

Diagnostic Significance of Paraneoplastic Neuronal Antibodies in Japanese Adults with Autoimmune Encephalitis: A Case Series Study in an Adult Epilepsy Clinic

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Purpose: Recently, the role of autoimmune mechanisms in epileptogenesis and intractability has attracted attention. The clinical features of autoimmune encephalitis (AE) vary; thus, diagnosis can be difficult and often delayed. Paraneoplastic neurological syndromes are usually associated with malignant cancers; however, paraneoplastic antibodies (PAs) can be present in the absence of overt cancers. In this single-center, retrospective study, we evaluated the diagnostic significance of PAs in adult Japanese patients with AE.

Patients and Methods: We retrospectively analyzed the medical records of all patients who visited our epilepsy clinic and underwent a thorough diagnostic evaluation for AE between April 2021 and October 2022. Patients who met the criteria for AE and presented to our epilepsy clinic with new-onset seizures or acute or subacute seizure aggravation were included. Data from the PA panel, including anti-amphiphysin (AMPH), CV2, paraneoplastic Ma antigen 2 (PNMA2), Ri, Yo, Hu, recoverin, SRY-related HMG-box gene 1 (SOX1), titin, zic4, glutamate decarboxylase 65 (GAD65), and Tr/DNER antibodies, were evaluated.

Results: Of 32 patients who were investigated, 6 (19.0%; 2 males, age: 38.0 ± 18.0 years) were positive for PAs (anti-AMPH: 1, PNMA2: 1, Yo: 1, recoverin: 2, SOX1: 1). No patients had malignant tumors. Serum anti-SS-A/Ro antibodies were detected in one patient, and the cerebrospinal fluid showed a slightly elevated protein level. Intravenous high-dose methylprednisolone was administered to four patients and was effective in three.

Conclusion: Approximately one-fifth of AE cases were attributable to PAs, although there were no signs of malignant tumors, in adult epilepsy clinics in Japan.

Plain Language Summary: Epilepsy affects more than 70 million people worldwide; however, its etiology remains unknown in approximately one-third of patients. Recently, autoimmune mechanisms of epilepsy have gained attention. However, diagnosing autoimmune encephalitis (AE) remains difficult, because patients with AE can show various symptoms. Paraneoplastic antibodies (PAs) cause one type of AE. Paraneoplastic neurological syndromes are usually associated with malignant cancers; however, patients can present with PAs but without cancer. In this study, we evaluated the diagnostic significance of PAs in adult Japanese patients with AE. We analyzed the medical records of all patients who visited our epilepsy clinic and were intensively evaluated for AE between April 2021 and October 2022. Patients with new-onset seizures or worsening acute or subacute seizures who also showed prominent AE symptoms, such as memory impairment, emotional lability, and involuntary movements, were enrolled. Data from the PA panel, including anti-amphiphysin (AMPH), CV2, paraneoplastic Ma antigen 2 (PNMA2), Ri, Yo, Hu, recoverin, SRY-related HMG-box gene 1 (SOX1), titin, zic4, glutamate decarboxylase 65 (GAD65), and Tr/DNER antibodies, were evaluated. Of 32 patients who were investigated, 6 (19.0%; 2 males, age: 38.0 ± 18.0 years) were positive for PAs (anti-AMPH: 1, PNMA2: 1, Yo: 1, recoverin: 2, SOX1:

1). No patients had malignant tumors. Intravenous high-dose methylprednisolone was administered to four patients and was effective in three. Thus, we conclude that approximately one-fifth of AE cases are attributable to PAs, even though there are no signs of malignant tumors, in adult epilepsy clinics in Japan.

Keywords: paraneoplastic neurological syndromes, paraneoplastic antibodies, autoimmune encephalitis, epilepsy, acute symptomatic seizures, malignant tumor

Introduction

Epilepsy affects more than 70 million people worldwide; however, its etiology remains unknown in approximately one-third of adult patients with epilepsy.^{1,2} Recently, the role of autoimmune mechanisms in epileptogenesis and intractability has garnered attention.³ A previous study of patients with “presumed autoimmune epilepsy” with intractable seizures as the chief complaint performed at an autoimmune neurology clinic showed that 62% responded to immunotherapy using high-dose intravenous methylprednisolone pulse therapy (IVMP) and intravenous high-dose immunoglobulin therapy (IVIg).⁴ In patients with cryptogenic new-onset status epilepticus (C-NORSE) and febrile infection-related epilepsy syndrome (FIRES), the overproduction of proinflammatory cytokines may underlie the pathogenesis and progression of refractory seizures. Several reports have demonstrated the clinical efficacy of IL-1 and IL-6 receptor antagonists for seizure suppression and outcome improvement in both the acute and chronic phases of C-NORSE and FIRES.^{5–7} Early immunotherapy including intravenous cyclophosphamide can improve the one-year functional status of patients with super-refractory C-NORSE.⁸ However, limited information is available on this topic.

The clinical features of autoimmune encephalitis (AE) are variable and include psychiatric symptoms, such as agitation and emotional lability, and autonomic symptoms, such as tachycardia and gastrointestinal dysfunction.^{9–11} These symptoms are easily considered nonspecific and often overlooked by neurologists and emergency care practitioners. New-onset seizures are a cardinal feature of AE. However, this condition cannot be evaluated in patients with epilepsy. Seizures of AE are characterized by “atypical”, “prolonged”, and “intractable” features intermingled with involuntary movements due to the involvement of deep structures of the brain, including the limbic system and basal ganglia, which can lead to an initial misdiagnosis of psychiatric or functional disorders.^{12,13} Ictal electroencephalography (EEG) can be normal or may show only slight changes.^{13,14} Although recent clinical diagnoses of AE emphasize the importance of early diagnosis and immunotherapy to improve clinical outcomes,^{9,11} these clinical obstacles cause diagnostic delays in AE.

Paraneoplastic neurological syndromes (PNS) are usually associated with cancer. However, onconeural and paraneoplastic antibodies (PAs) can also be present without overt cancer. The diagnostic algorithm for AE begins with possible AE, and PAs are part of the evaluation.⁹ When PAs are positive, autoimmune limbic encephalitis can be definitively diagnosed if two of these three features are present. Many PAs act on intracellular antigens. Encephalitis with antibodies against onconeural intracellular antigens, such as Hu, Ma2, collapsing response mediator protein 5, amphiphysin (AMPH), and glutamate decarboxylase 65 (GAD65), is mediated by cytotoxic T cells and causes poor clinical outcomes and intractable seizures or epilepsy.¹⁵ The 2016 AE guidelines identified epileptic disorders as differential diagnoses that should be excluded.⁹ Subsequently, in 2020, the International League Against Epilepsy (ILAE) proposed conceptual definitions of “acute symptomatic seizures secondary to AE” and “autoimmune-associated epilepsy”,¹⁶ because epileptic seizures and status epilepticus are strongly associated with the acute phase of AE, and epilepsy can occur as a consequence of AE. The ILAE states that acute symptomatic seizures are often related to antibodies against cell surface antigens, and seizures are usually terminated with the remission of encephalitis, whereas autoimmune-associated epilepsies are related to antibodies against intracellular antigens, and the outcome is poor for both seizures and cognition.¹⁶ In clinical practice, determining whether the underlying encephalitis/inflammation persists and causes recurrent medically intractable seizures is difficult. Additionally, recurrent seizures can aggravate inflammation via the activation of cytokines and microglia.¹⁵

In this study, we evaluated the diagnostic significance of PAs and the clinical characteristics of AE in adult Japanese patients in an epilepsy clinic.

Material and Methods

Patients

We retrospectively analyzed the medical records of patients who visited our adult epilepsy clinic and underwent a thorough diagnostic evaluation for AE between April 2021 and October 2022. The inclusion criteria and diagnostic procedures are shown in Table 1. Patients were included if they fulfilled all of the following inclusion criteria: 1) new-onset or acute/subacute aggravation of epileptic seizures, 2) acute/subacute symptoms associated with AE, 3) provision of informed consent for thorough diagnostic investigation, and 4) positive PAs. Patients who 1) refused to provide informed consent for diagnostic investigation or 2) could not complete a thorough evaluation were excluded. Diagnostic investigations included physical and neurological examinations; blood and cerebrospinal fluid (CSF) analyses; brain magnetic resonance imaging (MRI); whole-body computed tomography (CT); routine and long-term EEG with video

Table 1 Inclusion Criteria and Diagnostic Investigations

I. Inclusion criteria 1. Seizures / status epilepticus / epilepsy – New onset – Acute / subacute aggravation (with preexisting epilepsy) 2. Acute / subacute symptoms associated with AE – Memory disturbance – Psychiatric features – Involuntary movements – Autonomic nervous system disturbance – Sleep disturbance 3. Agreed with thorough diagnostic investigation 4. Positive PA Exclusion criteria 1. Refused to give informed consent for diagnostic evaluation 2. Incomplete evaluation	
II. Diagnostic investigations 1. General examination – General physical and neurological examinations – Blood test (complete blood cell count, conventional blood chemistry) – Cerebrospinal fluid – Brain MRI and whole-body CT imaging – Routine and long-term EEG with video record – Neuropsychology – Twelve-lead resting ECG – Thyroid disease – Diabetes mellitus – Infection (syphilis, hepatitis B virus, hepatitis C virus, herpes simplex virus, varicella zoster virus, cytomegalovirus, Epstein-Barr virus) 2. Extensive evaluations specialized to immune system – Systemic autoimmune diseases (systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis, antiphospholipid syndrome, Behçet's disease) – Neuroinflammatory diseases (multiple sclerosis, neuromyelitis optica) 3. Malignancy / benign tumors – Tumor markers 4. PA – Blood PA panel (anti-AMPH, CV2, PNMA2, Ri, Yo, Hu, recoverin, SOX1, titin, zic4, GAD65, Tr/DNER antibodies) 5. Antibodies for cell-surface antigens – Anti-NMDA receptor antibody	

Abbreviations: AE, autoimmune encephalitis; PA, paraneoplastic antibodies; MRI, magnetic resonance imaging; CT, computed tomography; EEG, electroencephalography; ECG, electrocardiography; AMPH, amphiphysin; PNMA2, paraneoplastic Ma antigen 2; SOX1, SRY-related HMG-box gene 1; GAD65, glutamate decarboxylase 65; NMDA, N-methyl-D-aspartate.

recording; and neuropsychology examinations. Intensive evaluations were performed, particularly with respect to the immune system. PA panel data, including anti-AMPH, CV2, paraneoplastic Ma antigen 2 (PNMA2), Ri, Yo, Hu, recoverin, SRY-related HMG-Box gene 1 (SOX1), titin, zic4, GAD65, and Tr/DNER antibodies, were investigated using immunoblotting (BML Inc., Tokyo). Additional examinations were conducted according to the type of positive PA.

Ethical Compliance

This study conformed to the World Medical Association Declaration of Helsinki and ICMJE Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals. The study protocol was submitted to the Ethics Committee of the National Hospital Organization Utano National Hospital and was exempt from ethics review board approval because of its retrospective, noninvasive, and anonymous design, including a small number of patients. Written informed consent to disclose clinical information for publication was obtained from all patients. We confirm that we have read the journal’s position on the issues involved in ethical publication and affirm that this report is consistent with these guidelines. Data supporting the findings of this study are available from the corresponding author upon request. This study was conducted in accordance with the case report (CARE) guidelines.

Therapies

Epileptic seizures and status epilepticus were treated with anti-seizure medications according to recommendations, ie, new-generation drugs without effects on hormonal metabolism and drug interactions, and continued even if seizures remained.^{17,18} In addition to antiseizure medications, IVMP (1,000 mg/day for 3–5 days) was administered for immunomodulation. Severe cases were repeatedly treated with immunotherapy with IVIg (400 mg/kg/day for five days), after obtaining written informed consent, followed by oral immunosuppressants using tacrolimus (0.5–3 mg/day). The psychiatric symptoms were treated with antipsychotics, antidepressants, sedatives, and psychological therapies.

Outcomes were measured as improvements in major AE symptoms according to each patient, including the number and severity of seizures, psychiatric symptoms, motor performance, cognitive performance, EEG, brain MRI, and antibody titers.

Results

Patient Demographics

Table 2 shows demographics of the patients who fulfilled the inclusion criteria. During the study period, 32 patients fulfilled inclusion criteria 1–3 and were diagnosed with possible AE.⁹ Six patients (19.0%; 2 males, age [mean ± SD]: 38.0 ± 18.0 years) were positive for PAs. Malignant tumors were not detected.

Two patients (Patients 4 and 5) experienced new-onset seizures characterized by atypical features of epileptic seizures, such as lasting for hours, hyperkinetic or negative motor symptoms with preserved consciousness, or variable

Table 2 Patient Demographics

Pt	Age	M/F	Epilepsy (y)	AE Symptoms	AE (m)	APE ² Score	ACES Score	Other Information
1	23	F	22	Memory disturbance, seizure worsening, sleep disturbance	36	5	4	FH: scirrhus stomach cancer (mother), pervasive developmental disorder, no retinal abnormality
2	48	F	38	Anxiety, depression, auditory hallucination, seizure worsening	72	6	3	HTLV-I carrier, PH: clinically isolated syndrome

(Continued)

Table 2 (Continued).

Pt	Age	M/F	Epilepsy (y)	AE Symptoms	AE (m)	APE ² Score	ACES Score	Other Information
3	23	M	13	Memory disturbance, seizure worsening	60	4	4	CSF protein 44 mg/dL (normal range: 10–40 mg/dL), anti-SS-A Ab 149 U/mL (< 7.0 U/mL), Sjögren's syndrome by salivary gland biopsy, no waxing/waning by peripheral motor nerve repetitive stimulation, WMS-R <64
4	46	F	-	New onset seizure (atypical: long, focal aware, hyperkinetic), anxiety, sleep disturbance	24	5	4	Mental retardation, no retinal abnormality
5	69	M	-	New onset seizure (atypical: variable types, focal aware negative motor), insomnia, memory disturbance	3	2	3	Gastric polyp
6	19	F	6	Seizure worsening, autonomic (orthostatic hypotension, hyperhidrosis)	24	4	3	Cu/Zn abnormality

Abbreviations: Pt, patient; M/F, male/female; AE, autoimmune encephalitis; APE² score, antibody prevalence in epilepsy and encephalopathy score; ACES score, antibodies contributing to focal epilepsy signs and symptoms score; FH, family history; HTLV-I, human T cell leukemia virus type 1; PH, past history; CSF, cerebrospinal fluid; Ab, antibody; WMS, Wechsler memory scale.

manifestations; however, they were diagnosed with focal epileptic seizures involving the cingulate gyrus. The remaining four patients had preexisting epilepsy and exhibited worsening seizures. In two patients (Patients 1 and 3), memory disturbance was the most disabling complaint that hindered their social adaptation. Psychiatric and autonomic symptoms were nonspecific. Five patients had AE durations of >2 years and antibody prevalence in epilepsy and encephalopathy score (APE²)¹¹ scores of ≥4. All patients had antibodies contributing to focal epilepsy signs and symptoms (ACES) scores of ≥3.¹⁹

PAs and Other Information

The PA findings were variable (anti-AMPH, 1; PNMA2, 1; Yo, 1; recoverin, 2; and SOX1, 1) (Table 3). The PA titers were relatively low but reproducibly detected. The PAs in the CSF were not examined.

Therapeutic Outcome

The combination of antiseizure medications was continued. IVMP was performed in four patients and was effective in three patients (Table 3).

Case Description

Patient 1 was a 23-year-old female who showed illustrative improvement with IVMP. She developed afebrile convulsions at age 1, focal impaired awareness seizures (FIAS) at age 12, and focal to bilateral tonic-clonic seizures (FBTCS) at age 14. She was treated with lamotrigine (LTG) but showed subacute worsening of seizures in frequency and memory disturbances, which caused difficulty in keeping up with university lectures at 19 years of age. At 21 years of age, she visited our clinic because of social maladaptation and difficulty finding employment opportunities. Neurological examination revealed postural myoclonus, numbness, and digit span errors. The patient's mother died of scirrhous stomach cancer in her 40s. The patient was positive for anti-recoverin antibodies (±). The Wechsler Adult Intelligence Scale-Third Edition showed a significant imbalance among sub-scores, especially in performance skills: verbal

Table 3 Fulfillment of Diagnostic Criteria and Therapeutic Outcome

Pt	Age	M/F	PA	AE Diagnosis (Graus et al, 2016) ⁹	PNS Diagnosis (Graus et al, 2004) ²⁰	PNS Diagnosis (Graus et al, 2021) ²¹	PNS-Care Score (Graus et al, 2021) ²¹	Antiseizure Medications	Immunotherapy	Outcome
1	23	F	Recoverin ±	Definite autoimmune limbic encephalitis	Possible PNS	Possible PNS	4	LTG	IVMPx6	Good
2	48	F	AMPH ±	Definite autoimmune limbic encephalitis	Definite PNS	Probable PNS	6	PHT, PB, LEV, CBZ, PER	IVMPx1, IVlgx17	Poor
3	23	M	SOX1 ±	Definite autoimmune limbic encephalitis	Definite PNS	Probable PNS	7	LEV, LTG, TPM, LCM	IVMPx3	Good
4	46	F	Recoverin I+	Definite autoimmune limbic encephalitis	Possible PNS	Possible PNS	4	DZP, LCM	IVMPx2	Good
5	69	M	Yo ±	Definite autoimmune limbic encephalitis	Definite PNS	Probable PNS	7	LCM	-	Good
6	19	F	PNMA2 2 +	Definite acute disseminated encephalomyelitis	Definite PNS	Probable PNS	7	LEV	-	Good

Abbreviations: Pt, patient; M/F, male/female; AE, autoimmune encephalitis; PA, paraneoplastic antibodies; PNS, paraneoplastic neurological syndromes; AMPH, amphiphysin; SOX1, SRY-related HMG-box gene 1; PNMA2, paraneoplastic Ma antigen 2; LTG, lamotrigine; PHT, phenytoin; PB, phenobarbital; LEV, levetiracetam; CBZ, carbamazepine; PER, perampanel; TPM, topiramate; LCM, lacosamide; DZP, diazepam; IVMP, high-dose intravenous methylprednisolone pulse therapy; IVlg, intravenous high-dose immunoglobulin therapy.

intelligence quotient (IQ), 102; performance IQ, 69; full-scale IQ, 86; verbal comprehension, 107; perceptual organization, 65; working memory, 103; and processing speed, 97. Brain MRI revealed amygdala enlargement on the right side, hippocampal atrophy on the left side, and a high-intensity lesion in the bilateral mesial temporal areas in a fluid-attenuated inversion recovery (FLAIR) sequence. Long-term video EEG monitoring showed a posterior-dominant rhythm at 10–11 Hz, an almost continuous low-frequency alpha burst at 8–9 Hz in the left temporal area, and sharp waves localized to the right temporal area. Although her total sleep time was 6 h and 20 min, the sleep architecture was disorganized, and abrupt arousal was frequently observed during the N3 and rapid eye movement stages (Figure 1). IVMP (1 g/day for 3 days) was administered once every several months for a total of 6 courses, and the patient's condition and EEG findings improved. She has been seizure-free and working full-time for more than two years. Whole-body CT, tumor marker analysis, and ophthalmological evaluation revealed no signs of malignant cancer or retinal abnormalities. However, anti-recoverin antibodies were repeatedly detected. The patient fulfilled the diagnostic criteria for definite autoimmune limbic encephalitis, when the subacute seizure worsening was considered a new appearance,⁹ and possible PNS, based on the 2004²⁰ and updated 2021 diagnostic criteria (PNS-Care score: 4).²¹

Patient 2 was a 48-year-old female who developed a confusional state with incoherent speech and memory loss for 2 days at the age of 11 years. Subsequently, she experienced focal aware seizures (FAS) manifesting visual loss and tinnitus, FIAS with head version, and FBTCs and was diagnosed with focal epilepsy arising from the right occipital lobe. Phenytoin and phenobarbital treatment suppressed the seizures to several FAS per month and rare FIAS. At 41 years old,

Sleep stage

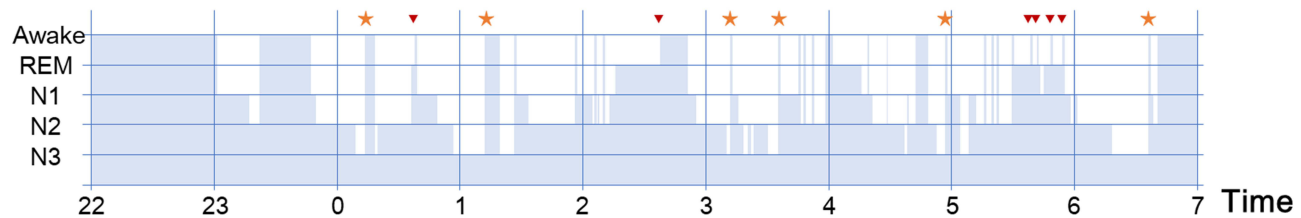


Figure 1 Hypnogram of Patient 1, showing disorganized sleep architecture with abrupt arousal during the N3 (yellow stars) and rapid eye movement (red triangles) stages.

she developed clinically isolated syndrome (CIS) with left facial pain due to a brainstem lesion²² and was treated with a half dose of IVMP. Oligoclonal bands (OCBs) in the CSF were negative, and MRI did not indicate dissemination in space based on the Barkhof criteria. The following year, her seizures became more frequent and severe, with convulsions, fear, photophobia, and psychiatric symptoms of depression, anxiety, and hallucinations. Add-on levetiracetam (LEV), carbamazepine, and perampanel were only partially effective. Topiramate (TPM), lacosamide (LCM), and LTG were administered but discontinued owing to adverse events. The patient was a carrier of the human T-cell leukemia virus type 1 (HTLV-1) but had no signs of adult T-cell leukemia or HTLV-1-associated myelopathy. EEG revealed focal abnormalities in the occipital and temporal areas. She had anti-AMPH antibodies ($\pm$). Malignant tumors, including small cell lung cancer (SCLC) and breast cancer, were not detected. A course of IVMP (1 g/day for 3 days) and 17 courses of IVIg (20 g/day for 5 days) were administered but were not effective. The patient fulfilled the diagnostic criteria for definite autoimmune limbic encephalitis,⁹ definite PNS,²⁰ and probable PNS (PNS-Care score: 6).²¹

Patient 3 was a 23-year-old male who had experienced suffocation by a fallen sliding screen at 7 months of age, followed by atonic seizures on several occasions. At 10 years of age, he developed FBTCS and FIAS, and an EEG revealed focal epileptiform discharges in the left parieto-occipital region. The seizures were relatively well controlled with a combination of clonazepam, LTG, LEV, and TPM, with one or two FIAS occurring per month. However, at the age of 18, he started having memory disturbances, which caused difficulty in keeping up with lectures and qualification examinations failure, and the FIAS durations increased. The Wechsler Memory Scale-Revised revealed the following memory impairments: verbal memory, <50; visual memory, 64; general memory, <50; attention/concentration, 64; and delayed recall, 50. Interictal EEG showed maximum left hemispheric epileptiform discharges in the left temporal or frontal areas, which had significantly widened (Figure 2). MRI showed bilateral atrophy of the mesial temporal structures (Figure 3). The patient had anti-SOX1 ($\pm$) and anti-SS-A (149 U/mL, normal range <7.0 U/mL) antibodies. CSF protein levels were slightly increased (44 mg/dL, normal range 10–40 mg/dL). The patient was diagnosed with Sjögren's syndrome based on a salivary gland biopsy (Figure 3). Whole-body CT and tumor marker analysis showed no signs of malignant tumors, including SCLC, and peripheral motor nerve repetitive stimulation showed no waxing/waning indicative of Lambert-Eaton myasthenic syndrome (LEMS). Three courses of IVMP (1 g/day for 3 days) improved the seizures to less than once every six months. He fulfilled the diagnostic criteria for definite autoimmune limbic

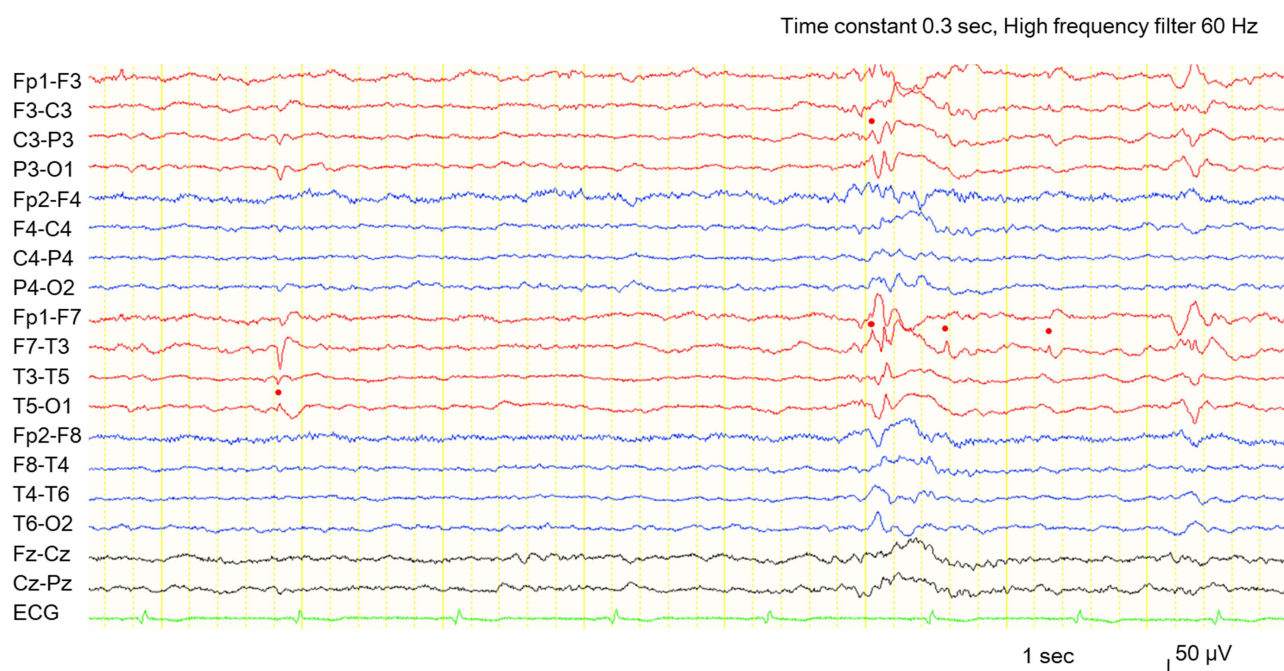


Figure 2 Interictal EEG of Patient 3, showing maximal left hemispheric epileptiform discharges in the left temporal or frontal regions (red dots).

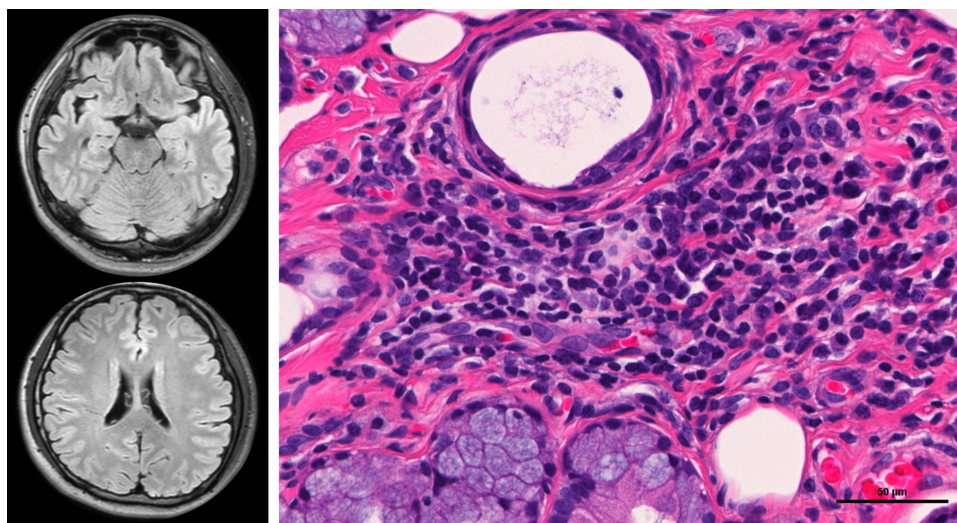


Figure 3 (Left) MRI of Patient 3, showing bilateral atrophy of the mesial temporal structures. (Right) Histopathology of the salivary gland biopsy in Patient 3, showing focal lymphocytic sialadenitis.

encephalitis when Sjögren's syndrome could be reasonably excluded,⁹ definite PNS,²⁰ and probable PNS (PNS-Care score: 7).²¹

Patient 4 was a 46-year-old female with mental retardation who graduated from high school for students with disabilities. At 44 years old, the patient developed FAS and FIAS, starting with strong anxiety, palpitations, tinnitus, and visual hallucinations, which worsened to falls and violent hyperkinetic seizures. The seizures occurred 3–4 times per week, and each seizure lasted for several hours. During the seizures, the patient was aware of the symptoms and surrounding situations but could not respond to or control dangerous movements. LCM and diazepam were effective in reducing the number of seizures to once per week and the duration to within one hour but could not suppress the seizures. Psychotropic and hypnotic medications were ineffective. Interictal EEG revealed abnormal waves in the frontal, temporal, and occipital regions. The patient tested positive for anti-recoverin antibodies (1+). MRI and CSF findings were unremarkable. Whole-body CT, tumor marker analysis, and ophthalmological evaluation revealed no signs of malignant cancer or retinal abnormalities. Two courses of IVMP (1 g/day for 3 days) improved the seizures to less than once per month, each lasting up to 30 min. Considering reproducible semiology, reactivity to antiseizure medications, and EEG abnormalities including temporal areas, the seizures were diagnosed as focal seizures involving the cingulate gyrus, though seemingly “atypical.” The patient fulfilled the diagnostic criteria for definite autoimmune limbic encephalitis,⁹ possible PNS,²⁰ and possible PNS (PNS-Care score: 4).²¹

Patient 5, a 69-year-old male, presented with FAS, with a cephalic aura, fainting sensation, and abrupt fall due to transient leg paresis, and FIAS, which caused a traffic accident over three months prior. The patient also complained of insomnia. Neurological examinations revealed time disorientation, calculation disturbances, and delayed recall memory impairment. The patient tested positive for anti-Yo antibodies (±), which were reproducible. Interictal EEG showed epileptiform discharges in the right frontotemporal area. MRI and CSF findings were unremarkable. Whole-body CT and tumor markers showed no signs of malignancy. Gastrointestinal endoscopy revealed gastric polyps; thus, regular examinations were planned. Antiseizure therapy with LCM suppressed the seizures, and immunotherapy was not performed. The patient was diagnosed with focal seizures involving the negative motor area in the cingulate gyrus. The patient fulfilled the diagnostic criteria for definite autoimmune limbic encephalitis,⁹ definite PNS,²⁰ and probable PNS (PNS-Care score: 7).²¹

Patient 6, a 19-year-old female, developed weekly episodes of dizziness, fading vision, and fainting at the age of 17 years, lasting for approximately one hour. At 11 years of age, she had several episodes of loss of consciousness. Neurological examinations revealed autonomic dysfunction with hyperhidrosis and orthostatic hypotension. The patient tested positive for anti-PNMA2 antibodies (2+). Interictal EEG revealed generalized spike-wave complexes, and MRI

revealed multiple white matter lesions. The CSF findings were unremarkable. Whole-body CT and tumor marker analysis showed no signs of malignancy. Blood tests showed a slight decrease in Cu and Zn levels, but no specific cause was found. The patient was treated with LEV, and the episodic symptoms were completely suppressed; thus, these episodes were diagnosed as FAS and FIAS. The patient fulfilled the diagnostic criteria for definite acute disseminated encephalomyelitis,⁹ definite PNS,²⁰ and probable PNS (PNS-Care score: 7).²¹

Discussion

Fulfillment of Diagnostic Criteria for AE and PNS

Autoimmune limbic encephalitis can be definitively diagnosed when all four of the following criteria are met: 1. subacute onset of working memory deficits, seizures, or psychiatric symptoms suggesting involvement of the limbic system; 2. bilateral brain abnormalities on T2-weighted FLAIR MRI that are highly restricted to the medial temporal lobes; 3. at least one of the findings of CSF pleocytosis or EEG with epileptic or slow-wave activity involving the temporal lobes; and 4. reasonable exclusion of alternative causes. In addition, if one of the first three criteria is not met, a diagnosis can be made only with the detection of antibodies against cell surface, synaptic, or onconeural proteins.⁹ In this study, five patients (Patients 1–5) were diagnosed with definite autoimmune limbic encephalitis based on the fulfillment of criterion 1 (memory disturbance [Patients 1, 3, and 5], new-onset seizures [Patients 4 and 5] or seizure worsening [Patients 1–3], and psychiatric symptoms [Patients 2 and 4]) and criterion 3 (EEG abnormalities involving the temporal areas [Patients 1–5] and detection of PAs [Patients 1–5]) (Tables 2 and 3). Patient 6 was diagnosed with definite acute disseminated encephalomyelitis based on diffuse lesions involving the white matter, a non-progressive course, and exclusion of alternative causes.⁹

The patients with AE and positive PA results showed no signs of malignant tumors. According to the 2004 diagnostic criteria for PNS, encephalomyelitis and limbic encephalitis comprised cardinal “classical” syndromes of the central nervous system, and “a neurological syndrome (classical or not) with well characterised onconeural antibodies (anti-Hu, Yo, CV2, Ri, Ma2, or amphiphysin), and no cancer” comprised “definite PNS.”²⁰ The PNS diagnostic criteria were updated in 2021 and include the concept of risk levels of phenotypes and antibodies and classify three levels of evidence for PNS: definite, probable, and possible.²¹ According to the 2021 PNS diagnostic criteria, limbic encephalitis (Patients 1–5) and encephalomyelitis (Patient 6) are high-risk neurological phenotypes. Anti-SOX1 (Patient 3), AMPH (Patient 2), Yo (Patient 5), and PNMA2 (Patient 6) antibodies are considered high-risk antibodies, leading to a diagnosis of probable PNS (Table 3). The association between anti-recoverin antibodies and cancer has not been established; thus, Patients 1 and 4 were diagnosed with possible PNS. However, anti-recoverin antibodies are associated with cancer with almost 100% specificity, are not detected in healthy individuals, and can be detected years before the clinical manifestation of cancer.^{23–26} Anti-recoverin antibodies are commonly associated with cancer-associated retinopathy; however, Patients 1 and 4 exhibited limbic encephalitis. Uncommon presentations of anti-recoverin antibodies have been reported, although they differ from limbic encephalitis. Bazhin et al revealed that 10% of patients with SCLC showed serum anti-recoverin antibodies without visual dysfunction.²⁷ A recent case report described an elderly woman who developed parkinsonism due to autoimmune basal ganglia encephalitis associated with anti-recoverin antibodies but without retinopathy or malignancy and was successfully treated with IVIg.²⁸

Patient 3 had definite autoimmune limbic encephalitis and anti-SOX1 antibodies and was diagnosed with Sjögren’s syndrome. Delalande et al reported that approximately 20% of patients with primary Sjögren’s syndrome manifest neurological symptoms, and 2.4% (2 out of 82 patients) present with encephalopathy.²⁹ Moreover, the relationship between neurological manifestations, especially those of the central nervous system, and the underlying Sjögren’s syndrome is still questionable.³⁰ A 26-year-old patient with AE and Sjögren’s syndrome who presented with progressive and intractable new-onset seizures and neuropsychiatric changes and tested positive for anti-neurochondrin antibodies in the CSF was successfully treated with immunotherapy.³¹ Therefore, it is unlikely that the AE symptoms in Patient 3 were attributable to Sjögren’s syndrome. As for clinical manifestations associated with anti-SOX1 antibodies, LEMS is the most common phenotype, followed by paraneoplastic cerebellar degeneration, sensory or sensorimotor polyneuropathy,

and paraneoplastic limbic encephalitis.³² This evidence strongly suggests that the limbic encephalitis in Patient 3 was associated with anti-SOX1 antibodies.

Multiple sclerosis (MS) is a demyelinating inflammatory disease of the central nervous system, and CIS is the first demyelinating event that can convert to MS. We previously investigated the conversion ratio from CIS to MS, including that in Patient 2, and found that patients with negative OCBs have significantly lower ratios than those with positive OCBs (9.5% vs 60.0%; $p=0.034$).²² Japanese patients with MS have a higher prevalence of epileptic seizures than the general population (6.3–8.3% versus 0.4–1.2%); however, we found only one patient with focal seizures originating from the temporal lobe, out of 63 patients with MS (1.5%).^{1,33,34} Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis can overlap with demyelinating disorders; however, Titulaer et al reported that none of the 30 patients with MS had anti-NMDAR antibodies.³⁵ Thus far, limited information is available on cases of anti-NMDAR encephalitis or autoimmune limbic encephalitis coexisting with MS.^{36–38} Anti-AMPH antibodies were initially reported in paraneoplastic stiff-person syndrome, but subsequent studies have demonstrated their relevance in limbic encephalitis.^{39–42} Therefore, we consider that the autoimmune limbic encephalitis in Patient 2 was associated with anti-AMPH antibodies unrelated to CIS.

Immunotherapy in AE with “Autoimmune Epilepsy”

In this study, 19% of patients with possible AE tested positive for PAs. Immunotherapy was attempted in two-thirds of the patients who were PA-positive and was effective in 75%. In a previous study that reported an overall response rate to immunotherapy of 62%, patients with antibodies against plasma membrane antigens showed a favorable response (87.5%), whereas only 33% of patients with anti-GAD65 antibodies against intracellular antigens responded well.⁴ Regarding PAs, one patient with anti-MA1/Ma2 antibodies did not respond; however, PAs were not fully investigated. The relatively good response in our patient may have been caused by milder seizures and more intensive therapy than in a previous report. In addition, considering that the anti-GAD65 antibody can cause fulminant and life-threatening AE with extremely intractable status epilepticus,⁴³ the type of causative antibody can widely influence the clinical course and prognosis of AE. From a practical perspective, information on antibody testing and reactivity to immunotherapy is not included in the diagnostic criteria for possible AE,⁹ and serum antibody titers do not always reflect the severity of neurological manifestations. The causal relationships among certain types of antibodies, neurological symptoms, and pathophysiological mechanisms should be further elucidated.

Impact of Sleep Disturbances in AE

Patients with AE often experience severe and prolonged sleep disturbances that worsen their neuropsychiatric symptoms and impair activities of daily living.⁴⁴ However, it is difficult to detect sleep symptoms in clinical practice. In this study, three of six patients with AE manifested sleep disorders, as revealed by intensive interviews. Patient 1 showed an abnormal sleep architecture with fragmentation, which was detected by long-term monitoring; however, the patient and their family were unaware of the problem until it was explained. An independent questionnaire and examination specializing in sleep would be useful for detecting and evaluating sleep quality.

Limitations

This study used an observational design to evaluate the importance of PA in an adult epilepsy cohort. The limitations of this study included the small number of patients, variable patient characteristics, incomplete investigation of antibodies against cell surface (membrane) antigens, short follow-up periods, and variable outcome measures. We demonstrated a wide range of clinical manifestations of AE and provided basic information for further studies.

Conclusion

Approximately one-fifth of the possible AE cases are attributable to PA, although there are no signs of malignancy, in adult Japanese patients.

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Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests for this work.

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