Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.3029-19
Intern Med 58: 3369-3378, 2019
http://internmed.jp

【 ORIGINAL ARTICLE 】

Clinical Characterization of Definite Autoimmune Limbic Encephalitis: A 30-case Series

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Abstract:

Objective Limbic encephalitis (LE) is an inflammatory condition of the limbic system that has an acute or subacute onset. Several types of antibodies are related to the onset of LE, including anti-N-methyl D-aspartate receptor (NMDAR) antibodies and voltage-gated potassium channel (VGKC)-complex antibodies. However, the characteristics and prevalence of LE remain unclear, especially in Asian cohorts, due to the rarity. We aimed to survey their characteristics.

Materials and Methods Data of 30 cases clinically defined as "definite autoimmune LE" (based on the standard criteria) were retrospectively collected. These patients were categorized into four subtypes: NMDAR (+) (n=8), VGKC (+) (n=2), antibodies related to paraneoplastic syndrome (n=2), and an antibody-negative group (uncategorized) (n=18).

Results LE is rare in Japan, and affected only 30 of 16,759 hospital patients (0.2%) over a ten-year period. The NMDAR (+) group showed distinctive symptoms, while the other three groups had similar indications. Brain MRI indicated significant medial temporal lobe atrophy at one year follow up after discharge. The prevalence of cognitive dysfunction as a complication was 64% (9/14). First-line immunotherapy resulted in a good outcome. A drastic improvement was seen from 4.0±1.1 to 1.1+ on the modified Rankin Scale. A good treatment outcome was observed in all groups (NMDAR, VGKC, and uncategorized), suggesting the importance of an early clinical diagnosis and the early initiation of treatment. Furthermore, we reviewed 26 cases that were clinically diagnosed as definitive autoimmune LE in previous case reports.

Conclusion Our findings show that the establishment of a clinical diagnosis based on the clinical criteria of definitive autoimmune LE is important for the initiation of immunotherapy.

Key words: autoimmune limbic encephalitis, NMDAR, VGKC, immunotherapy

(Intern Med 58: 3369-3378, 2019)
(DOI: 10.2169/internalmedicine.3029-19)

Introduction

Limbic encephalitis (LE) is an acute or subacute inflammatory condition localized to the structures of the limbic system in the region of hippocampus, amygdala, hypothalamus, cingulate gyrus, and limbic cortex (1). The symptoms are often similar to those of viral or bacterial encephalitis. However, LE generally shows good outcomes after comprehensive immunotherapy. First-line immunotherapy consists of steroids, intravenous immunoglobulins (IVIg), and plasmapheresis, while second-line immunotherapy consists of rituximab and cyclophosphamide (2, 3). Thus, in cases of LE, it is important to properly establish a diagnosis and initiate treatment. In 1960, Brierley et al. described the cases of three patients with specific inflammatory changes in the lim-

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Received: March 21, 2019; Accepted: July 3, 2019; Advance Publication by J-STAGE: August 21, 2019
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bic region (4). Subsequent research further characterized LE using neuro-imaging and discovered the related antibodies (1, 5). Specifically, LE was considered a classical paraneoplastic syndrome - a secondary disorder caused by cancer or benign tumors such as small cell lung cancer, testicular tumor, Hodgkin's disease, teratoma, or thymoma (6). Thus, LE is thought to be an autoimmune encephalitis syndrome related to antibodies that are specific for neuronal cell surface or synaptic proteins such as voltage-gated potassium channel (VGKC) complex antibodies, anti-N-methyl D-aspartate receptor (NMDAR) antibodies, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) antibodies, gamma-aminobutyric acid-B receptor ($GABA_BR$) antibodies, and anti-metabotropic glutamate receptor 5 (mGluR5) antibodies (1).

The NMDAR antibody is commonly seen in patients with LE. NMDAR is a hetero-tetramer comprised of two GluN1 subunits and two GluN2/3 subunits. The GluN1 subunit associates with LE involving NMDAR antibodies (7). Moreover, patients with LE who have NMDAR antibodies show distinctive clinical characteristics, including younger age at onset (<45 years), female predominance, psychosis or abnormal behavior, and associated teratoma (8). VGKC-complex antibodies, specifically leucine-rich glioma inactivated-1 (LGI-1), contactin-associated protein 2 (CASPR2), and contactin-2 are also related to LE. Among these, LGI-1 antibody is the most closely associated with LE, while CASPSR 2 is more closely related to neuromyotonia. Patients with LE related to VGKC-complex antibodies present with amnesia, seizures, psychosis, and cognitive decline (9). Thus, these biomarkers inform the differential diagnosis of patients with LE.

Recently, Graus et al. defined the clinical criteria for autoimmune LE (1). We retrospectively searched our medical records and collected the clinical information of 30 patients fulfilling the criteria for definite autoimmune LE. In addition, we collected 26 cases from 24 case reports or case series reported in Japan during an 11-year period (Table 1). We then compared these cases to our own to estimate the global prevalence of the various LE-related antibodies, as well as to evaluate therapies and the prognosis (10-33). In addition, some antibody tests are not commonly accessible in many hospitals in Japan. Furthermore, the global prevalence of each antibody in patients with LE remains unknown. In the present study, we surveyed the prevalence and clinical characteristics of patients with LE, categorized according to each antibody. Our study will expand the understanding of clinical characterization of patients with autoimmune LE.

Materials and Methods

This was a retrospective, multi-center study. The protocol was approved by the local ethics committee of each hospital (Juntendo University School of Medicine, Juntendo University Urayasu Hospital, and Juntendo Shizuoka Hospital). Firstly, we reviewed the medical records of 16,759 patients from the three hospitals: 8,854 patients from Juntendo University School of Medicine between 2007 and 2017, 4,377 patients from Juntendo Urayasu Hospital between 2010 and 2016, and 3,528 patients from Juntendo Shizuoka Hospital between 2007 and 2015. We excluded patients who had epilepsy or infectious encephalitis with apparent pathogens. The cerebrospinal fluid (CSF) of all patients was tested for herpes viruses by polymerase chain reaction and was negative in all cases. We diagnosed 30 patients with "definite autoimmune LE" based on the previously reported clinical criteria: (1) subacute onset of working memory deficits, seizures, or psychiatric symptoms suggesting limbic system involvement, (2) bilateral brain abnormalities on T2-weighted imaging (T2WI), fluid-attenuated, inversion recovery (FLAIR) magnetic resonance imaging (MRI) highly restricted to the medial temporal lobes, (3) pleocytosis in the CSF or on electroencephalography (EEG), with epileptic or slow-wave activity involving the temporal lobes, and (4) reasonable exclusion of alternative causes (1). Among the 30 cases of autoimmune LE, we collected the clinical information of each patient, as well as the laboratory data on VGKC-complex antibodies and anti-NMDAR antibodies. The methods for measuring the titers of VGKC-complex antibodies have been previously mentioned (34); in the present study, the cut-off value for VGKC-complex antibody positivity was >400 pmol/L. Due to technical limitations, we did not screen for anti-LGI-1 antibodies or anti-CASPR2 antibodies related to the VGKC complex. Regarding NMDAR antibodies, we measured the titers in the CSF or serum using either a quantitative analysis, as described previously (35), or a quantitative assay using anti-NMDA receptor antibodies (EUROIMMUN, Luebeck, Germany). In addition, we excluded cases with herpetic simplex virus limbic encephalitis, encephalitis related to HHV-6, and other types of LE, such as neuropsychiatric systemic lupus erythematosus, Hashimoto's encephalopathy. In the same manner, we collected cases previously reported in Japan. A statistical analysis was performed using the unpaired Student's *t*-test or Fisher's exact test. The GraphPad Prism®6 software program was used to perform the statistical analyses (GraphPad Software, San Diego, USA).

Survey of the cognitive functions at one-year after onset

We collected the clinical data of 14 patients who were followed-up for one year after discharge. These data included the same clinical parameters that were measured on admission. We divided the 14 patients into two groups: (1) cognitive decline after one year (n=9), and (2) no cognitive decline after one year (n=5). We then surveyed the differences between the two groups to ascertain the factors that were related to cognitive decline after one year. Cognitive decline was defined based on neurological findings, cognitive tests such as the Mini-Mental State Examination or the revised Hasegawa's dementia scale during hospitalization or

Table 1. Twenty-six Summarized Cases from 24 Case Reports of Patients Clinically Diagnosed with Definite Autoimmune LE during 10 Years from 2008 to 2018 in Japan.

Number	Reference	Gender	Age at onset	Pathogen or related disorders	Initial symptom	Related antibody
1	(10)	Woman	5	Stem cell transplantation	Seizure and altered mental status	GAD
2	(11)	Male	60	Nivolumab / Lung cancer	Drosiness and memeory disturvbance	Hu
3	(12)	Woman	41	none	Headache and moemory disturbance	LGI-1
4	(13)	Woman	68	none	Memory disturbance and peronality change	NMDAR
5	(14)	Male	24	none	Catatonia	NMDAR
6	(14)	Woman	60	none	confusiton, hallucination, delusion	NMDAR
7	(15)	Woman	35	Mature cystic teratoma	Psychosis and seizure	NMDAR
8	(16)	Woman	40	Mature cystic teratoma	Forgetfulness	NMDAR
9	(17)	Male	71	Gastric adenocarcinoma	Rapid deterioration in cognitive function	None
10	(18)	Woman	19	Ovarian teratoma	Psychosis and emotinal lability	NMDAR
11	(19)	Woman	42	none	Tonic clonic seizure	NMDAR
12	(19)	Woman	55	none	Emotionaly unstable	NMDAR
13	(20)	Male	53	none	Abnormal sensation	LGI-1
14	(21)	Woman	39	Ovarian teratoma	Hallucination and emotinal lability	NMDAR
15	(22)	Woman	65	none	Consiousness disturbance	VGKC
16	(23)	Woman	20	Ovarian teratoma	Psychosis and consciousness disturbance	NMDAR
17	(24)	Male	62	None	Personality changes and irritability	VGKC
18	(25)	Male	61	Small cell lung cancer	Seizure, confusion, personality changes	VGCC
19	(26)	Woman	63	Esophageal small cell carcinoma	Disorientation and emorinal disability	Hu
20	(27)	Woman	22	Mediastinal teratoma	Coma	Glu-R
21	(28)	Woman	33	Multiple screlosis	Consciousness disturbance and seizure	NMDAR
22	(29)	Woman	20	Ovarian teratoma	Consciousness disturbance and seizure	NMDAR
23	(30)	Woman	59	none	Consciousness disturbance and seizure	none
24	(31)	Woman	30	none	Headache, fever, disorientation	none
25	(32)	Male	54	Isaacs syndrome	Memory loss and insomnia	VGKC
26	(33)	Male	35	Testicula germ cell tumor	Diplopia, amnesia	Ma2
		Male : Female= 8 : 18	43.6±18.4, (5-71)			

GAD: glutamic acid decarboxylase, LGI-1: leucine-rich glioma inactivated-1, NMDAR: anti-N-methyl D-aspartate receptor, VGKC: anti-voltage-gated potassium channel, VGCC: voltage-gated calcium channel, Glu-R: Glutamate receptor

at one-year after discharge.

Survey of Japanese reports over the past 11 years

We searched the PubMed database (https://www.ncbi.nlm.nih.gov/pubmed) for Japanese patients in English-language reports published during the 11 years between 2008 and 2018 using the keywords: “limbic encephalitis” and “Japan.” We summarized the clinical data of each patient, as well as the antibodies that were detected. We could not collect the clinical details at the one-year because few of the previous case reports contained this information.

Results

General overview of our 30 patients

During the study period, LE was rare in Japan, affecting only 30 of 16,759 hospital patients (0.2%). The clinical details of all 30 patients are summarized in Table 2. Their mean age at the onset was 49.3±55.1 years [±standard deviation (SD); range 21-82]. The male:female ratio was 16:14. The mean duration from disease onset to hospital admission was 44.9±85.0 days (range: 0-365 days). The mean duration of hospital admission was 62.8±41.0 days (range: 8-180 days), which was significantly shorter in comparison to previous reports [134±113 (range: 30-420 days); p=0.003]. Symptoms such as fever or cough at onset occurred in 30% of patients (9/30). Tumors were detected in 26.7% of patients (8/30). The specific tumor types were as follows: ovarian teratoma (n=2), thymoma (n=2), brain tumor and nasopharyngeal cancer (n=1), nasopharyngeal cancer (n=1), testicular tumor (n=1), and uterus neuroendocrine tumor (n=1; previously reported by our group) (36). The prevalence of complicating tumors was significantly lower in comparison to previous reports (26.7%, 8/30 vs. 61.5%, 16/26; p=0.01). The prevalence of comorbid tumors in each antibody group was as follows: 3/8 NMDAR (+) patients, 0/2 VGKC (+) patients, 2/2 patients with antibodies related to paraneoplastic syndrome, and 2/16 uncategorized patients. Thus, NMDAR was more frequently associated with tumors in patients with LE than in uncategorized patients or those with VGKC.

The initial symptoms at onset were as follows: seizure (n=12), consciousness disturbance (n=10), psychosis (n=8), fever (n=4), character changes (n=4), hallucination (n=3), stereotyped behavior (n=2), memory disturbance (n=2), cognitive decline (n=2), ataxia (n=2), aphasia (n=2), and involuntary movements, hemiparesis, headache, gait disturbance, dyskinesia, depression, and delirium (n=1 each) (Fig. 1). EEG showed abnormalities in 19/27 patients (70%), including diffuse slow waves, sharp waves, poly-spikes, and spike and wave. In our 30 cases, the analysis of the patients' CSF revealed a relatively high cell count, 37.1±49.7/μL (range 0-176, normal value: under 5), and total protein level 55.9±45.3 mg/dL (range 17-177, normal value:15-45) (Table 3). When each of the NMDA antibody groups and the uncategorized group were compared, no significant differences were observed in the CSF cell count and total protein level. Furthermore, no differences were observed when the values of previous cases were compared with our own. The prevalence of intubation in our 30 patients was 30% (9/30). The prevalence of intubation among our patients was not significantly different from that in the 26 previously reported patients (32.0%, 8/25) (p=1.00) (Table 3).

Characteristics of patients with and without antibodies

Regarding antibody positivity, 8 of 21 patients (38.1%) had an anti-NMDAR antibody and were defined as "NMDAR (+)," while 2 of 12 patients (16.7%) had VGKC-complex antibodies and were categorized as "VGKC (+)." Two patients harbored antibodies related to paraneoplastic syndrome (anti-Ma2 antibody and anti-Yo antibody, respectively). The other 18 patients were placed in an uncategorized group, including 11 NMDAR (-) patients, eight VGKC (-) patients, and four patients who were not tested for NMDAR and VGKC-complex antibodies. Next, we compared the clinical symptoms among the following three groups: 1) all patients, 2) 8 patients in the NMDAR (+) group, and 3) 18 patients in the uncategorized group. In addition, we used the data of 26 previously reported cases (Table 2). The NMDAR (+) group showed distinctive characteristics including a female predominance (male:female ratio=1:7). The age at onset in the NMDAR (+) group (mean±SD: 35.8±6.9 years, range: 28-45 years) was significantly lower than that in the uncategorized group ($p<0.05$). The duration from disease onset to hospital admission in the NMDAR (+) group (mean±SD: 17.6±18.3 days) was shorter than in the uncategorized group (53.4±106 days). The duration of admission (approximately 50-70 days) did not differ among the groups to a statistically significant extent. The incidence of infection-like symptoms at the onset was high in the NMDAR (+) group (62.5%, 5/8). Furthermore, the rate of psychosis in the NMDAR (+) group was significantly higher than that in the uncategorized group (87.5% versus 11.1%; p=0.004). The VGKC (+) group and the group with paraneoplastic syndrome-associated LE could not be analyzed as due to the small number of patients.

The characteristics of patients with prolonged admission

Six cases, namely, patients 6, 11, 12, 16, 17, and 22, required more than 100 days of in-hospital care. Patient 6 had severe depression and abnormal behavior. Patient 11 had malignant cancer (large-cell neuroendocrine carcinoma) requiring prolonged in-hospital care and eventually died in the hospital. Patient 12 manifested repeated seizures complicated by severe liver cirrhosis and ascites leading to death. Patient 16 presented repeated seizures, prolonged cognitive decline, and visual hallucination, and a longer time was required to make an accurate diagnosis due to the patient's atypical symptoms. Patient 17 presented malignant syndrome, seizures, and prolonged consciousness disturbance. Patient 22 presented status epilepticus requiring a respirator. The patients who required long-term in-hospital care were most likely to have complications of repeated seizures, status epilepticus, or complications associated with the progression of primary disorders.

Table 2. **Clinical Overview and Comparative Evaluation of 30 Patients Diagnosed with Clinically Definite Autoimmune Limbic Encephalitis.**

		Our cases						Previous reports reported previously			Comparing among our cases	Ours*versus*previsous cases	
		(i) Total (n=30)	(ii) NMDAR (n=8)	(iii) VGKC (n=2)	(iv) Yo (n=1)	(v) Ma2 (n=1)	(vi) Uncategorized (n=18)	(vii) Total (n=26)	(viii) NMDAR (n=13)	(ix) VGKC (n=5)	(ii) vs. (vi)	(i) vs. (vii)	(ii) vs. (viii)
	Gender (Male : Female)	16 : 14	1 : 7	1 : 1	male	male	12 : 6	8 : 18	1 : 12	3 : 2	0.03	0.11	1.00
	Age at onset	49.3±55.1, 21-82	35.8±6.9, 28-45	53/53	78	61	52.5±16.0, 21-82	43.7±18.4, 5-71	36.7±16.2, 19-68	55.0±9.35, 41-65	0.01	0.24	0.88
	Days in admission	62.8±41.0, 8-180	76.9±30.1, 44-126	8/42	39	55	62.5±46.4, 16-180	134±113, 30-420	70.0±41.9, 30-180	NA	0.43	0.003	0.70
	Days from onset to admission	44.9±85.0, 0-365	17.6±18.3, 7-60	30/12	120	84	53.4±106, 0-365	74.2±148, 3-540	8.1±4.5, 3-14	NA	0.36	0.38	0.13
Diagnositc criteria for definite autoimmune limbic encephalitis	Subacute onset, rapid progression of less than 3 months	96.7%, 29/1	88%, 7/1	yes/yes	yes	yes	100%, 18/0	84.0%, 21/4	100%, 13/0	50.0%, 2/2	0.31	0.17	0.38
	Bilateral brain abnormalities on T2WI or RFLAIR, highly restricted to the medial temporal lobes	76.7%, 23/7	50%, 4/4	yes/no	no	no	100%, 18/0	58.3%, 14/10	27.3%, 3/8	80.0%, 4/1	0.005	0.24	0.38
	CSF pleocytosis or EEG with epileptic or slow wave activitiey	96.7%, 29/1	100%, 8/0	yes/yes	yes	no	100%, 18/0	73.7%, 14/5	77.8%, 7/2	50.0%, 1/1	1.00	0.03	0.47
	Resonalbe excluision of alternative causes	100%, 30/0	100%, 8/0	yes/yes	yes	yes	100%, 18/0	100%, 26/0	100%, 13/0	100%, 5/0	1.00	1.00	1.00
	Detected any antibodies	40.0%, 12/18	100%, 8/0	yes/yes	yes	yes	0%, 0/18	61.5%, 16/10	61.5%, 8/5	20.0%, 1/4	<0.0001	0.18	0.11
	Complication of any tumors	26.7%, 8/22	37.5%, 3/5	no/no	yes	yes	11.1%, 2/16	61.5%, 16/10	62.0%, 8/5	20.0%, 1/4	0.28	0.01	0.39
	Psychosis at onset	26.7%, 8/22	87.5%, 7/1	yes/no	no	no	11.1%, 2/16	60.0%, 15/9	75.0%, 9/3	60.0%, 3/2	0.0004	0.01	0.62
	Infection-like symptom at onset	30.0%, 9/21	62.5%, 5/3	no/no	no	no	22.2%, 4/14	87.5%, 13/12	61.5%, 8/5	0%, 0/4	0.08	0.11	1.00
Cerebrospinal fluid	Cell count (/uL, under 5)	37.1±49.7 (0-176)	101±180, 7-507	8/2	5	3	41.6±56.7 (0-176)	45.1±81.8, 0-330	75.4±115, 3-330	NA	0.21	0.70	0.75
	Total protein (mg/dL, range 15-45)	55.9±45.3 (17-177)	33.3±11.4, 17-48	43/41	50	58	67.1±53.6 (17-177)	119±181, 8-670	62.3±65.5, 21-160	NA	0.12	0.09	0.26

[mean±standard deviation (range)] or (percentage, positive findings/negative findings)

NMDAR: anti-N-methyl D-aspartate receptor, VGKC: anti-voltage-gated potassium channel

Intern Med 58: 3369-3378, 2019 DOI: 10.2169/internalmedicine.3029-19

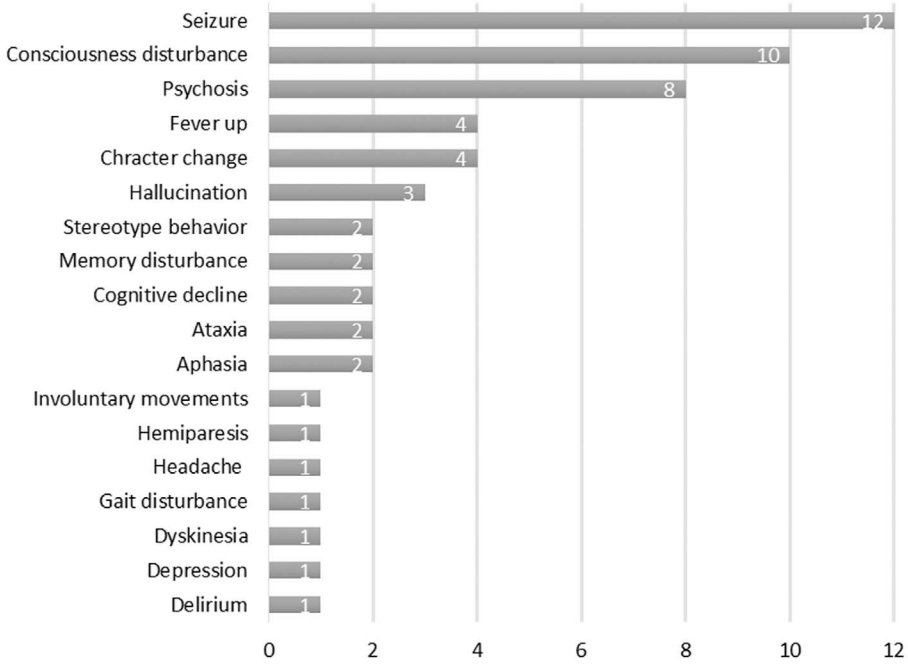

Figure 1. **The prevalence of symptoms at onset in our patients with autoimmune limbic encephalitis. The X-axis indicates the number of patients; the Y-axis indicates the symptoms at onset.**

Table 3. **Brain MRI Findings in the Medial Temporal Lobes at Two Points: Admission and One Year after Discharge.**

	(i) Total (n=30)	(ii) NMDAR (+) (n=8)	(iii) VGKC (+) (n=2)	(iv) Yo (n=1)	(v) Ma2 (n=1)	(vi) Uncategorized (n=18)
Admission						
(a) Bilateral brain abnormalities on T2WI or FLAIR, highly restricted to the medial temporal lobes	76.7%, 23/7	50%, 4/4	50%, 1/0	0	0	100%, 18/0
(b) Atrophic changes	0% (0/30)	0% (0/8)	0% (0/2)	0% (0/1)	0% (0/1)	0% (0/18)
One year after discharge						
(c) Bilateral brain abnormalities on T2WI or FLAIR, highly restricted to the medial temporal lobes	28.6%, 4/10	0%, 0/4	0/NA	0	NA	40.0%, 4/6
(d) Atrophic changes in the bilateral hippocampus	57.1%, 8/6	25.0%, 1/3	1/NA	0	NA	70.0%, 7/3
Cognitive dysfunction one year after discharge	64.3%, 9/5	75.0%, 3/1	1/NA	1	NA	50.0%, 4/4
p values (a) *vs.* (c)	0.003					0.0006
p values (b) *vs.* (d)	<0.0001					0.0001

Percentage, positive findings/negative findings

T2WI: T2-weighted image, FLAIR: fluid-attenuated inversion recovery, NMDAR: anti-N-methyl D-aspartate receptor, VGKC: anti-voltage-gated potassium channel

Comparison of the MRI findings on admission and at one year

In 23 of the 30 enrolled patients, brain MRI (T2WI or FLAIR) on admission showed bilateral abnormalities in the medial temporal lobes. None of the patients showed atrophic change in the same region (Table 3). At one year after discharge, brain MRI indicated that 28.6% (4/14) of the patients had areas of abnormal intensity in the medial temporal lobes, and that 57.1% (8/14) had atrophic changes. A comparative study of the follow-up findings showed significant changes between the disease onset and one year after the

Table 4. Selection and Efficacy of Treatments in the Present Study and Previous Cases.

		Our cases						Previous reports reported previously			Comparing among our cases	Ours *versus* previsous cases	
		(i) Total (n=30)	(ii) NMDAR (n=8)	(iii) VGKC (n=2)	(iv) Yo (n=1)	(v) Ma2 (n=1)	(vi) Uncategorized (n=18)	(vii) Total (n=26)	(viii) NMDAR (n=13)	(ix) VGKC (n=5)	(ii) vs. (vi)	(i) vs. (vii)	(ii) vs. (viii)
Treatments (percentage, effective/ non-effective)	Intravenous methylpredonisolone plus	93.3%, 28/2	100%, 7/0	Effective/ effective	NA	Effective	94.4%, 17/1	84.0%, 21/4	92%, 12/1	75.0%, 3/1	1.00	0.39	1.00
	Oral predonisolone	80.0%, 24/6	71.4%, 5/2	Effective/ effective	NA	Effective	83.3%, 15/3	32.0%, 8/17	38%, 5/8	25.0%, 1/3	0.60	0.0004	0.35
	Plasma exchange	30.0%, 9/21	28.6%, 2/5	NA/ effective	Non-effective	Effective	16.7%, 3/15	16.0%, 4/21	7.7%, 1/12	0%, 0/4	0.60	0.34	0.27
	Intravenous immunoglobulin	43.3%, 13/17	57.1%, 4/3	NA/NA	Non-effective	Effective	33.3%, 6/12	32.0%, 8/17	31%, 4/9	0%, 0/4	0.38	0.42	0.36
	Anti epileptic drugs	70.0%. 21/9	71.4%, 5/2	Effective/ NA	NA	Not assessed	77.8%, 14/4	29.2%, 7/17	31%, 4/9	33.3%, 1/2	1.00	0.006	0.16
	Respiratory management	30.0%, 9/21	37.5%, 3/5	NA/NA	NA	Not assessed	33.3%, 6/12	32.0%, 8/17	38.5%, 5/8	0%, 0/4	1.00	1.00	1.00

Percentage, positive findings/negative findings

IVIg: intravenous immunoglobulin, anti-epileptic drug, NMDAR: anti-N-methyl D-aspartate receptor, VGKC: anti-voltage-gated potassium channel

onset ($p<0.05$), regardless of the associated antibodies (Table 3).

Selection and efficacy of treatments

We selected intravenous methylprednisolone pulse therapy in 28 of 30 cases, oral prednisolone in 24 cases, plasma exchange in 9 cases, IVIg in 13 cases, and antiepileptic drugs in 21 cases (Table 4). In most cases, we initiated steroid therapy and did not commonly use rituximab or immunosuppressant drugs because their use for this purpose is off-label according to our health insurance system. Nine patients temporarily required respiratory management by intubation and a mechanical ventilator (30.0%, 9/30). The rates of each treatment did not differ among the antibody groups in our cohort (Table 4). Overall, the administration of medications resulted in good outcomes. The modified Rankin Scale indicated drastic improvements at three points: admission, discharge and one-year after discharge among 30 patients [4.0±1.1, range 2-5, 2.4±1.7 (±SD), range 0-6, and 1.1±1.3, range 0-5, respectively], for each of these comparisons between discharge and one year after discharge, the p value was <0.0001 (Table 5).

In comparison to previous cases, we were more likely to use anti-epileptic drugs and oral prednisolone after the first-line treatment. Previous investigations have commonly used other treatments, including surgery or chemotherapy to treat primary tumors or cancers. Generally, in previous cases as well as well as our own cases, intravenous methylprednisolone was the most common treatment, followed by oral prednisolone, IVIg, and plasma exchange in equal quantities. Among the 30 patients, we evaluated the prognosis at discharge from our hospital: 63.3% (19/30) of the patients improved, 23.3% (7/30) showed no change, 10% (3/30) worsened, and 3.3% (1/30) died. Four cases showed deterioration after one year of follow-up. The causes of deterioration were primary malignant cancer, progression of liver cirrhosis, and non-response to first-line immunotherapy.

Comparison of patients with and without cognitive decline at one year after discharge

In the survey carried out at one year after admission, the cognitive function could be evaluated in 14 patients. Nine patients showed cognitive decline, while 5 showed no cognitive decline (n=5). Patients with cognitive decline tended to have an older age of onset in comparison to those without cognitive decline (Supplementary material 1). None of the other parameters differed between the groups to a statistically significant extent.

The clinical outcome of each patient at discharge

The clinical outcome at discharge was categorized into 4 groups: improved, no changes, worsened and death. The prognoses, as determined from medical records, were as follows: improved, 63.3% (19/30); no changes, 23.3% (7/30); worsened, 6.6% (2/30); and death, 6.6% (2/30) (Fig. 2).

Prevalence of various antibodies related to LE in previous reports from Japan

We collected 26 patients from 24 case reports of patients clinically diagnosed with definite autoimmune LE (Table 1). The mean age at onset was 43.6±18.4 years (range 5-71). The male:female ratio was 8:18. The cause of LE was a malignant disorder in 9 patients (23.1%), teratoma in 7 patients (27.0%), other causes or comorbidity in 2 patients (7.7%), and undetermined in 11 patients (42.3%) (Supplementary material 2). The prevalence rates of the various antibodies were as follows: NMDAR (46.2%, 12/26), VGKC (19.2%, 5/26), Hu (7.7%, 2/26), glutamic acid decarboxylase (GAD)

Table 5. Alterations of the Modified Rankin Scale at Time of Admission, Discharge, and One Year after Discharge.

		Our cases						Previous reports reported previously			p values		
											Comparing among our cases	Ours *versus* previous cases	
		(i) Total (n=30)	(ii) NMDAR (n=8)	(iii) VGKC (n=2)	(iv) Yo (n=1)	(v) Ma2 (n=1)	(vi) Uncategorized (n=18)	(vii) Total (n=26)	(viii) NMDAR (n=13)	(ix) VGKC (n=5)	(ii) *vs.* (vi)	(i) *vs.* (vii)	(ii) *vs.* (viii)
Modified Rankin Scale	(a) at admission	4.0±1.1 (2-5)	3.8± 1.2, 2-5	5/5	4	4	4.0±1.2 (2-5)	3.8±1.0, 2-5	4.1±1.0, 3-5	3.8±1.0, 2-5	0.63	0.59	0.51
	(b) at discharge	2.4±1.7 (0-6)	3.0± 1.8, 1-6	1/4	4	3	1.9±1.7 (0-5)	1.6±2.1, 0-6	0.5±1.0, 0-3	1.6±2.1, 0-6	0.16	0.15	0.004
	(c) one-year after onset	1.1±1.1 (0-4)	0.8± 1.0, 0-2	0/NA	4	NA	1.0±0.7 (0-2)	2.1±2.7, 0-6	NA	0, 1, 4	0.58	0.18	NA
p values	(a) *vs.* (b)	<0.0001	0.33				0.0002	<0.0001	<0.0001	0.02			
	(a) *vs.* (c)	<0.0001	0.001				<0.0001	0.01	NA	0.08			

Mean±standard deviation (range)

NMDAR: anti-N-methyl D-aspartate receptor, VGKC: anti-voltage-gated potassium channel

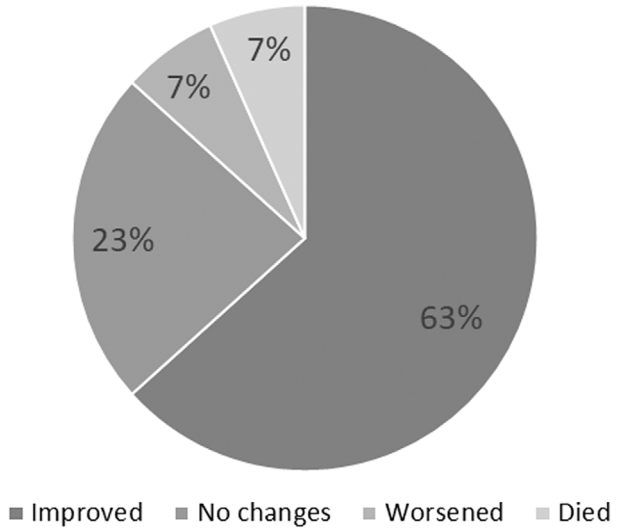

Figure 2. The prognosis of each of our 30 patients at hospital discharge.

(3.8%, 1/26), peptides of glutamate receptor subunits (GluR) (3.8%, 1/26), Ma2 (3.8%, 1/26), voltage-gated calcium channel (VGCC) (3.8%, 1/26), and undetermined (11.5%, 3/26) (Supplementary material 3).

Discussion

Thirty patients were retrospectively surveyed and clinically diagnosed with autoimmune definite LE with or without the relevant antibodies. The prevalence of definite autoimmune LE among the patients admitted to our hospitals was 0.2% during the 11-year study period. These 30 patients initially showed clinical symptoms at onset (which occurred in middle age), along with seizures, consciousness disturbance, psychosis, fever, and character changes. Areas of abnormal intensity were observed in the bilateral medial temporal lobes on brain MRI and abnormalities were observed on EEG. The mean duration of hospitalization was relatively long (approximately 60 days). However, most patients improved after first-line immunotherapy. At one year after discharge, the patients presented better outcomes. Intriguingly, various types of LE, including NMDAR, VGKC, and uncategorized, showed better outcomes in the evaluation of the modified Ranking Scale at one year after discharge. Thus, the results suggested the similarities of the symptoms, course, and response to treatment of LE patients, regardless of the associated antibodies that were identified.

Screening was not performed for other types of antibodies associated with LE [AMPA, GABA, GAD, Gly-R, or the subtypes of VGKC (e.g., LGI-1 and CASPR2)]. The prominent features of NMDAR (+) were highlighted in this comparative study. The NMDAR (+) group showed a female predominance, younger age at onset, shorter duration from onset to admission, higher prevalence of comorbid teratoma, and a higher rate of infection-like symptoms at onset. Thus, these characteristics may be useful to distinguish NMDAR (+) from other types of autoimmune LE. With regard to VGKC (+) LE, two positive patients presented with a middle-age onset, and this group showed good responses to first-line immunotherapies.

There have been many reports on autoimmune LE. Among the 501 NMDAR (+) LE patients reported from 200 centers in 35 countries, distinctive factors were seen, including a female predominance, relatively young age at onset (10s to 30s), and a high prevalence of behavioral abnormalities and cognitive dysfunction (2). Dyskinesia and other movement disorders, and a high prevalence of cerebrospinal fluid and electroencephalography abnormalities were observed. Half of the patients were complicated with mature or immature teratoma (37). Approximately 80% of the patients showed favorable outcomes after first-line immunotherapy. Second-line immunotherapies such as rituximab and cyclo-

phosphamide were useful for the patients who did not have a good response following first-line immunotherapy. First-line treatments yielded a good outcome after 24 months of follow-up in 97% of the enrolled patients. These findings largely match those of our NMDAR (+) patients. VGKC (+) LE patients frequently presented symptoms of seizures, psychiatric disturbance, dystonia, and cognitive impairment (in patients with the LGI-1 antibody) and amnesia, insomnia, dysautonomia, and neuromyotonia [(Morvan's syndrome) in patients with the CASPR2 antibody] (9). Overall, the co-occurrence of tumors is rare. Our patients with NMDA (+) or VGKC (+) shared similarities with these patients.

Importantly, in the series of patients from the present study, groups with undetermined antibody profiles were predominant (18/30). They commonly had better outcomes, with a good response to first-line immunotherapies and rarely had comorbid tumors (11.1%, 2/16). It follows that, with the exception of the NMDAR (+) group, most patients clinically diagnosed with definite autoimmune LE showed similar symptoms and progression, irrespective of antibody positivity or negativity. We therefore emphasize the importance of a correct clinical diagnosis, based on the standard criteria for autoimmune LE, as well as the immediate initiation of immunotherapy (1).

We summarized the cases of 26 patients that were reported in Japan over the past 11 years. This summary was not a serial screening study from a single cohort; thus, it may have included some bias. For instance, authors usually report cases that are atypical or in which patients are positive for antibodies. In line with this, the data indicated that the NMDAR antibody had the highest prevalence (50.0%, 13/26), followed by the VGKC-complex antibody (19.2%, 5/26). These values are close to those of the present study, where NMDAR and VGKC were detected in 38.1% (8/21) and 16.7% (5/26) of the patients, respectively. In all studies from Japan, NMDAR was the most common antibody, followed by VGKC. The prevalence of other antibodies, including Hu, GAD, Glu-R, Ma2, and voltage-gated calcium channel antibodies, seems to be low. Another retrospective study investigated the prevalence of antibodies among 190 Japanese patients with various types of autoimmune neurological disorders (including LE) over a 10-year period. The following LE-associated antibodies were detected: NMDAR (n=39), AMPA receptor (n=3), LGI-1 (n=3), GlyR (n=3), GABA (A) (n=2), GABA (B) (n=1), and unknown (n=6) (38). These findings suggest that the prevalence of the NMDAR antibody is likely to be high among patients with autoimmune neurological disorders. Further studies with larger cohorts and screening for all antibodies should be performed to confirm our findings. In the summarized data from previous reports that were included in this study, the comorbidities of autoimmune LE were: total (61.5%, 16/10), ovarian teratoma (19.2%, 5/26), thymoma (7.7%, 2/26), other tumors (34.6%, 9/26), and no complication of tumors (30.8%, 8/26). In contrast, the detection rate of related tumors in the present study was 26.7% (8/30). Overall, 20-60% of patients had autoimmune LE as a collateral effect of benign or malignant tumors. If such tumors are resected early, the outcomes would be more favorable and the incidence of recurrence would decrease (37); thus, it should be recommended that clinicians survey for tumors at onset. In our one-year follow-up survey, we identified factors that tended to predict prolonged cognitive decline at one year after discharge, which were older age at onset (Supplementary material 1).

A large study of 577 patients with NMDAR encephalitis demonstrated better outcomes after first-line immunotherapy (63.0%). Patients who received additional second-line immunotherapies, such as rituximab and cyclophosphamide, presented even better outcomes (2). However, the percentage of our patients who showed improvement after first-line treatment was still 63.0%; thus, clinicians should always ensure that they expand the availability of second-line immunotherapy in patients who do not respond to first-line therapy. As mentioned above, a non-response was related to the severity of the primary disorder or the type of complication. These factors also determine the patient's prognosis. Our study was associated with the following limitations: (1) a retrospective design, (2) a small number of patients due to the rarity of the disease, (3) partial screening tests for the LE-associated antibodies, and (4) bias from previous case reports. These factors may have influenced our results.

Conclusion

To conclude, we observed unique symptoms in 30 patients with clinically definite autoimmune LE with heterogeneous causes and prognoses. These findings emphasize the importance of making a clinical diagnosis of definite autoimmune LE and the prompt initiation of treatment. We hope to promote quick and easy-access screening tests for antibodies related to autoimmune LE.

The authors state that they have no Conflict of Interest (COI).

References

1. Graus F, Titulaer MJ, Balu R, et al. A clinical approach to diagnosis of autoimmune encephalitis. Lancet Neurol **15**: 391-404, 2016.
2. Titulaer MJ, McCracken L, Gabilondo I, et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. Lancet Neurol **12**: 157-165, 2013.
3. Iizuka T, Sakai F, Ide T, et al. Anti-NMDA receptor encephalitis in Japan: long-term outcome without tumor removal. Neurology **70**: 504-511, 2008.
4. Brierley J, Corsellis J, Hierons R, Nevin S. Subacute encephalitis of later adult life. Mainly affecting the limbic areas. Brain **83**: 357-368, 1960.
5. Dalmau J. NMDA receptor encephalitis and other antibody-mediated disorders of the synapse: The 2016 Cotzias Lecture. Neurology **87**: 2471-2482, 2016.
6. Gultekin SH, Rosenfeld MR, Voltz R, Eichen J, Posner JB, Dalmau J. Paraneoplastic limbic encephalitis: neurological symp-

toms, immunological findings and tumour association in 50 patients. Brain **123** (Pt): 1481-1494, 2000.

7. Gleichman AJ, Spruce LA, Dalmau J, Seeholzer SH, Lynch DR. Anti-NMDA receptor encephalitis antibody binding is dependent on amino acid identity of a small region within the GluN1 amino terminal domain. J Neurosci **32**: 11082-11094, 2012.

8. Dalmau J, Lancaster E, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R. Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. Lancet Neurol **10**: 63-74, 2011.

9. Vincent A, Bien CG, Irani SR, Waters P. Autoantibodies associated with diseases of the CNS: new developments and future challenges. Lancet Neurol **10**: 759-772, 2011.

10. Nagai K, Maekawa T, Terashima H, Kubota M, Ishiguro A. Severe anti-GAD antibody-associated encephalitis after stem cell transplantation. Brain Dev **41**: 301-304, 2019.

11. Matsuoka H, Kimura H, Koba H, et al. Nivolumab-induced limbic encephalitis with anti-Hu antibody in a patient with advanced pleomorphic carcinoma of the lung. Clin Lung Cancer **19**: e597-e599, 2018.

12. Takahashi Y, Mikami T, Suzuki H, et al. Development of moyamoya disease after non-herpetic acute limbic encephalitis: a case report. J Clin Neurosci **53**: 250-253, 2018.

13. Inoue T, Kanno R, Moriya A, et al. A case of paraneoplastic limbic encephalitis in a patient with invasive thymoma with anti-glutamate receptor antibody-positive cerebrospinal fluid: a case report. Ann Thorac Cardiovasc Surg **24**: 200-204, 2018.

14. Tsutsui K, Kanbayashi T, Takaki M, et al. N-Methyl-D-aspartate receptor antibody could be a cause of catatonic symptoms in psychiatric patients: case reports and methods for detection. Neuropsychiatr Dis Treat **13**: 339-345, 2017.

15. Hattori Y, Yamashita Y, Mizuno M, Katano K, Sugiura-Ogasawara M, Matsukawa N. Anti-N-methyl-D-aspartate receptor limbic encephalitis associated with mature cystic teratoma of the fallopian tube. J Obstet Gynaecol Res **43**: 412-415, 2017.

16. Terada A, Tasaki S, Tachibana T, et al. Two cases of acute limbic encephalitis in which symptoms improved as a result of laparoscopic salpingo-oophorectomy. Gynecol Minim Invasive Ther **6**: 34-37, 2017.

17. Uneno Y, Yokoyama A, Nishikawa Y, et al. Paraneoplastic limbic encephalitis in a human epidermal growth factor receptor-2-positive gastric cancer patient treated with trastuzumab-combined chemotherapy: a case report and literature review. Intern Med **55**: 2605-2609, 2016.

18. Shimoyama Y, Umegaki O, Agui T, Kadono N, Minami T. Anti-NMDA receptor encephalitis presenting as an acute psychotic episode misdiagnosed as dissociative disorder: a case report. JA Clin Rep **2**: 22, 2016.

19. Kuroda T, Futamura A, Sugimoto A, Midorikawa A, Honma M, Kawamura M. Autobiographical age awareness disturbance syndrome in autoimmune limbic encephalitis: two case reports. BMC Neurol **15**: 238, 2015.

20. Murata Y, Watanabe O, Taniguchi G, et al. A case of autoimmune epilepsy associated with anti-leucine-rich glioma inactivated subunit 1 antibodies manifesting electrical shock-like sensations and transparent sadness. Epilepsy Behav Case Rep **4**: 91-93, 2015.

21. Imai K, Fukuda T, Wada T, Kawanishi M, Yamauchi M, Hashiguchi Y, et al. Complete recovery from paraneoplastic anti-NMDAR encephalitis associated with a small ovarian teratoma following a laparoscopic salpingo-oophorectomy: a case report. Exp Ther Med **9**: 1723-1726, 2015.

22. Hiraga A, Watanabe O, Kamitsukasa I, Kuwabara S. Voltage-gated potassium channel antibody-associated encephalitis with claustrum lesions. Intern Med **53**: 2263-2264, 2014.

23. Omura T, Sonoda S, Nagata K, et al. Anti-NMDAR encephalitis: case report and diagnostic issues. Acute Med Surg **2**: 56-59, 2015.

24. Kanazawa K, Matsumoto R, Shimotake A, et al. Persistent frequent subclinical seizures and memory impairment after clinical remission in smoldering limbic encephalitis. Epileptic Disord **16**: 312-317, 2014.

25. Kaira K, Okamura T, Takahashi H, et al. Small-cell lung cancer with voltage-gated calcium channel antibody-positive paraneoplastic limbic encephalitis: a case report. J Med Case Rep **8**: 119, 2014.

26. Shirafuji T, Kanda F, Sekiguchi K, et al. Anti-Hu-associated paraneoplastic encephalomyelitis with esophageal small cell carcinoma. Intern Med **51**: 2423-2427, 2012.

27. Kawahara K, Miyawaki M, Anami K, et al. A patient with mediastinal mature teratoma presenting with paraneoplastic limbic encephalitis. J Thorac Oncol **7**: 258-259, 2012.

28. Uzawa A, Mori M, Takahashi Y, Ogawa Y, Uchiyama T, Kuwabara S. Anti-N-methyl D-aspartate-type glutamate receptor antibody-positive limbic encephalitis in a patient with multiple sclerosis. Clin Neurol Neurosurg **114**: 402-404, 2012.

29. Kawano H, Hamaguchi E, Kawahito S, et al. Anaesthesia for a patient with paraneoplastic limbic encephalitis with ovarian teratoma: relationship to anti-N-methyl-D-aspartate receptor antibodies. Anaesthesia **66**: 515-518, 2011.

30. Kishi M, Sakakibara R, Ogata T, Ogawa E. Transient phonemic paraphasia by bilateral hippocampus lesion in a case of limbic encephalitis. Neurol Int **2**: e8, 2010.

31. Shimazaki H, Ando Y, Nakano I, Dalmau J. Reversible limbic encephalitis with antibodies against the membranes of neurones of the hippocampus. BMJ Case Rep: 2009.

32. Takahashi H, Mori M, Sekiguchi Y, et al. Development of Isaacs' syndrome following complete recovery of voltage-gated potassium channel antibody-associated limbic encephalitis. J Neurol Sci **275**: 185-187, 2008.

33. Kimura M, Onozawa M, Fujisaki A, et al. Anti-Ma2 paraneoplastic encephalitis associated with testicular germ cell tumor treated by carboplatin, etoposide and bleomycin. Int J Urol **15**: 942-943, 2008.

34. Hart IK, Maddison P, Newsom-Davis J, Vincent A, Mills KR. Phenotypic variants of autoimmune peripheral nerve hyperexcitability. Brain **125** (Pt): 1887-1895, 2002.

35. Zhang Q, Tanaka K, Sun P, et al. Suppression of synaptic plasticity by cerebrospinal fluid from anti-NMDA receptor encephalitis patients. Neurobiol Dis **45**: 610-615, 2012.

36. Kobayashi M, Nishioka K, Takanashi M, et al. Anti-NMDA receptor encephalitis due to large-cell neuroendocrine carcinoma of the uterus. J Neurol Sci **383**: 72-74, 2017.

37. Dalmau J, Gleichman AJ, Hughes EG, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet Neurol **7**: 1091-1098, 2008.

38. Kaneko J, Kanazawa N, Tominaga N, et al. Practical issues in measuring autoantibodies to neuronal cell-surface antigens in autoimmune neurological disorders: 190 cases. J Neurol Sci **390**: 26-32, 2018.