2, sensitivity of 89.7%, and specificity of 75.8% (Figure 3A and Table 5). For the differentiation between GFAP-A and VE-G: GFAP-A diagnosis model = (serum K^+ ≤3.9 mmol/L×3) + (involvement of spinal cord ×3)—(nausea and vomiting ×4). ROC curve analysis showed that the model had an AUC of 0.875 (95% CI, 0.781–0.969), with a cutoff value of 3, sensitivity of 82.4%, and specificity of 80.8% (Figure 3A and Table 5).

TABLE 2 | The performance of continuous indicators for differentiating GFAP-A from AE-G and VE-G.

Indicators	AUC (95% CI)	Cutoff value	Sensitivity	Specificity	PPV	NPV	PLR	NLR
GFAP-A versus AE-G								
Onset age, years	0.687 (0.583–0.792)	50	0.577	0.771	0.731	0.627	2.520	0.549
CSF antibody titer	0.699 (0.576–0.821)	4	0.641	0.800	0.781	0.667	3.205	0.449
CSF WBC, $*10^6$	0.659 (0.539–0.779)	6	0.705	0.500	0.627	0.586	1.410	0.590
CSF protein, mg/L	0.740 (0.639–0.841)	700	0.627	0.810	0.805	0.654	3.300	0.460
CSF Cl^-, mmol/L	0.637 (0.523–0.750)	120	0.529	0.707	0.694	0.536	1.805	0.666
GFAP-A versus VE-G								
Course, days	0.779 (0.680–0.878)	14	0.688	0.800	0.805	0.696	3.440	0.390
Onset age, years	0.621 (0.506–0.736)	50	0.577	0.634	0.667	0.542	1.577	0.667
CSF antibody titer	0.667 (0.542–0.791)	4	0.641	0.639	0.658	0.611	1.776	0.562
LP pressure, mmH_2O	0.622 (0.507–0.737)	167	0.600	0.600	0.652	0.545	1.500	0.667
CSF WBC $*10^6$	0.670 (0.553–0.787)	46	0.545	0.795	0.750	0.608	2.659	0.572
Serum K^+, mmol/L	0.682 (0.564–0.801)	3.9	0.705	0.676	0.727	0.676	2.176	0.436

Note: The bolded numbers in the table indicate a *p*-value less than 0.05.

Abbreviations: AE-G, autoimmune encephalitis with GFAP-IgG; AUC, area under the ROC curve; CI, confidence interval; Cl–, chloride; CSF, cerebrospinal fluid; GFAP-A, autoimmune glial fibrillary acidic protein astrocytopathy; GFAP-IgG, glial fibrillary acidic protein immunoglobulin G; K+, potassium; LP, Lumbar puncture; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value; VE-G, viral encephalitis with GFAP-IgG; WBC, white blood cell.

TABLE 3 | Differences in GFAP-A versus AE-G/VE-G based on grouped indicators.

GFAP-A versus AE-G	GFAP-A ($n=52$)	AE-G ($n=48$)	*p*
Onset age ≥ 50 years	30.0/52.0, 57.7%	11.0/48.0, 22.9%	**< 0.001**
CSF antibody titer grade ≥ 4	25.0/39.0, 64.1%	7.0/35.0, 20.0%	**< 0.001**
CSF WBC ≥ $6*10^6$	32.0/44.0, 72.73%	19.0/36.0, 52.78%	0.065
CSF protein ≥ 700 mg/L	33.0/51.0, 64.71%	8.0/42.0, 19.05%	**< 0.001**
CSF Cl^- ≤ 120 mmol/L	25.0/51.0, 49.02%	11.0/41.0, 26.83%	**0.03**
GFAP-A versus VE-G	**GFAP-A ($n=52$)**	**VE-G ($n=41$)**	***p***
Course ≥ 14 days	33.0/47.0, 70.2%	8.0/40.0, 20.0%	**< 0.001**
Onset age ≥ 50 years	30.0/52.0, 57.7%	15.0/41.0, 36.6%	**0.043**
CSF antibody titer grade ≥ 4	25.0/39.0, 64.1%	13.0/35.0, 37.14%	**0.021**
LP pressure ≤ 167 mmH_2O	30/50, 60.0%	16/40, 40.0%	0.059
CSF WBC ≤ $46*10^6$	24/44, 54.55%	8/39, 20.51%	**0.001**
Serum K^+ ≤ 3.9 mmol/L	32.0/44.0, 72.7%	12.0/37.0, 32.4%	**< 0.001**

Note: The bolded numbers in the table indicate a *p*-value less than 0.05.

Abbreviations: AE-G, autoimmune encephalitis with GFAP-IgG; Cl–, chloride; CSF, cerebrospinal fluid; GFAP-A, autoimmune glial fibrillary acidic protein astrocytopathy; GFAP-IgG, glial fibrillary acidic protein immunoglobulin G; K+, potassium; VE-G, viral encephalitis with GFAP-IgG; WBC, white blood cell.

3.4 | Validation of Established Diagnostic Model by an Independent Cohort

These two diagnostic models were validated using an independent cohort of 138 GFAP-IgG-positive cases. ROC curve analysis for differentiating GFAP-A from AE-G yielded an AUC of 0.800 (95% CI, 0.717–0.883), with a cutoff value of 2, sensitivity of 76.9%, and specificity of 69.0%. ROC curve analysis for differentiating GFAP-A from VE-G yielded an AUC of 0.659 (95% CI, 0.534–0.785), and with a cutoff value of 1, sensitivity of 60.4%, and specificity of 66.7% (Figure 3B and Table 5).

TABLE 4 | Logistic regression results and indices of the scoring system for the diagnosis of GFAP-A versus AE-G and VE-G.

	β-coefficient	Standard error (SE)	Odds ratio (95% CI)	*p*	Score
GFAP-A versus AE-G					
CSF antibody titer grade ≥ 4	2.297	0.714	9.945 (2.456–40.269)	0.005	2
CSF protein ≥ 700 mg/L	2.555	0.709	12.871 (3.208–51.636)	0.002	3
Visual disturbances	−2.177	0.993	0.113 (0.016–0.794)	0.016	−2
GFAP-A versus VE-G					
Serum K^+ ≤ 3.9 mmol/L	2.763	1.000	15.852 (2.234–112.495)	0.006	3
Involvement of spinal cord	3.275	1.221	26.439 (2.414–289.625)	0.007	3
Nausea and vomiting	−3.896	1.532	0.020 (0.001–0.409)	0.011	−4

Abbreviations: AE-G, autoimmune encephalitis with GFAP-IgG; CI, confidence interval; CSF, cerebrospinal fluid; GFAP-A, autoimmune glial fibrillary acidic protein astrocytopathy; GFAP-IgG, glial fibrillary acidic protein immunoglobulin G; K+, potassium; VE-G, viral encephalitis with GFAP-IgG.

4 | Discussion

This study enrolled 141 GFAP-IgG-positive cases in China. Among the 48 AE-G cases, the positivity rates for AQP4-IgG, MOG-IgG, and NMDAR-IgG were 31:10:7. Globally, the incidence ratio of NMO, MOG-IgG encephalitis, and anti-NMDAR encephalitis is approximately 3:2:1 [12–14]. The probability of NMO accompanied by GFAP-IgG is close to that of anti-NMDAR encephalitis, whereas the probability of MOG-IgG encephalitis accompanied by GFAP-IgG is the lowest. However, the current incidence in China remains unclear and requires further investigation. EBV accounted for 51% of the 41 VE-G cases, suggesting that EBV infection more easily induced the production of GFAP-IgG, as previously indicated [15]. Our previous study has proven the close relationship between EBV and GFAP-A [6]. Furthermore, the combined proportion of HSV-1 (20%), HSV-4 (12%), and HSV-7 (2%) was 34% in this study. HSV is considered a triggering factor for AE [16], especially anti-NMDAR encephalitis; however, only one study has reported on the association between HSV and GFAP-A [9]. The relationship between HSV infection and GFAP IgG warrants further study.

The most prominent feature of GFAP-A, compared to AE-G, was a higher CSF GFAP-IgG titer and CSF protein content, with fewer visual disturbance symptoms. In autoimmune diseases, specific antibodies are often an essential diagnostic criterion, but GFAP-IgG can occur in various diseases. We previously reported that CD8+ T cells are critical immune cells in patients with GFAP-A [17], and GFAP-specific CD8+ T cells have been shown to induce multiple sclerosis-like encephalitis [18]. Therefore, we believe that the high titer of GFAP-IgG suggests that the immune response is specific to GFAP. Herein, patients with GFAP-A exhibited significantly higher CSF protein levels than those with AE-G. The median CSF protein level of GFAP-A was 870.0 mg/L, consistent with previous findings [4]. The median CSF protein in NMO is approximately 763 mg/L [19], and 646.5 mg/L [20] and 320 mg/L [21] in MOG-IgG encephalitis and anti-NMDAR encephalitis, respectively. Furthermore, the proportion of patients with CSF protein levels of > 600 mg/L was 3/25 for NMO and 3/33 for MOG-IgG encephalitis [22], whereas, for anti-NMDAR encephalitis, the proportion of patients with CSF protein levels of > 500 mg/L was 8/43 [21]. These findings demonstrate that the CSF protein content in GFAP-A is higher than that in AE-G, and a cutoff value of 700 mg/L can serve as an essential indicator for differentiating GFAP-A from AE-G.

Visual disturbance symptoms are important differential features between GFAP-A and AE-G. Visual symptoms are one of the most common manifestations of NMO and MOG-IgG encephalitis [23, 24] and are also common in anti-NMDAR encephalitis [25]. Early studies generally considered visual disturbances or optic neuritis as common symptoms of GFAP-A [1, 4], whereas a meta-analysis reported that the proportion of patients having GFAP-A with visual symptoms was approximately 25% (149/592) [26], significantly higher than the 11.54% reported in this study. This discrepancy may be due to our exclusion of patients with overlapping antibodies, whereas previous studies might have included cases of NMO or MOG-IgG encephalitis co-occurring with GFAP-IgG. Although GFAP-A is often considered similar to NMO and MOG-IgG encephalitis, it has fewer symptoms of focal lesions, especially visual disturbances.

The main differential points between GFAP-A and VE-G include the involvement of the spinal cord, serum K^+ ≤ 3.9 mmol/L, and fewer symptoms of nausea and vomiting. In previous studies, virus-induced myelitis was common, with HSV-1 being the most common infectious myelitis [27]. However, in this study, the VE-G showed a lower rate of spinal cord involvement (21.43%). We speculated that more concentrated brain inflammation was more likely to induce the production of GFAP-IgG; therefore, in VE-G, the involvement of the spinal cord is less common. Furthermore, a survey found that the ratio of encephalitis, meningitis, and encephalomyelitis in VE was close to 1.0:2.0:0.5 [28], which may explain why the VE-G showed higher rates of nausea and vomiting but had less spinal cord involvement. The median serum K^+ levels in all three groups were within the normal range (3.5–5.5 mmol/L). However, serum K^+ levels in the GFAP-A group were significantly lower than those in the VE-G group, and the proportion of patients with hypokalemia was higher in the GFAP-A group. Clinically, VE-G patients with more severe nausea and vomiting would theoretically be more prone to hypokalemia. However, our findings showed the opposite trend, suggesting that GFAP-A pathology may contribute more significantly to lower blood potassium levels.

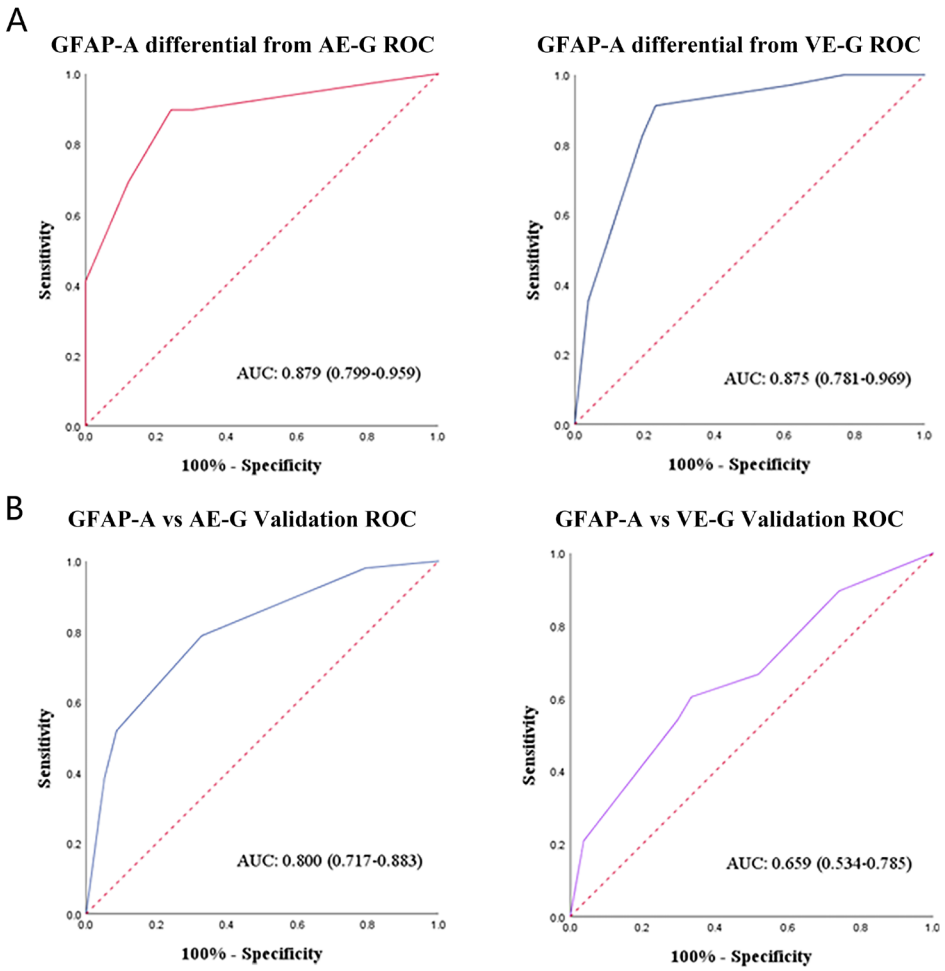

FIGURE 3 | ROC of diagnostic model for Differentiating GFAP-A from AE-G and VE-G. GFAP-A, autoimmune glial fibrillary acidic protein astrocytopathy; GFAP-IgG, glial fibrillary acidic protein immunoglobulin G; AE-G, autoimmune encephalitis with GFAP-IgG; VE-G, viral encephalitis with GFAP-IgG; AUC, area under the ROC curve. (A) ROC curve analysis showing the performance of diagnostic model in distinguishing GFAP-A from AE-G and VE-G in training cohort. (B) ROC curve analysis showing the performance of diagnostic model in distinguishing GFAP-A from AE-G and VE-G in validation cohort.

TABLE 5 | The performance of the diagnostic model for discriminating GFAP-A from AE-G and VE-G.

	AUC (95% CI)	Cutoff value	Sensitivity	Specificity	PPV	NPV	PLR	NLR
GFAP-A versus AE-G								
Training cohort	0.879 (0.799–0.959)	2	0.897	0.758	0.814	0.862	3.707	0.136
Validation cohort	0.800 (0.717–0.883)	2	0.769	0.690	0.684	0.778	2.481	0.335
GFAP-A versus VE-G								
Training cohort	0.875 (0.781–0.969)	3	0.824	0.808	0.848	0.778	4.292	0.218
Validation cohort	0.659 (0.534–0.785)	1	0.604	0.667	0.763	0.500	1.814	0.594

Abbreviations: AE-G, autoimmune encephalitis with GFAP-IgG; AUC, area under the ROC curve; CI, confidence interval; GFAP-A, autoimmune glial fibrillary acidic protein astrocytopathy; GFAP-IgG, glial fibrillary acidic protein immunoglobulin G; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value; VE-G, viral encephalitis with GFAP-IgG.

In the development of a diagnostic formula for differentiation between GFAP-A and VE-G, the titer grade of GFAP-IgG was not included. This may be attributed to the fact that, although the median GFAP-IgG titer grade in the VE-G group was 2 (range: 1–4), a significant proportion (37.14%) of cases exhibited a titer grade of ≥4, suggesting a bimodal distribution of GFAP-IgG

titer grade in VE-G. The underlying cause may be related to the type of viral infection. For instance, EBV infection may induce a higher titer of GFAP-IgG, similar to our previous study that demonstrated a correlation between EBV infection and GFAP-A [6]. In this study, we found that patients infected with EBV with antibody titer grade of 4 were 43.86%, which was greater than the 11.11% for HSV.

Unlike general AE, GFAP-IgG is not exclusively present in GFAP-A. In this study, GFAP-IgG expression is detected in numerous cases of AE, VE, and even those excluded from the study. Previous research has also identified GFAP-IgG as a potential biomarker for brain injury, with its titer being associated with the prognosis of brain injury [29, 30]. Therefore, the presence of GFAP-IgG cannot serve as the sole diagnostic criterion for GFAP-A. Upon analyzing the results of this study, we posit that utilizing the titer of GFAP-IgG as a differential diagnostic indicator for GFAP-A is a more rational approach.

In prior encephalitis research, the titer of NMDAR-IgG has been established to be positively correlated with the prognosis of anti-NMDAR encephalitis and the probability of concurrent teratoma [31]. This correlation may stem from the fact that teratomas induce cross-immune specificity targeting NMDAR, resulting in a higher CSF NMDAR-IgG titer in cases with teratomas. Similarly, a high titer of GFAP-IgG may indicate the specificity of the immune response to GFAP. Furthermore, research has proposed that a lower CSF NMDAR-IgG titer leading to a negative NMDAR-IgG CSF/serum antibody-specific index is one of the indicators for excluding anti-NMDAR antibody encephalitis [32]. Likewise, a low titer of GFAP-IgG may represent a byproduct of brain injury, although it is imperative to acknowledge that the titer of GFAP-IgG is influenced by a multitude of factors. Consequently, further standardized and in-depth research is warranted to elucidate GFAP-IgG's precise diagnostic role.

In addition, imaging is also an important reference for diagnosis. Previous studies reported that radial vascular enhancement on brain MRI is an important feature of GFAP-A [1, 4]. In this study, we verified this phenomenon through comparison to the other two groups, revealing that radial vascular enhancement on brain MRI had a sensitivity of 0.32 and a specificity of 0.93 in diagnosing GFAP-A in 141 cases. However, this feature was ultimately not included as an independent risk factor for GFAP-A. We attempted to incorporate MRI features into our diagnostic model (Figure S1, Tables 1 and 2), and found that it can improve the diagnostic value of the model, suggesting this observed MRI enhancement is an important reference standard for diagnosing GFAP-A.

This study has certain limitations; NMO and MOG-IgG encephalitis comprised a larger proportion in the AE-G group, which may have introduced a comparison bias. The incidence of these two diseases is higher in China, and they are more likely to be associated with GFAP-IgG positivity. However, it is precisely because of their high prevalence and propensity to co-occur with GFAP-IgG that studying the differences between GFAP-A and these conditions becomes more valuable. External validation showed that the discriminatory power of the VE-G diagnostic model was not satisfactory. This may be partly due to the similarity between GFAP-A and VE-G and that GFAP-A can occur concurrently with VE-G in clinical settings. Finally, this study focused solely on the clinical presentation and examination findings without describing the treatment and follow-up of these patients. These aspects should be addressed in future studies.

We identified the clinical characteristics of GFAP-A compared with those of AE-G and VE-G via comparative analysis of 141 cases positive for GFAP-IgG and constructed a differential diagnostic model that integrated clinical data to aid in the differential diagnosis of GFAP-A.

Author Contributions

Shifeng Zhang: conceptualization, methodology, data curation, writing – original draft. **Huilu Li:** writing – original draft, validation, methodology. **Peihao Lin:** investigation. **Ding Liu:** investigation. **Zhanhang Wang:** investigation. **Jie Yang:** investigation. **Shishuang Ruan:** investigation. **Yinan Zhao:** investigation. **Zhike Lan:** investigation. **Xiao Yang:** investigation. **Yue Wang:** investigation. **Yong You:** investigation. **Xiuling Wu:** investigation. **Haiyang Wang:** investigation. **Hongli Liu:** investigation. **Huan Yang:** investigation. **Huiyu Feng:** investigation. **Lu Zhang:** investigation. **Houshi Zhou:** investigation. **Qianhui Xu:** investigation. **Tengfei Ou:** investigation. **Yuhua Lu:** investigation. **Cong Gao:** investigation. **Wei Qiu:** investigation. **Junwei Hao:** conceptualization, writing – review and editing. **Youming Long:** conceptualization, funding acquisition, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data used for this manuscript are available upon reasonable request.

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Supporting Information

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