

Cytokine and Chemokine Profiles in Anti-LGI1 Encephalitis

Markers of Severity and Outcome

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Abstract

Background and Objectives

Anti-leucine-rich glioma-inactivated 1 (anti-LGI1) encephalitis is the most common antibody-mediated encephalitis in adults older than 50 years. In addition to antibody effects, cytokines and chemokines drive neuroinflammation in other autoimmune encephalitides. However, their role in anti-LGI1 encephalitis is underexplored. We evaluated cytokine profiles in serum and CSF, correlating them with acute severity and long-term outcome.

Methods

Cytokine/chemokine levels from 57 untreated patients with anti-LGI1 encephalitis were measured in serum and CSF (34 paired samples) with a bead-based multiplex assay and compared with those of patients with noninflammatory neurologic disorders (serum = 24; CSF = 21). Clinical information including degree of severity (modified Rankin Scale, mRS) and 12-month functional outcomes (resume previous activities and work) was assessed.

Results

Patients with anti-LGI1 encephalitis exhibited an increased proinflammatory profile in CSF and serum, with elevated levels of IL-1 β , IL-1RA, IL-6, IL-8, IL-10, IL-18, IL-35, IP-10, granzyme B, CX3CL1, MIG, TNF α , and SDF1. A higher CSF/serum IL-6 ratio correlated with disease severity at onset (mRS score >2: 1.67 [0.50–7.20] vs 0.39 [0.07–1.22], $p = 0.0069$). Elevated acute-phase serum IL-35 predicted poorer 12-month outcomes (81.34 vs 9.19 pg/mL in partial vs complete recovery, $p = 0.0003$). Increased B cell-related markers (IL-21, BAFF, APRIL, CXCL13) in CSF (all $p < 0.05$) were also observed.

Discussion

In this study, we show that the acute-phase IL-6 CSF/serum ratio and serum IL-35 levels in immunotherapy-naïve patients with anti-LGI1 encephalitis are associated with disease severity and poor outcomes, respectively, highlighting their potential as biomarkers for risk stratification and therapeutic targeting.

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Anti-LGI1 encephalitis Study Group coinvestigators are listed in appendix at end of the article.

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Supplementary Material

Glossary

anti-LGI1 = anti-leucine-rich glioma-inactivated 1; **IQR** = interquartile range; **mRS** = modified Rankin Scale; **NMOSD** = neuromyelitis optica spectrum disorder.

Introduction

Anti-leucine-rich glioma-inactivated 1 (anti-LGI1) encephalitis is the most common neuronal antibody-associated encephalitis in patients older than 50 years, presenting with seizures, memory deficits, and cognitive decline.¹ Early studies reported favorable response of memory deficits and seizures to first-line immunotherapy, mainly corticosteroids, and symptomatic treatments.² However, a recent prospective, longitudinal study showed that approximately two-thirds of patients still experience improved but persistent cognitive deficits, seizures, and sleep disturbances 1 year after disease onset.³ These persisting symptoms frequently hinder patients from resuming their normal activities or work.^{4,5}

The pathogenic role of LGI1 antibodies in anti-LGI1 encephalitis has been investigated in cultured neurons and animal models.⁶⁻⁸ These antibodies bind to the LGI1 protein and disrupt the interaction of its ligands: the metalloproteinase domain-containing proteins ADAM23 and ADAM22. This disruption promotes neuronal hyperexcitability and alters memory and behavior in animal models, supporting the disease-driving role of the antibodies. Multiple studies have linked anti-LGI1 encephalitis to HLA haplotypes DRB1*07:01-DQA1*02:01 and DRB1*07:01-DQA1*02:01-DQB1*02:02.^{9,10} However, despite advances in diagnosis and treatment, key gaps remain in understanding the disease's pathogenesis and immune mechanisms.

Cytokines, chemokines, and other immune-related soluble factors are important components of immune responses and autoimmunity. They orchestrate a complex network of signaling pathways that mediates the inflammatory response.¹¹ Altered production and regulation of these molecules drive autoimmune neurologic diseases by modulating immune cell recruitment, activation, and function, including microglia, astrocytes, and infiltrating lymphocytes.¹²⁻¹⁷ Furthermore, cytokines and chemokines are also involved in tissue repair and resolution of inflammation in the CNS.^{18,19}

These mediators have been extensively studied in multiple sclerosis²⁰⁻²² other autoimmune encephalitides,²³⁻²⁵ and neuromyelitis optica spectrum disorder (NMOSD),²⁶ leading to effective treatment strategies. For example, IL-6 inhibition with satralizumab has been shown to reduce the risk of relapses in patients with NMOSD (NCT02028884).²⁷ Furthermore, a new clinical trial on anti-LGI1 encephalitis is currently in the recruitment phase (NCT05503264).

In anti-LGI1 encephalitis, cytokine research is limited by small sample sizes, insufficient clinical data (e.g., long-term follow-up),

untargeted analyses, lack of CSF studies, restricted cytokine examinations, and rare serum-CSF comparisons,^{23,28,29} despite their proven importance.³⁰

We examined cytokine and chemokine profiles in patients with anti-LGI1 encephalitis with prospectively collected serum and CSF samples before immunotherapy, correlating findings with acute clinical features and long-term outcomes to explore immunopathologic mechanisms and identify severity biomarkers.

Methods

Study Group

Between December 2016 and December 2023, we diagnosed 115 patients with anti-LGI1 encephalitis. Antibodies in serum or CSF were detected with an in-house rat brain immunohistochemistry³¹ and confirmed by the cell-based assay (Euroimmun (Lübeck, Germany) and/or in-house CBA). Of these 115 patients, 57 with adequate clinical information and serum or CSF samples obtained before immunotherapy were selected for study. Overall, 94 samples were available: 45 CSF and 49 serum samples, including paired serum/CSF samples from 34 patients.

Clinical information was obtained by the authors or referring physicians using a structured written questionnaire. This included demographic data, symptom onset, sampling dates, and key initial symptoms: (1) memory and cognitive deficits, (2) seizures (faciobrachial dystonic seizures, other types of focal-onset and generalized seizures), (3) behavioral and psychiatric symptoms, and (4) sleep alterations. Additional data included blood biochemistry, CSF analysis, brain MRI, EEG and EMG findings, treatment details and timing, and symptom severity measured using the modified Rankin Scale (mRS) at nadir and the last visit.

Prospective 1-year follow-up data were available for 41 patients and included main symptoms during the disease course, mRS scores, and patients' ability to resume their previous activities or work (complete, good, partial or sub-optimal). Among the 16 patients without 1-year follow-up information, 11 were lost to follow-up and 5 died from unrelated causes.

Twenty-four serum and 21 CSF samples from age-matched participants with noninflammatory neurologic diseases served as controls. Participants' samples were coded, and clinical information was anonymized prior to analysis. The study was

approved by the Ethics Committee of Hospital Clínic of Barcelona.

Cytokine Measurement

Levels of sCD40L, fractalkine (CX3CL1), G-CSF, IL-1 α , IL-1 β , IL-1RA, IL-6, IL-8 (CXCL8), IL-10, IL-12 (p40), IL-17A, IL-18, IL-22, IP-10 (CXCL10), MCP-1 (CCL-2), MIG (CXCL9), TNF α , APRIL, BAFF, BCA1 (CXCL13), granzyme B, IL-21, IL-23, IL-35, SDF1 (CXCL12), MIP3A (CCL20), 6-Ckine (CCL21), and lymphotactin (XCL1) were determined with a Milliplex custom MAP Human Cytokine/Chemokine/Growth panel (MERCK Millipore Corporation, Billerica, MA) following the manufacturer's instructions and analyzed with LUMINEX xMAP100. All samples, standards, and quality controls were assayed in accordance with the manufacturer's instructions. Results were analyzed with Belysa Immunoassay Curve Fitting Software (Merck). All cytokine and chemokine concentrations are reported in pg/mL. Serum samples were measured in duplicate, whereas CSF samples were tested only once. All serum and CSF samples from both patients and controls were processed under standardized conditions. Samples were centrifuged and aliquoted within 2 hours of collection when obtained at our center and frozen at -80°C . Samples from collaborating hospitals across Spain followed the same protocol. All aliquots underwent a maximum of 2 freeze-thaw cycles prior to analysis. It is important to note that control samples were collected through the same clinical circuit and processed under the same preanalytical conditions as the patient samples, minimizing potential bias due to sample handling.

Statistical Analysis

All data are described as median and interquartile range (IQR) (25th, 75th percentiles) or absolute frequency and percentage for quantitative and qualitative variables, respectively. Because Gaussian distribution was not confirmed by Kolmogorov-Smirnov and Shapiro-Wilk normality tests, cytokine/chemokine levels were analyzed using nonparametric tests. Cytokine/chemokine levels were compared between anti-LGI1 and control groups, and among clinical subgroups of patients with anti-LGI1 encephalitis using the Mann-Whitney *U* test and Kruskal-Wallis test (post hoc analyses between groups were conducted with Bonferroni correction for multiple comparisons). Correlations between serum/CSF cytokine/chemokine levels at baseline and the functional outcome at 1-year follow-up were analyzed using the Spearman correlation coefficient (outcome measured using the mRS) and binary logistic regression (outcome dichotomized as good/complete recovery vs suboptimal/partial recovery). Outliers were detected by the Robust Outlier Test. Statistical analyses were performed with Jamovi (version 2.3.28) and IBM SPSS Statistics (version 26), and graphs were created using GraphPad Prism (version 10.4.1). All statistical tests were 2-sided with a type I error rate of 5%.

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the Ethics Committee of Hospital Clínic of Barcelona (HCB/2021/1017). Patients' samples were coded, and clinical information was anonymized prior to analysis. All participants were fully informed about study requirements and accepted data sharing and privacy policy before participating in the study.

Data Availability

All data analyzed in this study will be shared in anonymized form on reasonable request of a qualified investigator to the corresponding author.

Results

Clinical Features at Initial Evaluation

Demographic and clinical features of 57 patients with anti-LGI1 encephalitis are provided in Table 1. The median age was 68 years (IQR: 58–73 years), and 31 (54%) were male. At disease diagnosis, 44 patients (77%) had prominent short-term memory loss; 39 (68%) had seizures, including facio-brachial dystonic seizures in 25 (43%); and 37 (65%) had behavioral/psychiatric alterations. In addition, 27 patients (47%) had sleep disorders, and 21 of 42 (50%) had hyponatremia. CSF pleocytosis (>5 WBC/mm³) was detected in 4 of 44 (9%), MRI abnormalities in 34 of 57 (60%), and EEG alterations in 35 of 57 (62%). Overall, 27 patients (47%) met criteria for limbic encephalitis with bilateral MRI T2/FLAIR abnormalities in medial temporal lobes and CSF pleocytosis or EEG with epileptic or slow-wave activity involving the temporal lobes (Graus et al., 2016). The median mRS score at disease peak was 3 (IQR 2–3). All patients underwent comprehensive testing to rule out concomitant infections or other alternative causes of encephalitis, following standardized differential diagnosis protocols. None of the clinical, electrophysiologic, or radiologic variables analyzed demonstrated a significant association with clinical severity at initial evaluation (data not shown). We obtained treatment regimens from 42 patients. Of these, 40 received both first-line treatment (IVIG and/or corticosteroids) and second-line treatment (rituximab or cyclophosphamide). One patient received only first-line therapy, and another was treated exclusively with antiepileptic drugs. The median time from symptom onset to initiation of treatment was 10.9 weeks (IQR 5.2–32.8).

Clinical Features of Patients With Anti-LGI1 Encephalitis at 12-Month Follow-Up

Clinical data from the 41 patients with anti-LGI1 encephalitis who had available information at 1-year follow-up are presented in Table 2. Thirty-one patients (75%) exhibited cognitive/memory deficits, 17 (41%) showed persistent behavioral/psychiatric symptoms, 12 (29%) had seizures, and 8 (19%) had sleep alterations demonstrated by video-polysomnography. The median mRS score was 1 (IQR: 1–2). Twelve patients (29%)

Table 1 Clinical Findings of Patients With Anti-LGI1 Encephalitis Before Initiation of Immunotherapy and at the Time of Sampling

Patients with anti-LGI1 encephalitis before initial immunotherapy (n = 57) (%)	
Age at disease onset, y	68 (IQR, 58–73)
Sex	
Male	31 (54)
Female	26 (46)
Fulfilled criteria for LE	27/57 (47)
Cognitive and memory deficits	44/57 (77)
Behavioral and psychiatric symptoms	37/57 (65)
Seizures	39/57 (68)
Faciobrachial dystonic seizures	25/57 (43)
Sleep alterations	27/57 (47)
Worst mRS score	3 (IQR, 2–3)
Hyponatremia	21/42 (50)
CSF pleocytosis (WBC/μL)	4/44 (9)
Abnormal MRI (any type of inflammatory alterations)	34/57 (60)
Abnormal EEG	35/57 (61)

Abbreviations: IQR = interquartile range; LE = limbic encephalitis; LGI1 = leucine-glioma inactivated-1; mRS = modified Ranking Scale. Data are shown as median (IQR) and n (%).

reported complete recovery, 13 (32%) had mild deficits but were able to return to their previous activities and jobs, and 16 (39%) were unable to return to their premorbid activities. No association was found between clinical, electrophysiologic, or radiologic variables and the worst mRS score or functional outcome at 12-month follow-up (data not shown). We assessed the potential impact of treatment delay on 12-month outcomes by performing regression and correlation analyses with the mRS score at 12 months. These analyses did not yield statistically significant results ($p = 0.92$, Spearman $r = 0.02$, $R^2 = 0.003$).

Table 2 Clinical Findings in Patients With Anti-LGI1 Encephalitis at 12-Month Follow-Up

Patients with anti-LGI1 encephalitis at 12-mo follow-up (n = 41) (%)	
Cognitive and memory deficits	31/41 (75)
Behavioral and psychiatric symptoms	17/41 (41)
Seizures	12/41 (29)
Sleep alterations	8/41 (19)
Abnormal MRI (any type of inflammatory alterations and/or atrophy/sclerosis)	22/39 (56)
Abnormal EEG	3/37 (8)
mRS score at 12 mo	1 (IQR, 1–2)
Functional outcome (return to premorbid activities)	
Complete recovery (95%–100%)	12/41 (29)
Good recovery (75%–95%)	13/41 (32)
Partial recovery (50%–75%)	12/41 (29)
Suboptimal recovery (<50%)	4/41 (10)

Abbreviations: IQR = interquartile range; LGI1 = leucine-glioma inactivated-1; mRS = modified Ranking Scale. Data are expressed as median (IQR) or n (%).

Patients With Anti-LGI1 Encephalitis Exhibit a Highly Inflammatory Profile in the CNS and Periphery

Compared with controls, the CSF of patients with anti-LGI1 encephalitis showed significant differences in the levels of fractalkine/CX3CL1 ($p < 0.0001$), IL-10 ($p < 0.0001$), IL-18 ($p < 0.0001$), IL-6 ($p < 0.0001$), IL-8/CXCL8 ($p < 0.0001$), IP-10/CXCL10 ($p < 0.0001$), MIG/CXCL9 ($p < 0.0001$), TNF α ($p < 0.0001$), APRIL ($p < 0.0001$), BAFF ($p = 0.025$), BCA1/CXCL13 ($p < 0.0004$), granzyme B ($p = 0.0001$), and IL-21 ($p = 0.0309$) (eFigure 1, Table 3). No CSF differences were observed for the other indicated cytokines (not shown).

Compared with controls, serum samples of patients with anti-LGI1 encephalitis showed elevated levels of IL-10 ($p = 0.0062$), IL-18 ($p = 0.022$), IL-1 β ($p < 0.0001$), IL-6 ($p = 0.0009$),

IL-1RA ($p < 0.0001$), IP-10 ($p = 0.0245$), IL-35 ($p = 0.0483$), and SDF-1 ($p < 0.0001$) (eFigure 2). No significant serum differences were observed for the other indicated cytokines (not shown).

Cytokine and Chemokine Profiles Do Not Associate With Specific Symptoms in Anti-LGI1 Encephalitis

We investigated whether cytokine or chemokine analysis could distinguish clinical phenotypes of anti-LGI1 encephalitis. No significant differences were found across symptom-defined presentations before immunotherapy (i.e., cognitive, seizures, psychiatric, sleep, and autonomic dysfunction). Serum and CSF cytokine levels were similar regardless of clinical-radiologic features, including MRI alterations, limbic encephalitis, EEG changes, multiple symptoms, or 2 or fewer symptoms. We assessed whether the delay between symptom

Table 3 Cytokine and Chemokine Concentrations in Serum and CSF

	CSF		p Value
	LGI1 (n = 45)	NIND (n = 21)	
IL-10	0.83 (0.43–1.52)	0.09 (0.06–0.18)	<0.0001
IL-18	0.26 (0.14–0.39)	0.07 (0.01–0.13)	<0.0001
IL-6	2.6 (0.93–6.58)	0.46 (0.21–0.77)	<0.0001
IL-21	1.37 (0.76–4.39)	0.76 (0.48–1.23)	0.0053
GZMB	0.10 (0.10–0.50)	0.10 (0.10–0.10)	<0.0001
TNF α	3.17 (1.86–4.73)	0.51 (0.25–1.21)	<0.0001
APRIL	146.90 (79.76–228.9)	9.21 (2.77–18.27)	<0.0001
BAFF	157.80 (88.54–243.2)	98.86 (65.94–188.40)	0.0493
Fractalkine/CX3CL1	23.86 (17.75–35.30)	7.51 (6.00–13.61)	<0.0001
IL-8/CXCL8	31.42 (22.49–40.53)	8.79 (6.46–13.16)	<0.0001
IP-10/CXCL10	121.20 (62.66–193.50)	0.67 (0.67–7.42)	<0.0001
MIG/CXCL9	37.44 (13.99–68.81)	6.26 (4.34–15.90)	<0.0001
BCA1/CXCL13	0.34 (0.09–1.46)	0.03 (0.01–0.15)	0.0004
	Serum		p Value
	LGI1 (n = 49)	NIND (n = 24)	
IL-10	4.95(2.19–11.83)	1.26 (1.23–5.37)	0.0062
IL-18	87.81(46.99–169.70)	57.11(38.68–90.92)	0.022
IL-6	2.80(0.88–7.48)	0.88(0.88–1.27)	0.0009
IL-1b	10.07(7.14–13.50)	2.45(1.39–5.77)	<0.0001
IL-1RA	13.65(7.43–27.02)	4.07(1.71–7.42)	<0.0001
IL-35	10.13(4.39–25.87)	4.39(4.39–17.50)	0.0483
SDF1	3,686(3,289–4,323)	2,773 (2,432–3,268)	<0.0001
IP-10/CXCL10	101.9(61.66–198.8)	245.0(120.9–340.9)	0.0245

Abbreviations: LGI1 = leucine-glioma inactivated-1; NIND = noninflammatory neurologic disease. Data are presented as median values with interquartile range (IQR), expressed in picograms per milliliter (pg/mL).

onset and sample collection had any effect on cytokine/chemokine levels. The median delay was 3 weeks (IQR 0.8–6.5), but no significant correlation was observed with any measured cytokine or chemokine.

IL-6 CSF/Serum Ratio Is Associated With Clinical Severity

To assess the correlation between CSF/serum cytokine or chemokine ratios and acute disease severity, we analyzed paired CSF/serum samples from patients with anti-LGI1 encephalitis ($n = 34$). Patients with mRS score >2 ($n = 22$) exhibited an IL-6 CSF/serum ratio of 1.67 [IQR 0.50–7.20], whereas patients with mRS score ≤ 2 ($n = 12$) had a ratio of 0.39 [IQR 0.07–1.22] ($p = 0.0069$) (Figure 1).

Elevated IL-35 Predicts Poor Outcomes at 12-Month Follow-Up

We next examined acute-phase cytokine levels in relation to 1-year clinical outcomes. Compared with patients with good functional outcome (mRS score ≤ 2), those with worse outcomes (mRS score >2) had higher serum levels of IL-35 during the acute phase (81.34 pg/mL [IQR 22.76–97.47] vs 9.19 pg/mL [IQR 4.39–23.86]) ($p = 0.0003$) (Figure 2A). Furthermore, elevated serum IL-35 levels during the acute phase were associated with partial or suboptimal recovery at 12 months of follow-up (26.21 pg/mL [IQR 10.13–69.90] vs 7.54 pg/mL [IQR 4.39–20.09]) (Figure 2B). Descriptive analysis revealed a progressive increase in median IL-35 concentrations across mRS categories: 4.40 pg/mL for mRS score 0 ($n = 2$), 7.96 pg/mL for mRS score 1 ($n = 14$), 18.37 pg/mL for mRS score 2 ($n = 9$), and 26.21 pg/mL for mRS score 3 ($n = 5$), with extreme values observed in severe disability cases (546.51 pg/mL in mRS score 4 [$n = 1$] and 93.04 pg/mL in mRS score 6 [$n = 1$]). We

conducted a univariate logistic regression analysis of mRS scores and IL-35 levels that showed a statistically significant positive association between IL-35 levels and mRS scores (coefficient = 0.0111, $p = 0.008$). This indicates that higher IL-35 concentrations were associated with increased odds of greater clinical disability at follow-up.

Discussion

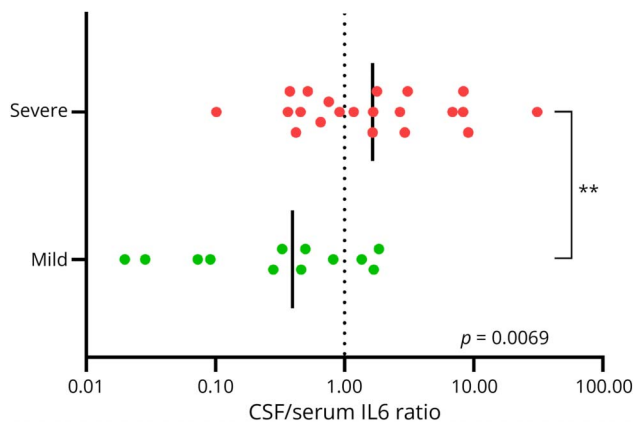
This study provides several novel findings regarding cytokines and chemokines in anti-LGI1 encephalitis and their potential use in a clinical setting. First, the disease is associated with a proinflammatory profile of cytokines and chemokines in both serum and CSF, with more prominent alterations in the CSF. Second, localized IL-6 production in CSF measured with the IL-6 CSF/serum ratio correlates with the clinical severity at diagnosis; third, elevated acute-phase serum IL-35 predicts poorer recovery at 12-month follow-up.

The increased levels of CSF cytokines, including IL-6, IL-10, IL-18, IP-10, TNF α , and CXCL13, are in line with the findings reported in other autoimmune encephalitides, such as anti-NMDAR encephalitis,^{24,32,33} and reveal a complex network of proinflammatory molecules, promoting immune cell recruitment and activation. The elevation of APRIL and BAFF, associated with T-B cell cooperation, suggests the involvement of T cells in the development of the disease, which is further supported by the strong association with the haplotypes DRB1*07:01-DQA1*02:01 and DRB1*07:01-DQA1*02:01-DQB1*02:02.^{9,10} IL-21, primarily produced by T follicular helper (T_{fh}) cells, plays a crucial role in driving B-cell differentiation and promoting the formation of long-lived plasma cells.³⁴ BCA1/CXCL13, a key chemokine for B-cell trafficking, facilitates the recruitment of B cells to the CNS.¹⁷ Elevated levels of IL-21 and CXCL13 in the CSF suggest enhanced B-cell migration, survival, and maturation, which may contribute to ongoing autoantibody production and disease persistence. In light of these findings, targeting chemokines/cytokines as an add-on therapy in anti-LGI1 encephalitis may help diminish the inflammatory environment in the CNS, as has shown promise in other CNS autoimmune diseases, such as NMOSD.²⁷

The CSF/serum cytokine ratio has proven to be a valuable marker for stratifying patients with distinct neurologic disorders.³⁰ A major strength of our study is the prospective collection of 34 paired serum-CSF samples prior to immunotherapy initiation, enabling the identification that patients with elevated IL-6 CSF/serum ratios tended to present with more severe disease at onset. Moreover, we also found that elevated IL-35 levels predicted a poorer outcome at the 12-month follow-up, as assessed by both the mRS and functional recovery (return to premorbid activities) regardless of treatment delay.

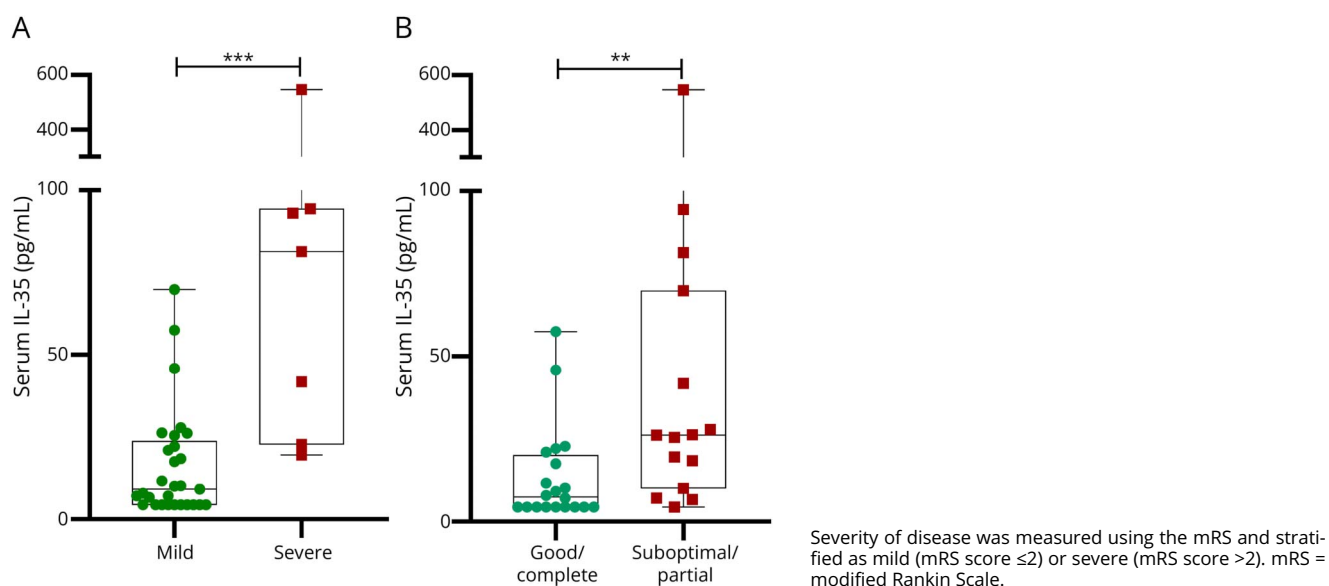
IL-35 is a heterodimeric cytokine primarily produced by regulatory T cells and involved in immune suppression and tolerance.³⁵ Elevated IL-35 levels have been reported in various

Figure 1 Box Plot Showing the IL-6 CSF/Serum Ratio in Patients With Anti-LGI1 Encephalitis Stratified by Disease Severity Based on the Modified Rankin Scale (mRS) Score



Patients with mRS score >2 ($n = 22$, red dots) exhibited a significantly higher IL-6 ratio (1.67 [IQR 0.50–7.20]) compared with those with mRS score ≤ 2 ($n = 12$, green dots) (0.39 [IQR 0.07–1.22], $p = 0.0069$). IQR = interquartile range; anti-LGI1 = anti-leucine-glioma inactivated-1.

Figure 2 Serum IL-35 Levels During the Acute Phase According to Disease Severity (A) or Ability to Return to Return to Premorbid Activities (B) at 12 Months of Follow-Up



autoimmune conditions, including systemic sclerosis, rheumatoid arthritis, and inflammatory myopathies.³⁶⁻³⁸ Conversely, decreased levels of IL-35 were observed in primary Sjögren syndrome, and a negative correlation between IL-35 and disease activity in systemic lupus erythematosus has been reported.^{39,40} These seemingly contradictory findings regarding IL-35 levels in different autoimmune diseases may reflect the complex and context-dependent role of this cytokine in immune regulation.

The lack of clinical or radiologic findings associated with disease severity or functional outcome suggests that the CSF/serum IL-6 ratio and serum IL-35 levels are independent indicators of disease severity and prognosis, respectively. The absence of correlation of clinical or radiologic findings with the outcome in our study is in line with previous published work,⁵ showing that failure to respond to first-line immunotherapy and clinical relapses were the only predictors of poor outcome. However, studies with smaller cohorts have previously reported that low cognitive reserve and bilateral hippocampal hyperintensity on initial MRI may independently predict long-term cognitive impairment.⁴

Nearly all patients with complete treatment data received both first-line and second-line immunotherapy, indicating a high degree of treatment homogeneity. This consistency in therapeutic approach suggests that the treatment regimen is unlikely to have significantly influenced patient outcomes. These findings further underscore the potential value of IL-35 as an independent prognostic biomarker for poor outcomes, reinforcing its role beyond the effects of treatment variability.

This study has limitations due to the rarity of anti-LGI1 encephalitis and its single-institution design. While a substantial

number of samples were analyzed, the cohort with long-term follow-up was relatively small. In addition, the lack of functional characterization of immune cells in the CSF restricts the ability to determine whether changes in cytokine levels are indicative of localized cellular activation or peripheral infiltration. By contrast, the centralized analysis of samples from patients prior to immunotherapy strengthens the validity of our findings.

The findings that the IL-6 CSF/serum ratio and serum IL-35 levels may serve as potential biomarkers for disease severity and outcomes could assist in risk-based patient stratification and the identification of new therapeutic targets. Prospective data analyses in larger patient cohorts are required to validate these findings.

Author Contributions

G. Muñoz-Sánchez: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. M. Guasp: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. A. Muñoz-Lopetegi: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. J.F. Luchoro: major role in the acquisition of data. E. Martínez-Hernández: major role in the acquisition of data. L. Naranjo: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. M.E. Erro: major role in the acquisition of data. Lorena Martín-Aguilar: major role in the acquisition of data. T. Iizuka: major role in the acquisition of data. M.M. Simabukuro: major role in the

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Disclosure

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Continued

Appendix (continued)

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