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ORIGINAL COMMUNICATION

Core cerebrospinal fluid biomarker profile in anti-LGI1 encephalitis

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Abstract

Objective To compare CSF biomarkers' levels in patients suffering from anti-Leucine-rich Glioma-Inactivated 1 (LGI1) encephalitis to neurodegenerative [Alzheimer's disease (AD), Creutzfeldt–Jakob's disease (CJD)] and primary psychiatric (PSY) disorders.

Methods Patients with LGI1 encephalitis were retrospectively selected from the French Reference Centre database between 2010 and 2019 and enrolled if CSF was available for biomarkers analysis including total tau (T-tau), phosphorylated tau (P-tau), amyloid-beta Aβ1-42, and neurofilaments light chains (Nf L). Samples sent for biomarker determination as part of routine practice, and formally diagnosed as AD, CJD, and PSY, were used as comparators.

Results Twenty-four patients with LGI1 encephalitis were compared to 39 AD, 20 CJD and 20 PSY. No significant difference was observed in T-tau, P-tau, and Aβ1-42 levels between LGI1 encephalitis and PSY patients. T-Tau and P-Tau levels were significantly lower in LGI1 encephalitis (231 and 43 ng/L) than in AD (621 and 90 ng/L, $p < 0.001$) and CJD patients (4327 and 55 ng/L, $p < 0.001$ and $p < 0.01$). Nf L concentrations of LGI1 encephalitis (2039 ng/L) were similar to AD (2,765 ng/L) and significantly higher compared to PSY (1223 ng/L, $p < 0.005$), but significantly lower than those of CJD (13,457 ng/L, $p < 0.001$). Higher levels of Nf L were observed in LGI1 encephalitis presenting with epilepsy (3855 ng/L) compared to LGI1 without epilepsy (1490 ng/L, $p = 0.02$). No correlation between CSF biomarkers' levels and clinical outcome could be drawn.

Conclusion LGI encephalitis patients showed higher Nf L levels than PSY, comparable to AD, and even higher when presenting epilepsy suggesting axonal or synaptic damage linked to epileptic seizures.

Keywords LGI1 encephalitis · CSF biomarkers · Epilepsy · Neuronal damage

Introduction

Patients with encephalitis associated with antibodies against Leucine-rich Glioma-Inactivated 1 (LGI1) frequently exhibit persistent cognitive impairments, evoking neurodegenerative processes [1, 2]. Whether these dementia-like features are

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a consequence of a neuronal damage or a functional synaptic disturbance is unclear. In the few neuropathological cases available, no massive neuronal loss was observed [3, 4]. However, longitudinal Magnetic Resonance Imaging (MRI) studies have shown that persistent cognitive deficits in some LGI1 encephalitis patients was accompanied by a pronounced hippocampal atrophy [2, 5] and an alteration of the hippocampal connectivity [1]. These imaging studies support a link between impaired cognitive functions and neuronal and axonal damages in LGI1 encephalitis.

The core neurodegenerative cerebrospinal fluid (CSF) biomarkers, i.e.Total tau (T-tau), phosphorylated tau (P-tau), and amyloid-β peptide (Aβ) levels are classically used to reflect Alzheimer's disease (AD) pathology [6]. More recently, CSF neurofilament light chain (Nf L) levels have been used to reflect axonal damages in different neurodegenerative and neuroinflammatory conditions [7–9]. However, CSF biomarkers have been analyzed in very few LGI1 encephalitis cases with conflicting results and without comparison to patients with classical neurodegenerative dementias, such as AD or Creutzfeldt–Jakob's disease (CJD) [10–12]. The main aim of this study was to report the CSF biomarker profiles of LGI1 encephalitis patients and to compare it to other neurodegenerative diseases. We also examined if these biomarkers may provide clues about the complex underlying pathophysiological mechanisms of LGI1 encephalitis.

Methods

Patients' selection

Patients with LGI1 encephalitis were retrospectively selected from the database of the Centre de Référence National pour les Syndromes Neurologiques Paranéoplasiques (French reference center for paraneoplastic syndromes and autoimmune encephalitis). Patients fulfilled the current diagnostic criteria for definite autoimmune limbic encephalitis [13] and the detection of anti-LGI1 IgG was performed in the serum and/or CSF by indirect immunofluorescence on rat brain sections and cell-based assay with human embryonic kidney cells (HEK 293) transfected with the LGI1 protein, as previously described [14].

Among adult LGI1 encephalitis patients with clinical onset between 2010 and 2019, those for whom at least one CSF sample had been tested for core neurodegenerative biomarkers—T-tau, P-tau, Aβ1-42 proteins—and/or Neopterin in the regional reference Neurochemistry department (Hospices Civils de Lyon, Groupement Hospitalier Est, Lyon) were included.

For CSF biomarker profile comparison, control, typical and rapidly progressive AD, and CJD populations were selected from the Neurochemistry Unit database. The diagnosis of AD cases had been validated in multidisciplinary consultation meetings according to international diagnosis criteria including evidence of the AD pathophysiological process [15]. Rapidly progressive AD was defined by a decrease of 3 or more points in the Mini-Mental State Examination (MMSE) score per 6-month period [16]. All CJD cases were sporadic and had been confirmed by autopsy according to the updated WHO diagnostic criteria [17]. Psychiatric cases were non-neurological patients suffering from depressive syndrome associated with a cognitive complaint, an absence of progression during the 2-year follow-up, and a normal CSF biomarker profile [18].

Clinical and paraclinical data of LGI1-E patients

Detailed clinical data on acute disease stages were obtained at the time of biological diagnosis and data regarding the clinical course of the disease were collected during follow-up examinations. An acute onset was defined as sudden installation (in less than one day). A subacute onset was defined by a delay of 3 months or less between the first symptoms and the clinical nadir. A progressive onset was defined by more than 3 months between the first symptom and the clinical nadir.

The initial clinical presentation was also registered: cognitive impairment, behavioral changes, generalized or focal seizures, facio-brachial dystonic seizures (FBDS), and sleep disturbances. The type of cognitive impairment (anterograde amnesia and/or executive dysfunctions) was also specified, as well as the presence of psychiatric or behavioral changes, such as apathy, disinhibition, impulsivity, or loss of empathy. FBDS were defined as very brief daily attacks with a dystonic posture of the arm, accompanied or not by a facial contraction with a possible leg participation [19]. Movements described as frequent twitches or pseudo-myoclonus affecting the arm and ipsilateral face were assimilated to FBDS.

Information about immunosuppressive treatments undergone during the course of the disease– intraveinous immunoglobulin (IVIg), glucocorticoids, cyclophosphamide, rituximab—was collected.

The clinical disability was assessed using the modified Rankin Scale (mRS) at the initial stage and at 24 months. MMSE scores at diagnosis and at 6, 12 and 24 months of follow-up were collected.

The results from the initial ancillary examinations (CSF analysis, sodium blood levels, EEG analysis) were compiled. Concerning CSF analysis, pleocytosis was defined as increased cell count > 5 leucocytes/µl in CSF and intratechal synthesis was defined as the presence of specific oligoclonal bands in the CSF. The presence of uni- or bilateral MRI hyper T2 signals in temporomesial areas was recorded, as well as the brain metabolic changes assessed by 18

Fluoro-Deoxy-Glucose Positron Emission Tomography (18F-FDG PET).

Cerebrospinal fluid biomarkers analysis

All CSF samples were collected in a standardized polypropylene tube (Sarstedt ref. 62.610.201) and stored at −80 °C until analyses. CSF neopterin concentrations were determined as previously described using high-performance liquid chromatography coupled with fluorimetric detection. The cut-off value used was 5 nmol/L [20].

The CSF concentrations of Aβ1-42, T-tau, and P-tau181 were routinely measured using the standardized commercially available sandwich ELISA kit (INNOTEST®) according to the manufacturer's instructions (Fujirebio, Ghent, Belgium). For each CSF sample, Aβ1-42, T-tau, and P-tau181 biomarkers were simultaneously analyzed, the cut-off values defining a positive AD CSF biomarker profile were: T-tau ≥ 350 ng/L, P-tau181 ≥ 60 ng/L, and Aβ1-42 ≤ 700 ng/L [21]. When there was still CSF available for further analysis, concentrations of Nf L were measured in one batch and with maximum two freeze-thraw cycle for each sample using commercially Uman Diagnostics Nf L ELISA kits (NF-light® ELISA #10–7001, Umea, Sweden) according to the recommended standard operating procedure [22]. The Neurochemistry Unit is involved in quality control schemes organized by The Alzheimer's Association QC program for CSF and blood biomarkers and managed by the Goteborg University.

Biostatistical analysis

Statistical comparisons were made with R-statistic®. Categorical variables were compared with the Fisher's test. Median comparisons were perfomed using the Wilcoxon–Mann–Whitney test considering the limited sample size, and multiple mean comparisons were performed using the Kruskal–Wallis test. Linear correlation between two continuous variables was assessed using the Spearman test. p values < 0.05 were considered as significant.

Results

Characteristics of patients with LGI1 encephalitis

A total of 24 patients with LGI1 encephalitis were enrolled in the study between May 2010 and January 2019. The group was composed of 10 females (42%), with a median age of 69 years (range 56–86). Five LGI1 encephalitis patients (21%) had a history of autoimmune disease, and 4 (17%) presented with preexisting cognitive complaints. The median delay from symptoms onset to diagnosis was 100 days (range 4–716). The onset was acute in 9 (39%), subacute in 10 (44%), and progressive in 4 (17%) patients. Initial symptoms included anterograde mnesic disorders in 19 (83 %), behavioral disorders in 14 (61%), epileptic seizures in 14 (61%), and FBDS in 12 (52%) patients. Sleep complaints were registered in 7 (30%) patients.

A brain MRI was performed at the initial stage of the encephalitis in 22/24 (91%) patients. On T2-weighed sequences, a hypersignal of the medial temporal lobe was observed in 9 (41%) patients. Among the 22 patients with available serum sodium concentration data, 18/22 (82%) presented hyponatremia (<135mmol/L). A moderate CSF pleocytosis (range 5–18 white cells/mm^3) was detected in 6/19 (32%) patients, and intrathecal synthesis was present in 3/15 (20%) patients. Initial EEG abnormalities were described in 14 (60%) of 23 patients with available recordings; they consisted of focal or diffuse slowing (n = 8), and/or ictal activity (n = 11).

LGI1 encephalitis diagnosis was based on the detection of LGI1 antibodies (Abs) in the serum in 18/24 (75%) patients, and/or in the CSF in 21/24 (88%) patients. In two cases, only the serum was positive. LGI1-Abs remained detectable in the serum of 9 out of 12 patients upon long-term follow-up. The median (range) delay from the first reported symptoms to sampling and first immunologic assessment was 113 (4–725) days for CSF and 77 (4–716) days for sera.

Details about immunosuppressive treatments were available for 23 patients. The median (IQR) delay between the first related symptoms and the first treatment was 3.8 months (1.6–5.7). Only 1 out of the 23 patients never received immunotherapy, whereas 20/23 (87%) received IVIg and 19/23 (83%) received oral or parenteral glucocorticoids during the course of the disease. Twelve (52%) patients received cyclophosphamide and 10/23 (43%) received rituximab.

Upon initial assessment, the mRS score was determined for 23 patients; among them, only 5/23 (22%) had a mRS score <3. At 24 months of disease course, the mRS score was determined for 17 LGI1 encephalitis patients. Among them, 13/17 (76%) had a mRS score of 0, 1 or 2. One patient died 70 days after the first symptoms because of encephalitis complications (status epilepticus), despite early treatment (at 40 days).

Upon initial evaluation, the median MMSE score for 20 patients was 23 (range 11–30). Fourteen LGI1 patients were evaluated at 12 months with a median MMSE score of 27 (range 12–30), and 9 were evaluated at 24 months with a median MMSE score of 28 (range 21–30). Detailed characteristics of patients and paraclinical data for each case are provided in Table 1.

Table 1 Detailed clinical and paraclinical data of all 24 LGI1 encephalitis patients

Patient	Sex, age	Clinical	Hyponatremia	Before treatment	Delay (months)	LGI1 first serology	T-tau (ng/L)	P-tau (ng/L)	Aβ 1–42 (ng/L)	14.3.3	Nf L (ng/L)	Neopt (nmol/L)	IT	MMSE at 6 months	mRS at 24 months
1	M, 65	Ep, Psy, Mn, sleep	Yes	Yes	0–3	+ on CSF, N.a. on ser	383	–	554	Neg	–	7	IVIg, CTC	–	1
				Yes	0–3		307	59	1145	Neg		5			
2	M, 68	FBDS	No	Yes	0–3	+ on ser,− on CSF	133	28	827	Neg	2758	3	IVIg, CTC	–	0
3	M, 69	Ep, Psy, Mn, sleep	No	Yes	4–6	+ on ser,− on CSF	209	39	956	Neg	2039	6	IVIg, CTC	30	
4	F, 75	Mn	No	No	0–3	+ on ser and CSF	182	37	976	–	1490	6	No treatment	–	
5	F, 56	Psy, Mn	Yes	Yes	4–6	+ on ser and CSF	1200	22	–	Neg	–	–	IVIg, CTC, CPH, Rit	–	3
				No	7–18		57	20	–	Neg	4869	–			
6	M, 57	Mn, sleep	Yes	Yes	0–3	+ on CSF, N.a. on ser	187	31	917	Neg	884	7	IVIg, CTC	25	1
				Yes	4–6		820	47	–	Int	5630	–			
7	M, 62	Psy, Mn, sleep	Yes	Yes	19–23	+ on ser and CSF	170	34	916	Neg	1577	–	IVIg, CPH, Rit	–	3
				No	> 24		–	–	–	Neg	982	5			
				No	> 24		160	32	1063	–	–	4			
				No	> 24		–	–	–	–	–	18.7			
8	M, 63	Ep, FBDS, Psy, Mn	Yes	Yes	0–3	+ on ser and CSF	431	58	1336	Neg	1790	4	IVIg, CTC, CPH	26	0
9	F, 73	Ep, FBDS, Psy, Mn, sleep	Yes	Yes	4–6	+ on CSF, N.a. on ser	177	33	672	Neg	3855	6	IVIg, CTC	11	0
10	F, 73	Ep, Psy, Mn,	Yes	Yes	0–3	+ on CSF, N.a. on ser	238	44	359	Neg	1944	–	IVIg, CTC	–	3
11	M, 73	Ep, FBDS, Psy, Mn	Yes	Yes	4–6	+ on ser and CSF	201	42	1099	Neg	934	4	IVIg, PE, CTC, CPH, Rit	25	3
				No	7–18		–	–	–	–	–	3			
12	M, 72	Psy, Mn, sleep	Yes	Yes	0–3	+ on ser and CSF	77	20	684	Neg	1106	3	IVIg, CTC, CPH, Rit	21	1
				Yes	4–6		–	–	–	–	–	4			
13	M, 79	Ep, FBDS, Mn	Yes	Yes	0–3	+ on CSF, N.a. on ser	673	54	1205	Neg	4273	8	IVIg, CTC, CPH	28	1
				No	7–18		389	43	–	–	2701	–			
14	F, 59	FBDS, Psy,	Yes	Yes	0–3	+ on ser and CSF	–	–	–	Neg	–	3	IVIg, CTC, CPH, Rit	–	5

Table 1 (continued)

Patient	Sex, age	Clinical	Hyponatremia	Before treatment	Delay (months)	LGI1 first serology	T-tau (ng/L)	P-tau (ng/L)	Aβ 1–42 (ng/L)	14.3.3	Nf L (ng/L)	Neopt (nmol/L)	IT	MMSE at 6 months	mRS at 24 months
15	F, 63	Ep, FBDS	Yes	No	0–3	+ on ser and CSF	2050	45	1025	Int	32,084	9	IVIg, CTC, CPH	27	2
				No	7–18		371	64	1242	Neg	4987	–			
				No	19–23		–	–	–	Neg	2871	6			
16	F, 71	Ep, FBDS, Psy, Mn	Yes	Yes	0–3	+ on ser and CSF	364	69	1306	Neg	2701	8	IVIg, CTC	–	1
17	F, 74	Ep, FBDS, Psy, Mn	Yes	Yes	0–3	+ on ser and CSF	340	56	572	Neg	4015	7	IVIg, CPH, Rit	10	3
				Yes	4–6		–	–	–	–	–	5			
				No	19–23		366	62	467	–	1561	–			
18	M, 62	N.a	N.a	Yes	0–3	+ on CSF, N.a. on ser	–	–	–	Neg	313	3	N.a	–	–
19	M, 65	FBDS, Mn	N.a	Yes	0–3	+ on CSF, N.a. on ser	–	–	–	Neg	2079	6	IVIg, CTC	25	1
20	M, 84	FBDS, Mn	Yes	Yes	0–3	+ on ser and CSF	–	–	–	Neg	–	8	IVIg, CPH	–	6
21	F, 74	Ep, FBDS, Mn	Yes	Yes	4–6	+ on CSF, N.a. on ser	425	50	1199	Neg	–	14.4	CTC, Rit	22	
22	M, 61	Ep, FBDS, Psy, Mn, sleep	Yes	Yes	4–6	+ on ser and CSF	136	29	1151	Neg	–	3.4	IVIg, CTC, CPH, Rit	26	–
				No	7–18		143	33	900	Neg	–	4.6			
23	F, 86	FBDS	No	Yes	0–3	+ on ser, − on CSF	387	56	432	Neg	–	3.1	CTC, Rit	–	–
				Yes	0–3		–	–	–	–	–	3.2			
24	M, 74	Ep, Psy, Mn	Yes	Yes	0–3	+ on ser and CSF	223	44	1240	–	–	–	IVIg, CTC, CPH, Rit	26	–

Clinical and paraclinical information at first admission including age, sex, first symptoms, hyponatremia (Natremia < 135 mmol/L), anti-LGI1 antibody (CSF/serum) positivity, cell count, as well as MMSE score at 6 months and modified Rankin scale (mRS) at 24 months when available and the cumulative immunosuppressive treatment (IT). CSF Biomarker concentrations including Total tau protein (T-tau), phosphorylated tau (P-tau), β-amyloid peptide 1–42 (Aβ42), Neopterin (Neopt) and neurofilament light chain (Nf L) are assessed at variable times of the follow-up. To specify the time of sampling, a range in months from the first symptom is indicated along with its completion before or after the immunosuppressive treatment

Ep epileptic seizures, *FBDS* facio-brachial dystonic seizures, *Mn* anterograde mnesic disorders, *Psy* psychiatric/behavioral disorders, *Sleep* sleep complaints, *ser* serum, "–" unavailable numeric data, *N.a.* unavailable qualitative information

CSF biomarker profiles of LGI1 encephalitis

A total of 41 CSF samples from the 24 patients with LGI1 encephalitis were analyzed (Table 2). The median delay between the first symptoms and the first CSF sampling was 73 days (IQR 34–134) as detailed for each patient in the Table 1. Twenty-one out of 24 (88%) patients had their first sampling within six months after disease onset. When several samples were available for a patient, we included only the first for assessment of biomarker levels.

The initial T-tau concentration was available for 20 patients (median at 231 ng/L) and 8/20 (40%) had a T-tau level higher than the AD cut-off value. The initial P-tau-181 median concentration (n =20) was 43 ng/L, and only 1/20 (5%) patient had a P-tau-181 higher than the AD cut-off value. The initial Aβ1-42 median level (n = 19) was 956 ng/L, and 6/19 (32%) patients had Aβ1-42 concentrations below the AD cut-off value. No LGI1 encephalitis patient presented a CSF biomarker profile consistent with AD diagnosis as defined in the methods section. Thirteen out of 24 (54%) of LGI1 patients had at least one abnormal CSF core AD biomarker level. CSF levels of neopterin were slightly higher than the cut-off value for 11/18 (61%) patients. The initial Nf L median level (n = 17) was 2,039 ng/L.

A total of 17 patients were sampled at least twice during follow-up and, when these data were analyzed altogether, there was no significant difference between the first and last CSF biomarker levels (data not shown). However, biomarkers levels differed greatly between first and last sampling for a few patients of our cohort. In particular, 2 patients (case 5 and case 15, Table 1) presented high initial T-tau and Nf L levels that drastically decreased over time.

Comparisons of CSF biomarker profiles between patient groups

Eighteen typical AD (tAD), 21 rapidly progressive AD (rpAD), 20 sporadic CJD, and 20 non-neurological psychiatric patients (PSY) were compared to LGI1 encephalitis patients (Table 2, Fig. 1). There was no significant difference in sex ratio between the LGI1 encephalitis and other patients' groups. Patients from PSY group were younger than LGI1 encephalitis, AD and CJD patients ($p < 0.05$).

Initial T-tau protein concentrations in the CSF were similar in PSY patients and LGI1 encephalitis but significantly lower in these two groups compared to tAD, rpAD, and CJD patients ($p < 0.001$), and the same was observed for P-tau levels.

Initial Aβ1-42 levels were significantly higher in LGI1 encephalitis patients compared to tAD and rpAD patients, but were similar to CJD and PSY patients. No correlation was found between Aβ1-42 and neopterin levels and

Table 2 Demographic and initial CSF biomarker levels of LGI1 encephalitis, psychiatric controls, typical and rapid AD, and CJD patients

	LGI1-E (n=24)	PSY (n=20)	tAD (n=18)	rpAD (n=21)	CJD (n=20)	p value
Demography						
Age, years, median (range)	69 (56–86)	62 (43–79)	73 (53–83)	74 (57–85)	68 (50–88)	$<0.05^{*1}$
Female (%)	14 (58)	11 (55)	10 (56)	15 (71)	13 (65)	NS
CSF core biomarkers						
T-tau, No median, ng/L (IQR)	20 231 (181–397)	20 204 (64–248)	18 528 (426–665)	21 707 (555–900)	20 4327 (2277–6749)	$<0.001^{*2,*3,*4}$
P-tau, No median, ng/L (IQR)	20 43 (33–55)	20 38 (20–46)	18 74 (66–97)	21 97(75–127)	20 55(47–68)	$<0.001^{*2,*3}<0.01^{*4}$
Aβ1 – 42, No median, ng/L (IQR)	19 956 (678–1175)	20 1010 (434–1144)	18 571 (441–606)	21 562 (452–693)	20 712 (581–1081)	$<0.01^{*2,*3}$
Nf L, ng/L, No median, ng/L (IQR)	17 2039 (1490–3855)	20 1223 (291–1566)	18 2616 (2044–6003)	21 2974 (2189–2974)	20 13457 (10301–18540)	$<0.05^{*1}<0.001^{*4}$

LGI1-E LGI1 encephalitis, *PSY*: psychiatric controls, *tAD* typical Alzheimer's disease, *rpAD* rapidly progressive Alzheimer's disease, *CJD* Creutzfeldt–Jakob's disease patients, *IQR* interquartile range, *NS* not significant

$^{*1}p$ value for LGI1-E vs PSY comparisons

$^{*2}p$ value for LGI1-E vs tAD comparisons

$^{*3}p$ value for LGI1-E vs rpAD comparisons

$^{*4}p$ value for LGI1-E vs CJD comparisons

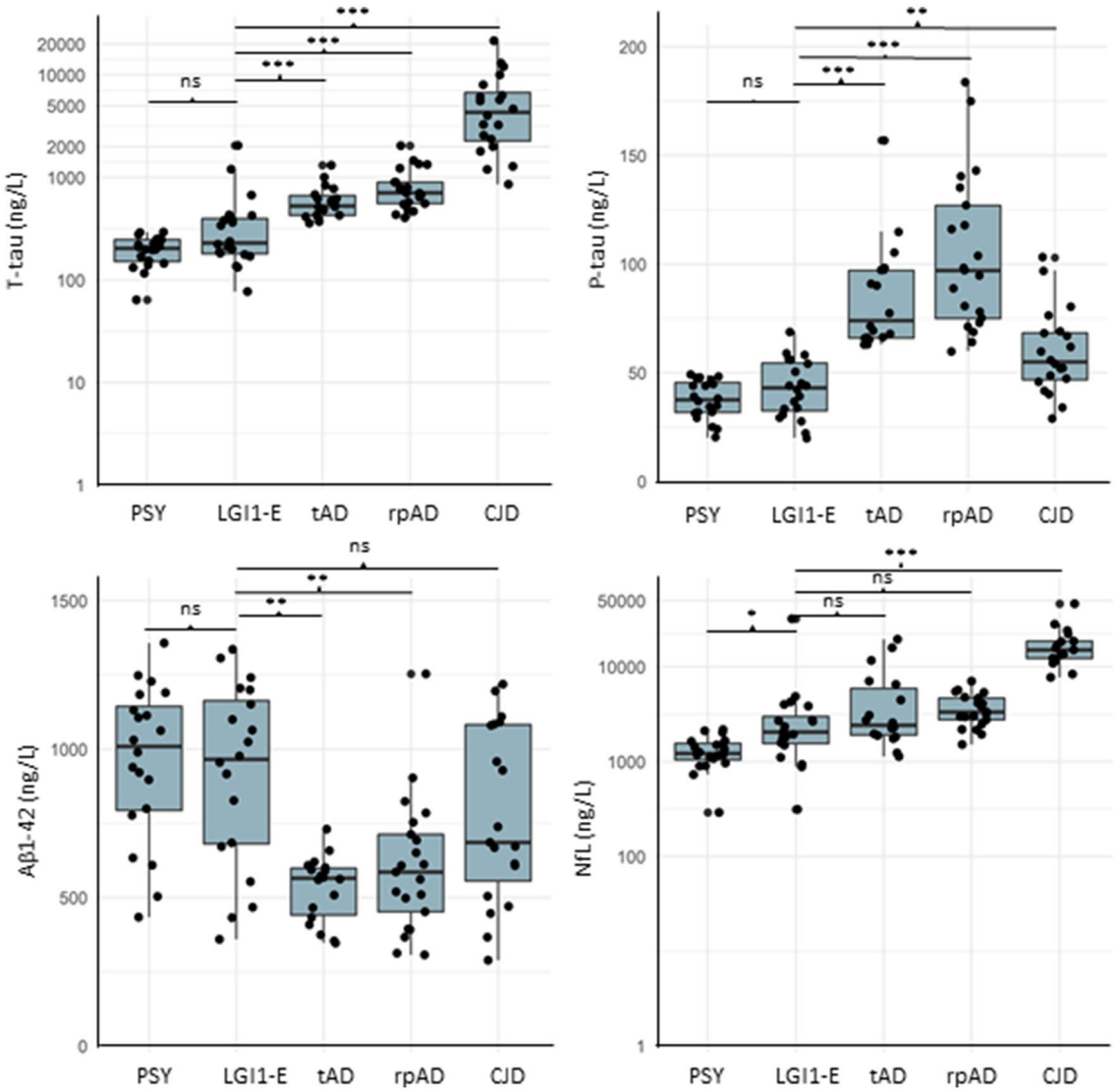

Fig. 1 Box plot showing core CSF biomarker levels in LGI1 encephalitis (LGI1-E), psychiatric control (PSY), typical Alzheimer's disease (tAD), rapidly progressive AD (rpAD), and sporadic autopsy-confirmed Creutzfeldt–Jakob's disease (CJD) patients. LGI1-E data concern the initial biomarker samples. *PSY* non-neurological psychiatric controls, *LGI1-E* anti-LGI1-associated encephalitis; *tAD* typical Alzheimer's disease; *rpAD* rapidly progressive Alzheimer's disease; *T-tau* total tau protein; *P-tau* 181 phosphorylated tau protein; *Aβ1-42* Aβ1-42 peptid; *Nf L* neurofilament light chain; *ns* non-significant; $*p<0.05$; $**p<0.01$; $***p<0.001$. All biomarkers levels here are reported in ng/L. T-tau and Nf L levels are displayed on a base10 logarithmic scale

means of Aβ1-42 levels did not differ between patients with negative versus positive neopterin CSF levels ($p = 0.6$, data not shown).

On the contrary, Nf L concentrations in the CSF were significantly higher in LGI1 encephalitis patients compared to PSY group, but significantly lower than those of CJD patients. However, comparison to tAD and rpAD patients did not reach statistical significance.

Clinico-biological correlations in LGI1 encephalitis patients

Patients with LGI1 encephalitis who presented clinical or electrophysiological generalized or focal seizures before CSF biomarkers sampling were compared with LGI patients without epileptic seizures. Nf L concentrations were significantly higher in LGI1 epileptic patients

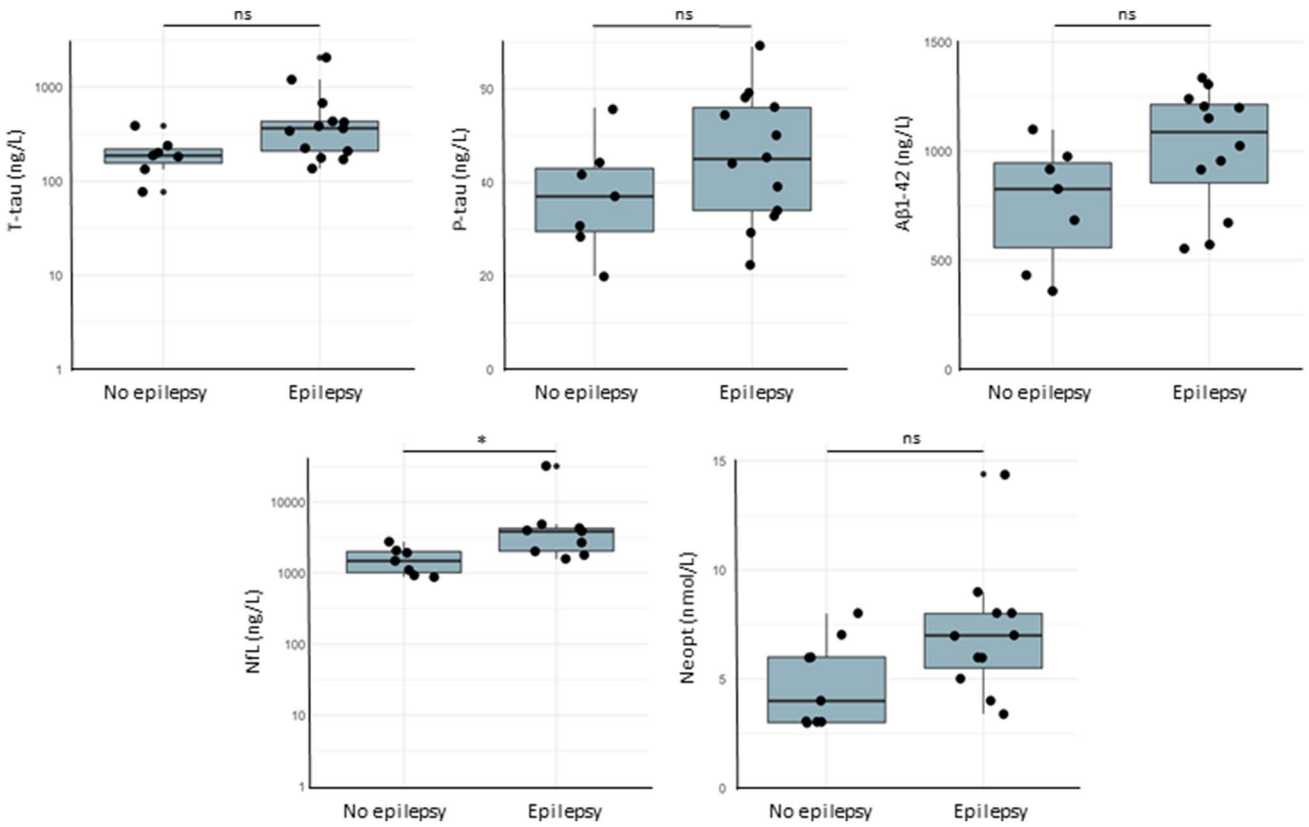

Fig. 2 Box plot showing CSF biomarker levels in LGI1 encephalitis according to the presence or absence of epileptic seizures before sampling. All biomarker levels are expressed in ng/L except for Neopterin (nmol/L). T-tau and Nf L levels are displayed on a base10 logarithmic scale. *ns* non-significant.*$p < 0.05$. *T-tau* total tau protein; *P-tau* 181 phosphorylated tau protein; *Aβ1-42* Aβ1-42 peptid; *Nf L* neurofilament light chain, *Neopt* neopterin

compared to encephalitic patients without epileptic seizures ($p = 0.02$; Table 3 and Fig. 2). There was also a trend towards higher CSF neopterin levels in the epileptic group ($p = 0.051$). Nf L levels did not differ between non-epileptic LGI1 patients and PSY group ($p = 0.06$).

No differences in CSF biomarkers were observed between LGI1 encephalitis patients presenting with FBDS or not (Table 3 and Fig. 3). There was also no difference in the levels of CSF biomarkers according to the presence of temporo-mesial T2 hypersignal on MRI or brain metabolic changes on 18F-FDG PET (data not shown). No effect of immunosuppressive treatment initiation on CSF biomarkers was detected (Table 3). The initial Aβ1-42 levels in the CSF were positively correlated with the MMSE score at 6 ($p < 0.01$) and 12 months ($p = 0.01$) after the disease onset. No other prognostic correlation was found between any CSF biomarkers and the MMSE scores or the mRS scores at first clinical assessment and during follow-up.

Discussion

In our cohort of 24 LGI1 encephalitis patients, CSF T-tau, P-tau, and Aβ1-42 levels did not differ from levels detected in patients with psychiatric conditions associated with non-neurological cognitive impairment. Moreover, no LGI1 patient presented with CSF biomarker profile consistent with AD pathology. However, Nf L concentrations of LGI1 encephalitis were significantly higher compared to PSY and similar to AD, but significantly lower than those of CJD. Among the LGI1 encephalitis, higher levels of Nf L were observed in patients presenting with epileptic seizures before sampling.

The comparison of our data with existing literature is rendered difficult by the heterogeneity of previous studies often reporting CSF biomarkers level in several antibody-mediated encephalitis. The very small number of LGI1 encephalitis patients included in those series (3 to 11

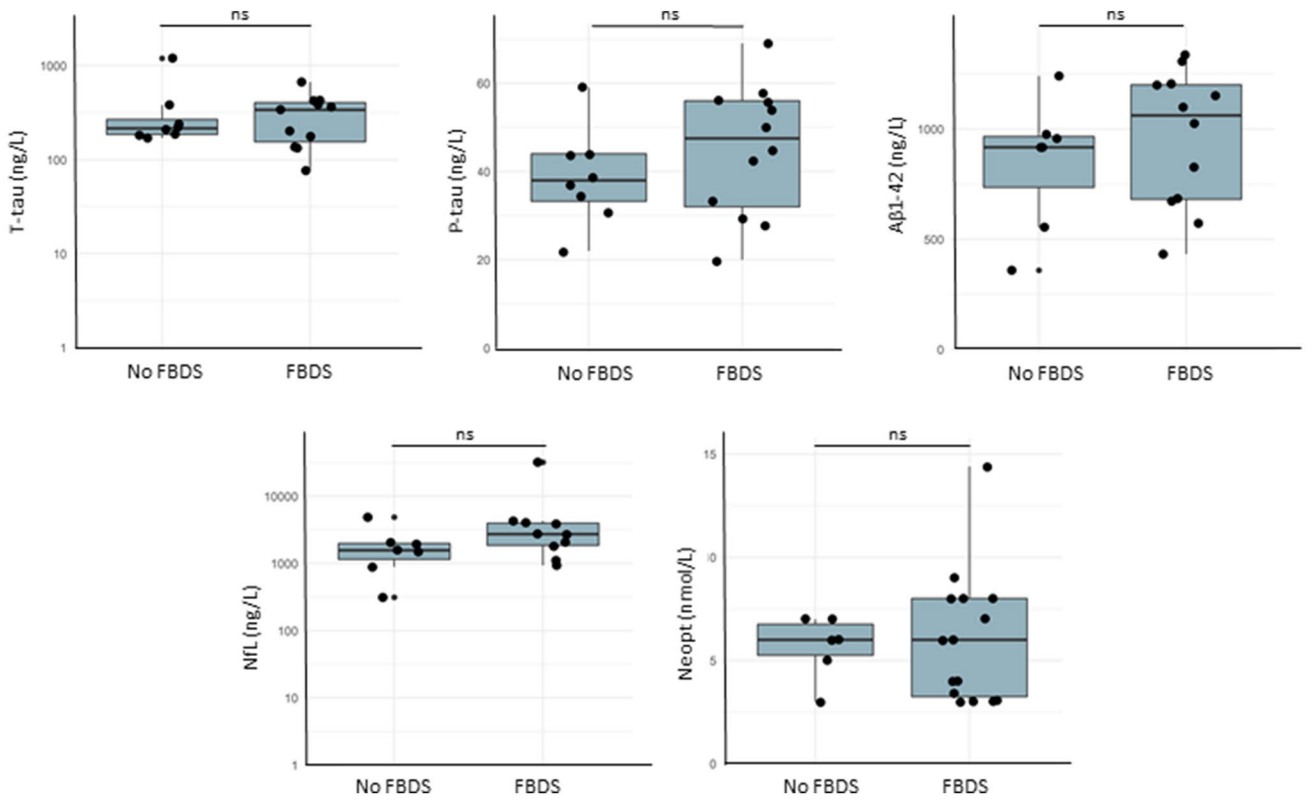

Fig. 3 Box plot showing CSF biomarker levels in LGI1 encephalitis according to the presence or absence of facio-brachial dystonic seizures (FBDS) before sampling. All biomarker levels are expressed in ng/L except for Neopterin (nmol/L). T-tau and Nf L levels are displayed on a base10 logarithmic scale. *ns* non-significant. *T-tau* total tau protein; *P-tau* 181 phosphorylated tau protein; *Aβ1-42* Aβ1-42 peptid; *Nf L* neurofilament light chain; *Neopt* neopterin

in each) does not allow conclusions to be drawn in that respect [10–12]. Nonetheless, taking into account the limited sample size and potential interlaboratory variability, our results were consistent with previous studies [10–12].

CSF T-tau levels did not differ between LGI1 patients and PSY patients. These results are consistent with normal CSF T-tau levels assessed in 11 LGI1 or CASPR2 encephalitis cases described elsewhere [11], suggesting either that neuronal integrity is maintained during the acute phase of the encephalitic disease or that CSF T-tau levels is not a reliable biomarker to reflect a possible neuronal damage in LGI1 patients.

Interestingly, CSF Nf L levels were higher in LGI1 patients than in PSY patients. Even if an estimated two percent per year increase of neurofilaments has been described in the general population, difference observed in our study between PSY and LGI1 groups overwhelmed largely the age effect on Nf L values [23]. Levels were corresponding to those found in AD patients without reaching levels observed in CJD patients. Such an increase in Nf L concentrations has been previously reported in a cohort of 25 antibody-mediated encephalitis including 9 LGI1 patients [24], but data for the LGI1 encephalitis subgroup were not available. Knowing the implication of Nf L in the synaptic structure, these increased Nf L levels in LGI1 patients support the hypothesis of ongoing axonal and/or synaptic damages [25]. This observation is consistent with the frequence of temporomesial atrophy on MRI and the incomplete cognitive recovery in most cases [1, 26]. LGI1 encephalitis patients presenting with epileptic seizures before sampling had higher CSF Nf L concentrations than LGI1 seizure-naive patients for which Nf L levels were similar to controls. This correlation between seizures and Nf Llevels is observed at an individual scale for few patients in our cohort. Indeed, two patients displaying important decrease in Nf L levels during disease course had experienced epileptic seizures before their first sampling (cases #13 and #15; 15 days between seizures and the first CSF sampling). Moreover, CSF Nf L levels remained very high for these two patients even after a second sampling 198 days and 284 days without seizures, respectively. Conversely, one patient with normal initial Nf L levels and great increase at day 28 had a first epileptic seizure 24 hours before the second sampling (case #6). These data did not permit to determine if there is a minimal time delay between seizure and sampling to avoid the effect of seizures on CSF concentrations.

Table 3 Biomarker profile of LGI1 encephalitis patients according to their clinical features

	Total LGI1-E ($n=24$)	Pre-T LGI1-E ($n=22$)	Post-T LGI1-E ($n=10$)	With epilepsy ($n=13$)	Without epilepsy ($n=9$)	With FBDS ($n=15$)	Without FBDS ($n=8$)
T-tau, No median, ng/L (IQR)	20 231 (181–397)	18 231 (189–386)	7 160 (120–366)	13 364 (209–431)	7 187 (158–220)	12 352 (167–427)	8 216 (186–274)
P-tau, No median, ng/L (IQR)	20 43 (33–55)	18 43 (32–56)	7 33 (27–53)	13 45 (34–56)	7 37 (30–43)	12 48 (32–56)	8 38 (33–44)
Aβ1-42, No median, ng/L (IQR)	19 956 (678–1175)	17 917 (672–1199)	5 900 (762–1063)	12 1088 (855–1214)	7 827 (558–947)	12 1062 (681–1201)	7 917 (735–966)
Neopterin, No median nmol nmol/L (IQR)	20 6.0 (3.3–7.3)	18 6.0 (3–7)	3 4.6 (3.8–8.2)	11 7.0 (5.5–8.0)	9 4.0 (3.0–6.0)	15 6.0 (3.3–8.0)	6 6.0 (5.3–6.8)
Nf L, No median, ng/L (IQR)	17 2039 (1490–3855)	14 1992 (1224–2744)	4 1555 (1124–2416)	9 3855* (2039–4273)	7 1490 * (1020–2012)	10 2730 (1862–3975)	7 1577 (1187–1992)

"Total LGI1-E" column indicates the initial biomarker samples of all LGI1-E patients

"Pre-T LGI1-E" column indicates first pre-therapeutic biomarkers of LGI1-E patients

"Post-T LGI1-E" column indicates the post-therapeutic biomarker samples of LGI1-E patients

"With epilepsy" column indicates the initial biomarker samples of LGI1-E patients (1 sample per patient) with epileptic seizure preceding sampling

"Without epilepsy" column indicates the initial biomarker samples of LGI1-E patients (1 sample per patient) without epileptic seizures preceding sampling

"With FBDS" column indicates the initial biomarker samples of LGI1-E patients (1 sample per patient) with FBDS preceding sampling

"Without FBDS" column indicates the initial biomarker samples of LGI1-E patients (1 sample per patient) without FBDS preceding sampling

LGI1-E LGI1 encephalitis, *FBDS* facio-brachial dystonic seizures, *IQR* interquartile range, *No* number of assessable cases

*Indicates significant difference ($p<0.05$) between LGI1-E with preceding epilepsy vs LGI1-E without preceding epilepsy

These results suggest that Nf L CSF levels could reflect their synaptic release due to direct disturbances of synaptic integrity by seizures [27], and/or to the presence of an active neuro-inflammation, as suggested by the trend towards higher CSF neopterin levels in the epileptic group [28, 29]. Conversely, a possible explanation for higher Nf L levels in LGI1 encephalitis patients even without epileptic clinical features could be the occurrence of infraclinical or unreported ictal events.

Contrary to epileptic seizures, no significant impact of facio-brachial dystonic seizures (FBDS) on CSF biomarker level was detected, highlighting the uncertainty regarding the nature and origin of this hallmark of LGI1 encephalitis. Although some studies have pointed toward a cortical origin of FBDS, their epileptic nature is stillunder debate [14, 19, 30]. The frequent concomitanceof FBDS and focal epilepsy seizures is a major confounding factor in assessing its mechanisms [19].

A quarter of LGI1 patients had an isolated abnormal CSF concentration of Aβ1-42 protein, suggesting that an amyloid pathology might occur in some LGI1 encephalitis cases. Moreover, there was a positive correlation between Aβ1-42 levels and the MMSE score at 6 and 12 months of evolution in our LGI1 cohort. The decrease of Aβ1-42 levels in the CSF of elderly patients has been shown to correlate with abnormal extracellular amyloid depositions in post-mortem studies [31]. Thus, this amyloid pathology may interfere with the cognitive recovery of LGI1 encephalitis. Indeed, brain amyloid deposition has been correlated to late onset epilepsy in the general elderly population [32]. Therefore, we can hypothesize that this amyloid alteration could promote a certain degree of neuronal hyperexcitability and thus contribute to refractoriness of the disease and poor cognitive outcome.

Several issues arise from the retrospective design of our study. First, there is a notable variability in the time from disease onset to first CSF sampling which accurately reflects diagnostic delay frequently experienced by LGI1 encephalitis patient before referral in expert center. Nevertheless, the majority (88%) of patients' first CSF sampling occurred within 6 months after the first symptoms and we deemed probable that they were still going through the active phase of the disease. However, it could account partly for the variability of our results and impair our ability to draw conclusions from these data. A second issue concerns the indication of core biomarkers analyses which

are not systematically undergone in diagnostic work-up of a suspicion of limbic encephalitis and were thus addressing in a specific clinical concern about patient's cognition. In that respect, it brings in potential confounding factors that cannot be overcome in a retrospective study.

To further explore the potential predictive values of these CSF biomarkers on the patient's functional outcome, prospective studies are necessary. In this perspective, there is a strong need for validated scales assessing the cognitive functions and quality of life in patients suffering from autoimmune encephalitis [33]. Indeed, the mRS scale is neither designed nor appropriate to evaluate the cognitive consequences on daily living autonomy in encephalitis patients. In addition, MMSE scale, the most frequently used in routine evaluation, does not accurately assess neither the cognitive executive functions nor the behavioral or thymic disorders that nevertheless constitute a major outcome.

In LGI1 patients presenting with a subacute cognitive disorder, cognitive impairment may sometimes represent, later on, the only remaining symptoms suggesting a neurodegenerative disease. In this condition, CSF biomarkers are usually sampled for diagnosis purpose.

None of the CSF samples from LGI1 encephalitis patients displayed a typical AD pattern or a massive neuronal lysis evoking CJD, but it is noteworthy that more than half of the patients had at least one abnormal level of one CSF core AD biomarker. Such atypical but pathological profiles in patients presenting with subacute cognitive disorders could be falsely considered as suggestive of a neurodegenerative condition by physicians, misleading LGI1 encephalitis. Thus, every patient presenting with subacute cognitive impairment with altered CSF AD biomarkers but without typical AD profile should be tested for anti-LGI1 antibodies to avoid any delay in diagnosis of this treatable disease.

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Data availability Data related to the current article are available from the corresponding authors on reasonable request.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethics approval and consents Written consent was obtained from all patients and the present study (NCT-04001270) was approved by the institutional review board of the Université Claude Bernard Lyon 1 and Hospices Civils de Lyon. CSF samples were stored with authorization (declaration number DC-2008-304) of the Ministère de la Santé (French ministry of health).

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