Am J Transl Res 2025;17(10):7980-7990
www.ajtr.org /ISSN:1943-8141/AJTR0165180

Original Article

Cerebrospinal fluid biomarkers for predicting immunotherapy response in autoimmune encephalitis

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Received April 9, 2025; Accepted September 15, 2025; Epub October 15, 2025; Published October 30, 2025

Abstract: Objectives: This study aimed to investigate the predictive value of cerebrospinal fluid (CSF) biomarkers for immunotherapy response in patients with autoimmune encephalitis (AE). Methods: We conducted a retrospective analysis of 80 patients with AE who were hospitalized at Zhongshan Hospital of Xiamen University between January 2014 and July 2024. Treatment response was evaluated using a combined assessment of the modified Rankin Scale (mRS) and the Clinical Assessment Scale in Autoimmune Encephalitis (CASE) scores. Patients were classified into good response (n = 52, 65.0%) and poor response (n = 28, 35.0%) groups according to predefined criteria. Feature selection was performed using the Boruta algorithm and least absolute shrinkage and selection operator regression, followed by multivariable logistic regression to identify independent predictors. Diagnostic performance was evaluated using receiver operating characteristic curves and area under the curve (AUC) analysis. Results: Following systematic feature selection and multicollinearity assessment, three independent predictors of poor immunotherapy response were identified: intrathecal immunoglobulin G (IgG) synthesis rate (OR = 1.57, 95% CI: 1.03-2.45, P = 0.037), peak CASE score (OR = 1.22, 95% CI: 1.07-1.42, P = 0.004), and CSF albumin concentration (OR = 1.00, 95% CI: 1.00-1.01, P = 0.005). Among individual biomarkers, intrathecal IgG synthesis rate demonstrated the highest discriminatory ability (AUC = 0.734), followed by CSF albumin (AUC = 0.722). The combined predictive model showed significantly superior diagnostic performance compared to any single biomarker (AUC = 0.896). Conclusions: Elevated intrathecal IgG synthesis, peak CASE score, and CSF albumin concentration are independent predictors of poor response in patients with AE.

Keywords: Autoimmune encephalitis, immunotherapy response, cerebrospinal fluid biomarkers, clinical assessment scale in autoimmune encephalitis, intrathecal IgG synthesis

Introduction

Autoimmune encephalitis (AE) encompasses a group of central nervous system inflammatory disorders mediated by autoantibodies directed against neuronal surface antigens or synaptic proteins [1]. With continuous advances in antibody detection technologies and deepening clinical understanding, the recognition rate of AE has shown a sustained upward trend [2]. Currently, standard treatment strategies for AE employ a stepwise immunotherapy approach: first-line therapies include corticosteroids, intravenous immunoglobulin (IVIG), and plasma exchange; second-line treatments encompass immunosuppressive agents such as rituximab, cyclophosphamide, and azathioprine [3, 4]. Although timely immunotherapy can significantly improve clinical outcome in most patients, a substantial proportion continue to face poor treatment response, functional disability, or even death [5]. Furthermore, individual variability in immunotherapy response is significant, and the dynamic evolution of symptoms during disease progression adds complexity to prognostic assessment [6]. Therefore, identifying reliable predictors of treatment response holds significant clinical value for optimizing individualized therapeutic strategies and improving patient outcome.

Traditionally, the modified Rankin Scale (mRS) has been widely used to assess functional status in patients with AE. However, the mRS pri-

https://doi.org/10.62347/OVOU1056

marily focuses on motor function assessment and fails to comprehensively capture the core symptoms characteristic of AE patients, including cognitive dysfunction, psychiatric behavioral abnormalities, and seizures. To address this limitation, Lim and colleagues developed the Clinical Assessment Scale in Autoimmune Encephalitis (CASE) in 2019, which encompasses nine neurological domains and provides more precise quantification of disease severity and treatment response in AE, offering a more sensitive and comprehensive tool for clinical evaluation [7].

Cerebrospinal fluid (CSF) biomarkers play a crucial role in the diagnosis and prognostic assessment of AE [8]. CSF cytology, protein concentrations, and immunoglobulin indices are closely associated with disease severity and treatment response. The albumin quotient (QAlb), as a sensitive indicator of blood-brain barrier (BBB) integrity, often suggests compromised barrier function when abnormally elevated, while intrathecal immunoglobulin G (IgG) synthesis reflects the intensity of local immune responses within the central nervous system. However, despite the value these indicators show in reflecting disease severity and predicting treatment outcome, systematic studies examining them as predictors of immunotherapy response remain lacking.

Based on this background, the present study aimed to integrate the CASE and mRS scoring systems and employ machine learning algorithms to identify clinical and CSF biomarker predictors of immunotherapy response in patients with AE. We hypothesized that CSF biomarkers could predict treatment response in the early treatment phase, thereby providing objective evidence for early risk stratification and individualized treatment decision-making.

Materials and methods

Study design and participants

This was a single-center retrospective cohort study that consecutively enrolled patients with AE hospitalized at Zhongshan Hospital of Xiamen University, from January 1, 2014, to July 1, 2024. The study included 80 patients meeting inclusion criteria for AE, comprising two subgroups: antibody-positive AE and antibody-negative probable AE. This study adhered to the ethical principles of the Declaration of Helsinki and received approval from the institutional medical ethics committee (approval number: xmzsyyky2024-097). Given the retrospective nature of the study and anonymized data processing, the ethics committee waived the requirement for written informed consent.

The diagnostic criteria for antibody-positive AE were based on the detection of specific autoantibodies against neuronal surface antigens or synaptic proteins in serum and/or CSF. The diagnostic criteria for antibody-negative probable AE followed the clinical diagnostic criteria proposed by Graus et al. in 2016 [9]. Patients were required to meet all of the following conditions simultaneously: (1) Age ≥ 14 years; (2) Acute or subacute onset with symptom progression < 3 months; (3) Clinical presentation including one or more of the following symptoms: working memory deficits, altered consciousness, psychiatric behavioral abnormalities, new-onset seizures, speech dysfunction, movement disorders, gait abnormalities, or limb weakness; (4) Meeting at least one of the following auxiliary examination abnormalities: seizures that cannot be explained by previous epilepsy history; CSF white cell count > 5 cells/mm^3; CSF-specific oligoclonal bands positive or IgG index > 0.85; abnormally elevated protein concentration; magnetic resonance imaging (MRI) showing abnormal signals consistent with AE characteristics.

Exclusion criteria included: (1) Laboratory-confirmed infectious encephalitis; (2) Pre-existing toxic/metabolic encephalopathy, brain tumors, vitamin deficiency syndromes, alcohol-related encephalopathy, or primary epilepsy diagnosed before AE onset; (3) Primary psychiatric disorders; (4) Other specific immune-mediated encephalitis or encephalopathy; (5) Patients with incomplete clinical data or who did not complete serum and CSF antibody testing.

Data collection

All clinical data were systematically collected through the hospital's electronic medical record system. Baseline characteristics included demographic features (age at symptom onset, sex), clinical comorbidities (autoimmune diseases, active malignancy), antibody status, and clinical manifestations (prodromal symptoms, altered consciousness, seizures, psychiatric

Table 1. Computational formulas for inflammatory markers and blood-brain barrier function data

Biomarker	Formula
Neutrophil-to-lymphocyte ratio (NLR)	Neutrophil count ÷ Lymphocyte count
Monocyte-to-lymphocyte ratio (MLR)	Monocyte count ÷ Lymphocyte count
Neutrophil percentage-to-albumin ratio (NPAR)	Neutrophil percentage ÷ Serum albumin
Albumin quotient (QAlb)	(CSF albumin ÷ Serum albumin) × 1000
Intrathecal IgG synthesis	$\{Q_{IgG} - 0.8 \times [Q_{Alb}^2 + (15 \times 10^{-6})]^{0.5} + 1.8 \times 10^{-3}\} \times IgG_{Serum}$

Unit standardization: All calculations performed using standardized units. CSF albumin and IgG concentrations in mg/L; serum albumin and IgG concentrations in g/L. Unit conversions: CSF data (mg/L) ÷ 10 = mg/dL; serum parameters (g/L) × 100 = mg/dL. Age-specific albumin quotient thresholds: QAlb normal ranges: < 40 years: < 6.5×10^{-3}; 40-60 years: < 8.0×10^{-3}; > 60 years: < 9.3×10^{-3}. Abbreviations: CSF, cerebrospinal fluid; IgG, immunoglobulin G.

symptoms, cognitive impairment, memory dysfunction, movement disorders, and gait abnormalities and ataxia). We also collected brain MRI findings (limbic inflammation, other encephalitis patterns) and EEG examination results (pathologic EEG changes, focal or generalized slow waves, or epileptiform discharges). Treatment information included the use of first-line immunotherapy agents (IVIG, corticosteroids, plasma exchange) and combination therapy regimens. We collected complete blood count results from fasting venous blood samples obtained within 24 hours of admission in the morning (white blood cells, neutrophils, monocytes, and neutrophil percentage). Serum chemistry data were also collected, including serum protein concentration, albumin, and serum immunoglobulin levels. All patients underwent lumbar puncture as early as possible after admission, and we collected CSF pressure, routine CSF analysis, chemistry data, and quantitative immunoglobulin detection results.

Additionally, we calculated a series of composite inflammatory markers, including neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), and neutrophil percentage-to-albumin ratio (NPAR). Blood-brain barrier function was assessed using quantitative analysis of QAlb. Evaluation of central nervous system immune responses was achieved by calculating the intrathecal IgG synthesis, with the calculation formula shown in **Table 1**.

Antibody testing

Neuronal autoantibody testing for all patients was performed at Guangzhou KingMed Diagnostics using combined cell-based assays and tissue immunofluorescence methods. Neuronal surface antibodies tested included NMDAR, LGI1, CASPR2, AMPAR, GABAAR, GABABR, DPPX, GlyR, and IgLON5. Intracellular antibodies included GAD65, SOX1, Hu, Yo (PCA-1), Ri (ANNA-2), Ma1, Ma2, CV2, and amphiphysin. All serum and CSF specimens were strictly collected within 24 hours of patient admission and before initiation of immunomodulatory treatment.

Classification of disease severity and treatment response

Patient disease status was assessed using both mRS and CASE scores, with recordings obtained at three time points: admission, peak disease severity, and discharge. The mRS primarily evaluates overall functional status and degree of disability, with particular emphasis on motor function. The CASE scale encompasses nine neurological functional domains, including seizures, cognitive function, psychiatric behavior, consciousness status, language ability, movement/muscle tone, balance/coordination, brainstem function, and muscle strength. Each domain is scored from 0 to 3 points, with a total score ranging from 0 to 27 points.

Based on the mRS score at peak disease severity, we classified disease severity into three grades: severe (mRS score 4-5), moderate (mRS score 2-3), and mild (mRS score 0-1). Treatment response assessment comprehensively considered the degree of improvement in both mRS and CASE scores, categorizing patients into three groups: good response, partial response, and no response. Based on these assessments, patients were further divided into good response and poor response groups, with the poor response group encompassing patients with partial response or no response.

Specifically, the treatment response assessment criteria were as follows: (1) Good response: Patients with severe disease achieved mRS score reduction to ≤ 3 points with concurrent CASE score reduction; moderate patients achieved mRS score reduction to ≤ 2 points with concurrent CASE score reduction; mild patients maintained mRS scores of 0-1 points with concurrent CASE score reduction. (2) Partial response: Patients with severe disease demonstrated CASE score reduction but mRS scores remained ≥ 4 points; moderate patients demonstrated CASE score reduction but mRS scores remained ≥ 3 points. (3) No response: Patients of all severity levels showed no change or increase in CASE scores.

Statistical analysis

All statistical analyses were performed using R software (version 4.2.2). The Shapiro-Wilk test was used to assess normality of continuous variables. Normally distributed continuous variables were presented as mean ± standard deviation, with between-group comparisons performed using independent samples t-tests. Non-normally distributed continuous variables were presented as median (interquartile range), with between-group comparisons performed using the Mann-Whitney U test. Categorical variables were presented as frequency (percentage), with between-group comparisons performed using Pearson's chi-square test or Fisher's exact test.

We employed the Boruta algorithm for initial screening of potential predictive variables for poor treatment response. Based on the initial screening results, least absolute shrinkage and selection operator (LASSO) regression was used for further optimization of variable selection to avoid overfitting issues. Prior to constructing the multivariable logistic regression model, variance inflation factor (VIF) and tolerance were used to diagnose multicollinearity among candidate variables, with variables showing VIF ≥ 10 or tolerance ≤ 0.1 excluded from analysis (Supplementary Table 1). Multivariable logistic regression modeling was ultimately employed to identify independent predictive factors. Model performance evaluation was completed by plotting receiver operating characteristic (ROC) curves and calculating the area under the curve (AUC) with 95% confidence intervals. All statistical tests were two-sided, with $P < 0.05$ considered significant.

Results

Patient characteristics

A total of 80 patients with AE were included in this study and divided into two groups based on treatment response: good response (n = 52, 65.0%) and poor response (n = 28, 35.0%). The median age at symptom onset was 34 years overall, with 34 male patients (42.5%; **Table 2**). The proportion of antibody-positive AE was slightly higher in the good response group (P = 0.090). Disease severity assessment revealed that the poor response group had significantly higher CASE scores at admission compared to the good response group (P = 0.003), with the difference becoming even more pronounced at peak disease severity (P = 0.005). Correspondingly, mRS scores demonstrated similar differential patterns at both admission and peak disease severity (both $P < 0.005$). Clinical manifestation analysis showed that altered consciousness (P = 0.004) and cognitive impairment (P = 0.034) occurred significantly more frequently in the poor response group. EEG examination results indicated that diffuse slow wave activity was more commonly observed in the poor treatment response group (P = 0.013).

The selection of immunotherapy regimens showed no significant differences between the two groups. Corticosteroids (85.71% vs. 92.31%) and IVIG (67.86% vs. 69.23%) were the most commonly used first-line therapeutic agents. The application rate of combination immunotherapy was comparable between groups (60.71% vs. 65.38%, P = 0.942). Plasma exchange, as a second-line treatment modality, had relatively low utilization rates with no significant difference between groups (14.29% vs. 7.69%, P = 0.584). The combination of IVIG and corticosteroids represented the predominant treatment approach (46.43% vs. 57.69%), and the distribution of treatment regimen selections showed no significant differences (P = 0.703).

CSF biomarkers are elevated in poor response patients

Laboratory examination results revealed significant differences between the two patient groups in both peripheral blood and CSF measurements (**Table 3**). Peripheral blood analysis demonstrated that serum IgG concentration was significantly higher in the poor response

Table 2. Baseline demographics and clinical characteristics stratified by treatment response

Variable	Total (n = 80)	Poor response (n = 28)	Good response (n = 52)	t/z/χ^2	*P* value
Age at symptom onset, years	34 (20, 60)	40 (19, 64)	34 (20, 57)		0.431
Male sex	43 (53.75)	13 (46.43)	30 (57.69)	0.93	0.335
Comorbidities					
Autoimmune disorders	12 (15.00)	6 (21.43)	6 (11.54)	0.73	0.393
Active malignancy	9 (11.25)	4 (14.29)	5 (9.62)	0.07	0.795
Antibody-positive AE	50 (62.50)	14 (50.00)	36 (69.23)	2.87	0.090
Severity assessment					
CASE score at admission	5.0 (3.0, 8.5)	8.5 (4.5, 14.0)	4.5 (3.0, 6.5)	-2.93	0.003
Peak CASE score	6.0 (3.0, 9.5)	9.0 (5.0, 16.5)	5.0 (3.0, 8.0)	-2.83	0.005
mRS score at admission	3.0 (1.0, 4.0)	4.0 (2.5, 5.0)	2.00 (1.0, 4.0)	-2.93	0.003
Peak mRS score	3.0 (1.0, 4.0)	4.0 (2.5, 5.0)	2.00 (1.0, 4.0)	-3.17	0.002
Clinical manifestations					
Prodromal symptoms	19 (23.75)	9 (32.14)	10 (19.23)	1.68	0.196
Seizures	38 (47.50)	14 (50.00)	24 (46.15)	0.11	0.742
Memory dysfunction	14 (17.50)	5 (17.86)	9 (17.31)	0.00	> 0.99
Psychiatric symptoms	44 (55.00)	16 (57.14)	28 (53.85)	0.08	0.777
vAltered consciousness	15 (18.75)	10 (35.71)	5 (9.62)	8.14	0.004
Movement disorders	24 (30.00)	9 (32.14)	15 (28.85)	0.09	0.759
Gait abnormalities and ataxia	11 (13.75)	4 (14.29)	7 (13.46)	0.00	> 0.99
Cognitive impairment	32 (40.51)	16 (57.14)	16 (31.37)	4.49	0.034
Neuroimaging findings					
Limbic encephalitis	24 (30.00)	8 (28.57)	16 (30.77)	0.04	0.838
Non-limbic encephalitis	12 (15.00)	5 (17.86)	7 (13.46)	0.04	0.844
Electroencephalography					
Abnormal EEG	55 (68.75)	22 (78.57)	33 (63.46)	1.93	0.164
Diffuse slowing	31 (38.75)	16 (57.14)	15 (28.85)	6.14	0.013
Focal slowing	7 (8.75)	2 (7.14)	5 (9.62)	0.00	> 0.99
Epileptiform discharges	19 (23.75)	4 (14.29)	15 (28.85)	2.13	0.144
Immunotherapy regimens					
IVIG therapy	55 (68.75)	19 (67.86)	36 (69.23)	0.02	0.899
Corticosteroid therapy	72 (90.00)	24 (85.71)	48 (92.31)	0.30	0.441
Plasma exchange	8 (10.00)	4 (14.29)	4 (7.69)	0.30	0.584
Combined first-line therapy	51 (63.75)	17 (60.71)	34 (65.38)	0.01	0.942
Therapy regimen				-	0.703
IVIG alone	8 (10.00)	4 (14.29)	4 (7.69)		
Corticosteroids alone	21 (26.25)	7 (25.00)	14 (26.92)		
IVIG + corticosteroids	43 (53.75)	13 (46.43)	30 (57.69)		
Corticosteroids + PLEX	4 (5.00)	2 (7.14)	2 (3.85)		
IVIG + corticosteroids + PLEX	4 (5.00)	2 (7.14)	2 (3.85)		

Data presentation: Continuous variables are expressed as mean ± SD or median (IQR) based on distribution normality. Categorical variables are presented as number (percentage). Abbreviations: AE, autoimmune encephalitis; CASE, Clinical Assessment Scale in Autoimmune Encephalitis; EEG, electroencephalography; IVIG, intravenous immunoglobulin; mRS, modified Rankin Scale; PLEX, plasma exchange.

group compared to the good response group (P < 0.001). CSF analysis indicated that patients in the poor response group had significantly higher CSF total protein concentration (P < 0.001), CSF albumin concentration (P = 0.001), and CSF IgG concentration (P < 0.001) com-

Table 3. Laboratory data and biomarkers stratified by treatment response

Variable	Total (n = 80)	Poor response (n = 28)	Good response (n = 52)	t/z/χ^2	*P* value
Peripheral blood					
WBC count	8.32 ± 2.83	8.52 ± 2.97	8.22 ± 2.78	0.46	0.646
Neutrophil count	5.62 (4.00, 7.71)	5.78 (3.94, 7.91)	5.30 (4.04, 7.71)	-0.37	0.709
Lymphocyte count	1.70 ± 0.67	1.56 ± 0.67	1.77 ± 0.66	-1.30	0.197
Monocyte count	0.53 (0.44, 0.68)	0.51 (0.37, 0.70)	0.54 (0.45, 0.68)	-0.54	0.586
Neutrophil percentage	69 (60, 77)	71 (61, 81)	69 (58, 74)	-1.29	0.197
Total protein	69 ± 8	70 ± 8	69 ± 8	0.71	0.482
Albumin	40.2 ± 4.9	39.3 ± 5.6	40.7 ± 4.4	-1.21	0.228
Serum IgG	11.5 (10.1, 13.1)	13.7 (11.2, 23.1)	11.0 (10.0, 12.0)	-3.34	< .001
NLR	3.3 (2.1, 5.4)	4.0 (2.2, 6.5)	3.0 (2.0, 5.1)	-0.92	0.356
MLR	0.32 (0.23, 0.48)	0.34 (0.24, 0.60)	0.31 (0.22, 0.43)	-0.89	0.372
NPAR	1.70 (1.39, 1.99)	1.95 (1.40, 2.12)	1.64 (1.39, 1.92)	-1.69	0.091
Cerebrospinal fluid					
CSF total protein	424 (326, 613)	583 (411, 734)	367 (276, 535)	-3.56	< .001
CSF albumin	228 (143, 381)	339 (249, 442)	182 (130, 333)	-3.25	0.001
CSF lactate	1.74 (1.47, 2.03)	1.88 (1.64, 2.18)	1.67 (1.44, 1.99)	-1.83	0.068
CSF chloride	125.4 (123.5, 127.8)	125.4 (123.6, 128.1)	125.6 (123.4, 127.6)	-0.12	0.908
CSF glucose	3.76 (3.43, 4.21)	3.96 (3.48, 4.56)	3.71 (3.39, 4.06)	-1.17	0.244
CSF WBC count	4 (2, 28)	3 (1, 18)	5 (3, 36)	-1.37	0.169
CSF IgG	37 (26, 54)	51 (38, 59)	31 (21, 46)	-3.34	< .001
Albumin quotient	30 (37.50)	16 (57.14)	14 (26.92)	7.09	0.008
Intrathecal IgG synthesis	11.5 (10.2, 13.9)	14.3 (10.9, 19.5)	11.2 (10.0, 12.1)	-3.43	< .001
Elevated CSF pressure	23 (28.75)	10 (35.71)	13 (25.00)	1.02	0.313

Reference ranges: Serum - WBC 3.5-9.5 × 10^9/L, neutrophils 1.8-6.3 × 10^9/L, lymphocytes 1.1-3.2 × 10^9/L, total protein 65-85 g/L, albumin 40-55 g/L, IgG 7.0-16.0 g/L. CSF - WBC 0-5 cells/μL, total protein 150-450 mg/L, albumin 100-300 mg/L, glucose 2.5-4.0 mmol/L, chloride 120-130 mmol/L, lactate 1.1-2.4 mmol/L. Albumin quotient age-specific thresholds: < 40 years < 6.5 × 10^{-3}, 40-60 years < 8.0 × 10^{-3}, > 60 years < 9.3 × 10^{-3}. Abbreviations: AE, autoimmune encephalitis; CSF, cerebrospinal fluid; IgG, immunoglobulin G; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; NPAR, neutrophil percentage-to-albumin ratio; WBC, white blood cells.

pared to the good response group. The intrathecal IgG synthesis rate was also significantly elevated in the poor response group (P < 0.001). The proportion of patients with abnormal albumin quotient was significantly higher in the poor response group (P = 0.008).

Seven key variables identified for treatment response

We employed the Boruta algorithm for initial feature selection, with the maximum number of iterations set to 100, which identified seven important predictive variables (**Figure 1**): serum IgG concentration, intrathecal IgG synthesis, peak CASE score, CSF total protein concentration, CSF albumin concentration, admission CASE score, and CSF IgG concentration. Subsequently, we performed LASSO regression analysis on the seven variables selected by the Boruta algorithm. Using 10-fold cross-validation, we selected optimal parameters from 100 candidate λ values for model training (**Figure 2**). LASSO regression retained five key predictive variables at the optimal λ value: serum IgG concentration, intrathecal IgG synthesis, peak CASE score, CSF total protein concentration, and CSF albumin. Multicollinearity diagnosis revealed high collinearity between CSF total protein and CSF albumin (VIF ≥ 10), ultimately leading to the exclusion of CSF total protein while retaining CSF albumin concentration.

Three independent biomarkers predict poor response

A logistic regression model for treatment response prediction was constructed using four variables (**Table 4**). Univariate analysis demonstrated that all candidate variables were signifi-

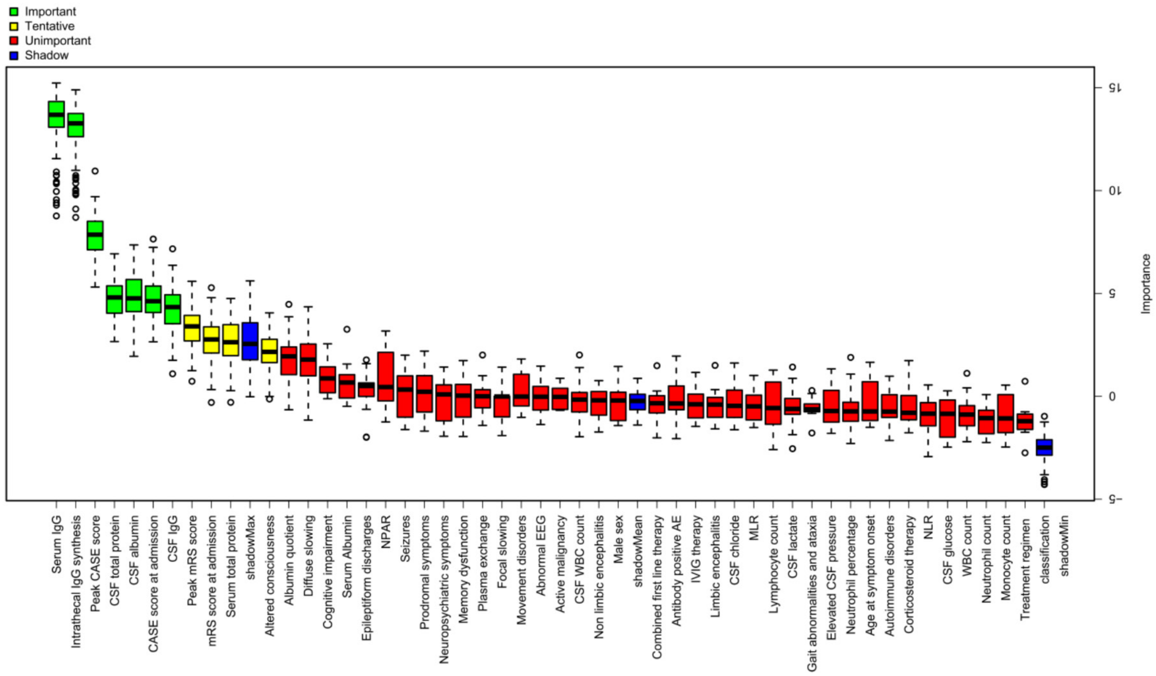

Figure 1. Boruta algorithm feature selection results for treatment response prediction variables. Boruta algorithm feature importance analysis identifying significant predictors of poor immunotherapy response in autoimmune encephalitis patients (maximum iterations = 100). Variables are color-coded by classification: green (confirmed important), yellow (tentative), red (unimportant), and blue (shadow variables). Seven important variables were identified: serum IgG concentration, intrathecal IgG synthesis, peak CASE score, CSF total protein concentration, CSF albumin concentration, admission CASE score, and CSF IgG concentration. Abbreviations: CASE = Clinical Assessment Scale in Autoimmune Encephalitis; CSF = cerebrospinal fluid; IgG = immunoglobulin G.

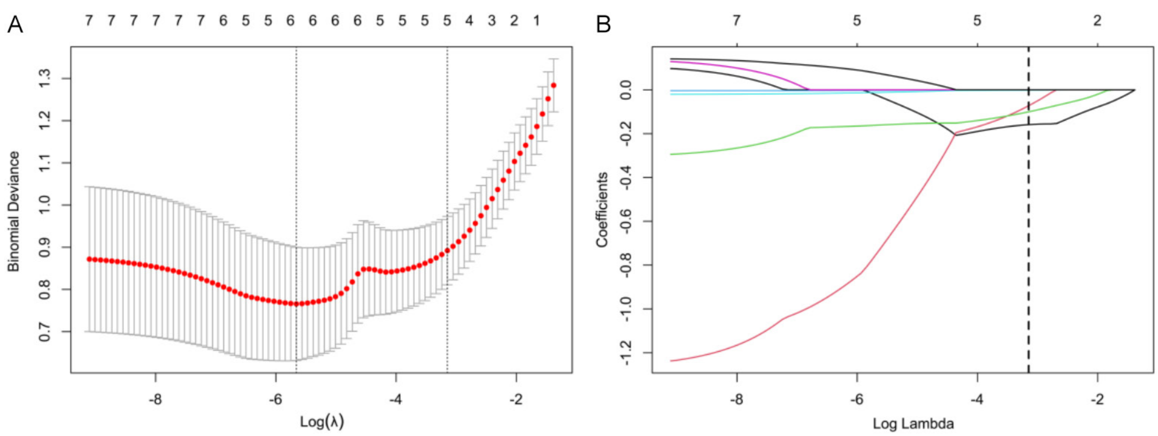

Figure 2. Least Absolute Shrinkage and Selection Operator (LASSO) regression analysis for variable selection. LASSO regression analysis results for optimizing biomarker selection in predicting poor immunotherapy response. A. Cross-validation curve showing binomial deviance (±1 standard error) as a function of log(λ) values. B. Coefficient path plot showing how regression coefficients for each variable change as log(λ) increases.

cantly associated with poor response (all $P < 0.05$). Multivariable logistic regression analysis revealed that three independent predictors maintained statistical significance in the model: intrathecal IgG synthesis (OR = 1.57, 95% CI: 1.03-2.45, $P = 0.037$), peak CASE score (OR = 1.22, 95% CI: 1.07-1.42, $P = 0.004$), and CSF albumin (OR = 1.00, 95% CI: 1.00-1.01, $P = 0.005$).

Biomarker diagnostic performance analysis

ROC curve analysis demonstrated (**Figure 3**) that the intrathecal IgG synthesis exhibited the

Table 4. Univariable and multivariable logistic regression analysis of cerebrospinal fluid biomarkers for predicting poor response in autoimmune encephalitis

Characteristic	Univariable		Multivariable	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Serum IgG	1.38 (1.17-1.76)	0.002	1.13 (0.82-1.68)	0.498
Intrathecal IgG synthesis	1.47 (1.21-1.91)	< .001	1.57 (1.03-2.45)	0.037
Peak CASE score	1.19 (1.08-1.33)	< .001	1.22 (1.07-1.42)	0.004
CSF total protein	1.00 (1.00-1.01)	0.032	1.00 (1.00-1.01)	0.005

Abbreviations: CASE, Clinical Assessment Scale in Autoimmune Encephalitis; CI, confidence interval; CSF, cerebrospinal fluid; IgG, immunoglobulin G; OR, odds ratio.

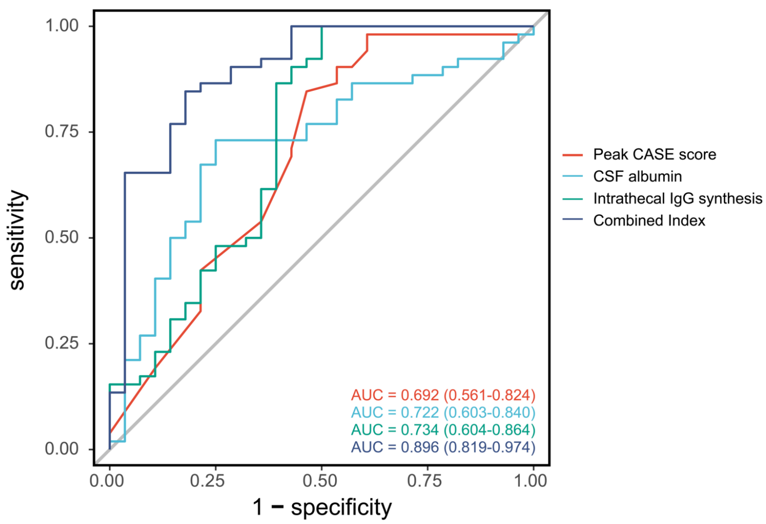

Figure 3. Receiver Operating Characteristic (ROC) curve analysis of cerebrospinal fluid biomarkers for predicting poor immunotherapy response in autoimmune encephalitis. ROC curves demonstrating the diagnostic performance of individual biomarkers and the combined predictive model for identifying poor immunotherapy response in patients with autoimmune encephalitis.

best diagnostic performance, with an AUC of 0.734, followed by CSF albumin with an AUC of 0.722. The peak CASE score showed relatively lower diagnostic efficacy, with an AUC of 0.692. The combined model displayed significantly superior diagnostic performance, achieving an AUC of 0.896, markedly exceeding the diagnostic efficacy of any single biomarker.

Discussion

This study classified patients into good and poor immunotherapy response groups based on changes in mRS and CASE scores before and after treatment. Univariate logistic regression analysis revealed that altered consciousness, cognitive impairment, peak CASE score, serum IgG levels, CSF albumin concentration, abnormal QAlb, and elevated intrathecal IgG synthesis rate were all significantly associated with poor immunotherapy response (all P < 0.05). Following Boruta and LASSO selection, further multivariable logistic regression analysis demonstrated that peak CASE score, intrathecal IgG synthesis, and CSF albumin concentration remained significantly associated with poor short-term immunotherapy outcomes in patients with AE.

Altered consciousness has historically been recognized as an important predictor of poor prognosis in AE [10, 11]. Yang et al. [10] found in a cohort study of 121 patients with AE that altered consciousness was an independent risk factor for poor prognosis (mRS > 2). However, in our multivariable analysis, altered consciousness did not reach statistical significance. This discrepancy in results may stem from previous studies primarily focusing on the impact of clinical manifestations and imaging features on prognosis, without incorporating detailed laboratory biomarker analyses. Nevertheless, clinical practice still requires special attention to AE patients presenting with altered consciousness. These patients often require prolonged bed rest, significantly increasing the risk of complications such as deep vein thrombosis, pulmonary infections, and urinary tract infections, which not only increases the economic burden on patients but also adds to the diagnostic and therapeutic challenges for healthcare teams. Therefore, arousal therapy should be considered for such patients to restore consciousness as early as possible, to reduce the incidence of related complications.

QAlb, as a sensitive indicator reflecting BBB integrity, has been confirmed in multiple previous studies to be closely associated with pathological manifestations such as decreased consciousness levels and elevated CSF protein concentrations, with patients showing abnormally elevated QAlb often demonstrating poor response to first-line immunotherapy [12-14]. However, in our multivariable logistic regression analysis, the association between QAlb and immunotherapy response did not reach statistical significance. This discrepancy may stem from differences in efficacy assessment methods between studies as an important contributing factor. Previous studies predominantly employed mRS as a single assessment tool, whereas our study innovatively integrated dynamic changes in both mRS and CASE scores for comprehensive evaluation. This multidimensional assessment approach may exert different influences on the predictive value of QAlb.

Since the formal release of the CASE scale in 2019, its accuracy and effectiveness for assessing disease severity in patients with autoimmune encephalitis have been validated in multiple independent studies [15, 16]. Cai et al. [15] confirmed a strong correlation between CASE scores and mRS scores (r = 0.8) in a study of 143 patients with AE. Similarly, Zhang et al. [16] found a high positive correlation between CASE scores and mRS scores (r = 0.85) in a cohort study involving 176 patients with AE, and demonstrated that CASE scores had good predictive value for functional status at 1 year post-discharge. These research findings are highly consistent with our study results, further confirming the important value of the CASE scale in predicting short-term immunotherapy response in patients with AE, providing clinicians with a standardized, objective assessment tool to quantify disease severity and predict treatment outcomes.

Despite the excellent performance of the CASE scale in comprehensive assessment, our clinical observations have identified limitations in certain specific clinical contexts. Particularly when evaluating patients with AE presenting primarily with single or few symptoms, the CASE scale may not adequately capture subtle disease changes. For example, in patients presenting primarily with seizures, even when seizure frequency decreases significantly or seizure severity improves markedly, the change in total CASE score may be insufficient to reflect this clinically meaningful improvement due to other neurological domain scores remaining relatively stable. Similarly, for patients presenting primarily with ataxia, small but clinically relevant improvements in balance/coordination function may be masked by other scoring components, resulting in reduced sensitivity of the CASE scale.

Notably, our study confirmed that elevated CSF albumin concentration and increased intrathecal IgG synthesis are closely associated with poor immunotherapy response in patients with AE. Protein and IgG levels in cerebrospinal fluid are important biochemical indicators for assessing central nervous system immune responses [17]. Under physiological conditions, protein and IgG content within the central nervous system is extremely low, with albumin originating entirely from blood circulation [18]. Therefore, elevated CSF protein concentration primarily reflects increased BBB permeability and abnormal penetration of blood-derived immunoglobulins and other exogenous proteins, indicating the presence of abnormal immune-inflammatory responses in the central nervous system.

Furthermore, under normal physiologic conditions, IgG in CSF has dual origins: intrathecally synthesized immunoglobulins and circulating immunoglobulins synthesized by the liver and transported across the BBB. Intrathecal IgG synthesis refers to immunoglobulin G produced locally within the central nervous system, primarily by activated B cells and plasma cells, which differs fundamentally in pathologic significance from IgG passively transported from blood circulation into CSF. Traditional methods for assessing intrathecal IgG synthesis include the IgG index and 24-hour intrathecal IgG synthesis rate, but these indicators are based on an important assumption: that the transport capacity of albumin and IgG across the BBB is independent of blood-brain barrier functional status. Therefore, when BBB permeability is abnormal, these traditional indicators may produce false-positive results and cannot accurately reflect the true intensity of immune responses within the central nervous system. In contrast, the intrathecal IgG synthesis proposed by Reiber employs a more precise calculation method that corrects for individual differences in blood-brain barrier permeability through QAlb [18]. The core principle of this method is to compare the actually measured CSF IgG concentration with the IgG concentration that should theoretically exist through passive diffusion across the blood-brain barrier alone. This approach can more accurately distinguish between intrathecally synthesized and blood-derived IgG, thereby objectively reflecting the true level of local immune responses within the central nervous system.

Elevated intrathecal IgG synthesis reflects multiple complex pathophysiologic processes. First, sustained activation of B cells and plasma cells within the central nervous system leads to excessive production of autoantibodies directed against neuronal surface antigens or synaptic proteins, which directly participate in disease pathogenesis [19]. Second, dysregulation of immune regulatory mechanisms, particularly regulatory T cell dysfunction, may be an important factor contributing to the persistence of autoimmune responses [20, 21]. Additionally, antibody- and/or complement-mediated cytotoxic effects are considered the primary mechanisms of neuronal damage mediated by surface antigen antibodies. Excessive autoantibody production, massive release of proinflammatory cytokines, and chronic inflammatory responses triggered by immune complex deposition play crucial roles in AE disease progression [22]. The synergistic effects of these pathologic mechanisms may explain the close association between elevated intrathecal IgG synthesis and poor response in patients with AE, suggesting that the intensity of intrathecal immune responses may be an important factor influencing immunotherapy efficacy.

This study has the following limitations that should be considered in result interpretation. First, as a single-center retrospective study, CASE and mRS scores relied entirely on electronic medical record documentation, potentially leading to incomplete information recording or inter-rater variability, which may affect scoring reliability and accuracy. Second, the relatively limited sample size restricted the statistical power and precluded more in-depth subgroup analyses or model validation. Third, this study analyzed all AE subtypes collectively and did not adequately explore heterogeneity in treatment response predictors among patients with different antibody types of AE, possibly masking subtype-specific predictive indicators. Furthermore, although we conducted detailed analyses of various first-line immunotherapy combinations and found no significant statistical associations between treatment regimens and prognosis, this does not exclude actual effects of treatment regimens on outcomes. Different patients received different treatment regimens, including variations in drug selection, treatment duration, and combination therapies, and these factors may significantly impact treatment response. Future studies should employ standardized treatment protocols and randomized controlled trial designs, or establish propensity score-matched cohort studies to more accurately assess the independent predictive value of biomarkers.

Conclusion

This study identified three independent predictors of poor immunotherapy response: elevated intrathecal IgG synthesis, increased peak CASE score, and elevated CSF albumin concentration. The combined predictive model demonstrated excellent diagnostic performance, significantly superior to any single indicator. These cerebrospinal fluid biomarkers facilitate identification of high-risk patients in the early treatment phase, providing objective evidence for early treatment intensification and individualized treatment decision-making.

Acknowledgements

This work was supported by the Fujian Key Clinical Specialty Discipline Construction Program (050172).

Disclosure of conflict of interest

None.

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Supplementary Table 1. Multicollinearity assessment of variables included in the multivariable logistic regression models

Variable	Tolerance[a]	VIF[a]	Tolerance[b]	VIF[b]	Tolerance[c]	VIF[c]
Serum IgG	0.53914672	1.854783	0.08036318	12.443509	0.5469138	1.828442
Intrathecal IgG synthesis	0.46574959	2.147077	0.08236514	12.141059	0.4699429	2.127918
Peak CASE score	0.90344981	1.106868	0.88464279	1.130400	0.9033015	1.10705
CSF total protein	0.07227269	13.836485	0.87218331	1.146548	-	-
CSF albumin	0.07143326	13.999081	-	-	0.8163227	1.225006

Multicollinearity was assessed using variance inflation factor (VIF) and tolerance values before inclusion in multivariable Logistic regression models. VIF values < 10 and tolerance values > 0.1 indicate acceptable levels of multicollinearity. All variables demonstrated acceptable multicollinearity levels across all modeling approaches. a Model including all five variables: Serum IgG, Intrathecal IgG synthesis, Peak CASE score, CSF total protein, and CSF albumin. b Model excluding CSF albumin. c Model excluding CSF total protein.