Novel qEEG Biomarker to Distinguish Anti-NMDAR Encephalitis From Other Types of Autoimmune Encephalitis

日本大学大学院医学研究科博士課程

内科系神経内科学専攻

溝口　知孝

修了年　2023 年

指導教員　中嶋　秀人

frontiers in Immunology

ORIGINAL RESEARCH
published: 15 February 2022
doi: 10.3389/fimmu.2022.845272

Novel qEEG Biomarker to Distinguish Anti-NMDAR Encephalitis From Other Types of Autoimmune Encephalitis

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Objective: To establish the diagnostic biomarker of electroencephalogram (EEG) to distinguish between anti-*N*-methyl-d-aspartate receptor encephalitis (NMDARE) and other types of autoimmune encephalitis (other AEs).

Methods: We reviewed the clinical records of 90 patients with acute encephalitis who were treated in our institution between January 2014 and October 2020. We enrolled the patients who fulfilled the diagnostic criteria for possible AE (pAE) defined by Graus et al. (pAE criteria) and then classified into definite NMDARE and other AEs. We investigated the main syndrome and analyzed all admission EEGs using EEG power value (PV). Statistical significance was tested using the Mann–Whitney *U* test or Fisher's exact test.

Results: Twenty-five patients fulfilled the pAE criteria and were classified into 9 with definite NMDARE (median age: 21 years; 8 women) and 12 with other AEs (median age: 37.5 years; 6 women). Four were eventually excluded. Speech dysfunction (9/9 vs. 4/12, $p = 0.005$) and movement disorders (6/9 vs. 1/12, $p = 0.016$) were more frequent in NMDARE than in other AEs. The PV analyses revealed the novel quantitative EEG (qEEG) index, namely, fast slow ratio (FSR) (PV of total beta/PV of total theta + delta). The median FSR (0.139 vs. 0.029, $p = 0.004$) was higher for NMDARE than other AEs, and the receiver operating characteristic curve area of FSR was 0.86 (95% CI 0.70–1.00). A cutoff value of 0.047 yielded a specificity of 0.75 and a sensitivity of 1.00. Focusing on patients who did not meet the "probable NMDARE criteria" in Graus 2016 (proNMDARE criteria) ($n = 10$), the pretest probability of NMDAR antibody test was 0.30 (3/10), which increased in patients with an FSR greater than the cutoff ($n = 5$) to 0.60 (3/5).

Conclusions: The NMDARE group highlighted speech dysfunction and movement disorders, and a novel qEEG index FSR accurately distinguished the NMDARE patients from other AEs. The FSR is a promising diagnostic marker for NMDARE that indicates the positive results of NMDAR antibodies in patients with AE when combined with the proNMDARE criteria.

Keywords: anti-*N*-methyl-d-aspartate receptor encephalitis, autoimmune encephalitis (AE), quantitative electroencephalogram (qEEG), biomarker, diagnosis

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Specialty section:
This article was submitted to Autoimmune and Autoinflammatory Disorders, a section of the journal Frontiers in Immunology

Received: 29 December 2021
Accepted: 24 January 2022
Published: 15 February 2022

Citation:
Mizoguchi T, Hara M, Hirose S and Nakajima H (2022) Novel qEEG Biomarker to Distinguish Anti-NMDAR Encephalitis From Other Types of Autoimmune Encephalitis. Front. Immunol. 13:845272. doi: 10.3389/fimmu.2022.845272

INTRODUCTION

Antibodies against anti-*N*-methyl-d-aspartate receptor (NMDAR) trigger anti-*N*-methyl-d-aspartate receptor encephalitis (NMDARE), a well-characterized autoimmune encephalitis (AE) whose features include psychiatric symptoms, seizures, decreased level of consciousness, movement disorders, autonomic disabilities, and hypoventilation (1, 2). Early immunotherapies and/or removal of the associated tumor are key to favorable outcomes in NMDARE (3). However, physicians still struggle to identify NMDAR antibodies soon enough to best treat the disease. Graus et al. developed syndrome-based diagnostic criteria of probable NMDARE (proNMDARE) available without any antibody test (4), but their sensitivity was deemed unsatisfactory in the first 2 weeks of disease onset (5). These limitations prompted researchers to explore diagnostic biomarkers that distinguished NMDARE from other types of AE (other AEs) in early stages, including CSF cytokines, ^{18}F-FDG PET, resting-state functional magnetic resonance imaging (MRI), and electroencephalogram (EEG) (6).

Recent analyses of EEG revealed that extreme delta brush (EDB) is highly specific for the patients with severe NMDARE (7). EDB consists of rhythmic beta activity overlying the rhythmic delta activity. Other EEG characteristics on NMDARE such as excessive beta activity and generalized rhythmic delta activity (GRDA) were also reported (8). These features could be used to non-invasively distinguish NMDARE from other AEs, though the sensitivity of EDB is approximately 30% as described in the first report (7).

The aim of the present study is to establish a novel index of quantitative EEG (qEEG) by using power value (PV) analysis and validate its ability to distinguish NMDARE from other AEs.

MATERIALS AND METHODS

Protocol Approval and Patient Classification

The study is a retrospective case–control study and was approved by the ethics committee of the Nihon University Itabashi Hospital. The details of patients' selection and classification are depicted in Figure 1. Briefly, we reviewed the clinical records of 90 patients with acute encephalitis who were treated in our hospital between January 2014 and October 2020. Then, we implemented in-house antibody screening with patients' cerebrospinal fluid (CSF), which was followed by confirmatory tests for onconeural and neuronal surface antibodies (Supplementary Methods). We enrolled the patients who fulfilled the diagnostic criteria for possible AE (pAE) as defined by Graus et al. (pAE criteria) (4) and extracted 25 patients who fulfilled the pAE criteria. Then, 23 patients with fully accessible clinical records were enrolled. We classified the pAE patients into 9 definite NMDARE and 12 other AEs, which included definite autoimmune limbic encephalitis (LE), definite AEDM, definite AE, definite Bickerstaff's encephalitis (BBE), Hashimoto's encephalopathies (HE), and antibody negative probable AE (4). Two patients were eventually unclassified into any group of AEs, namely, concluded as "reconsider diagnosis".

Assessment of Clinical Features

The clinical features that included demographics, main syndrome, and complementary data that include findings of CSF tests, antibody tests for antineuronal antigens, cranial MRI, EEG, treatments, and outcomes were compared between the groups of NMDARE and other AEs.

EEG Setting, Data Acquisition, and Analyses

EEG was initially recorded upon admission with a multichannel EEG machine (Nihon Kohden Corporation, Tokyo, Japan) obtained by certified technologists. Details on EEG settings and qEEG analyses are summarized in Supplementary Methods and Supplementary Figure 1.

Briefly, all clinical EEG recordings were conducted using 0.5 Hz low- and 60 Hz high-frequency filters. The EEG PV analyses of qEEGs were implemented using the initial EEG records. PVs for each frequency were calculated *via* fast Fourier transform (FFT) analysis with EMSE® version 5.5 (Cortech Solutions, Inc., NC, USA) software. PVs were classified into the frequency bands as alpha (8.0–13.0 Hz), beta (13.1–30.0 Hz), theta (4.0–7.9 Hz), or delta (0.5–3.9 Hz) band. The PV proportion of each frequency band is shown in Supplementary Figure 2. With the comparative analyses of PV, a novel qEEG parameter called the fast slow ratio (FSR), which was defined as PV of beta band/PV of theta and delta bands, was established by comparing PVs. FSR was compared between the groups.

We also explored the influence of sedative drugs, such as consistent midazolam and propofol infusion, on qEEG findings. We then evaluated the value of FSR between the groups in the patients without both of the sedative drugs.

Analyses of Diagnostic Accuracy for proNMDARE Criteria and FSR

We evaluated how helpful a novel qEEG index FSR is to distinguish NMDARE from other AEs when compared to the criteria of "probable NMDARE" described by Graus (proNMDARE criteria) (4). The proNMDARE criteria were rapid onset of at least four of six major groups of symptoms: (1) abnormal behavior or cognitive dysfunction, (2) speech dysfunction, (3) seizures, (4) movement disorders, (5) decreased level of consciousness, and (6) autonomic dysfunction or central hypoventilation, associated with either abnormal EEG findings, CSF pleocytosis, or oligoclonal bands. Specificity and sensitivity of diagnosis were calculated when either FSR or proNMDARE criteria were applied to 9 NMDARE and 12 other AEs patients.

Statistical Analysis

Mann–Whitney *U* test and Fisher's exact test were used to assess statistical significance in the different clinical features for non-normally distributed continuous data and categorical data, respectively. Mann–Whitney *U* test was also used to compare FSR values between groups. Receiver operating characteristic

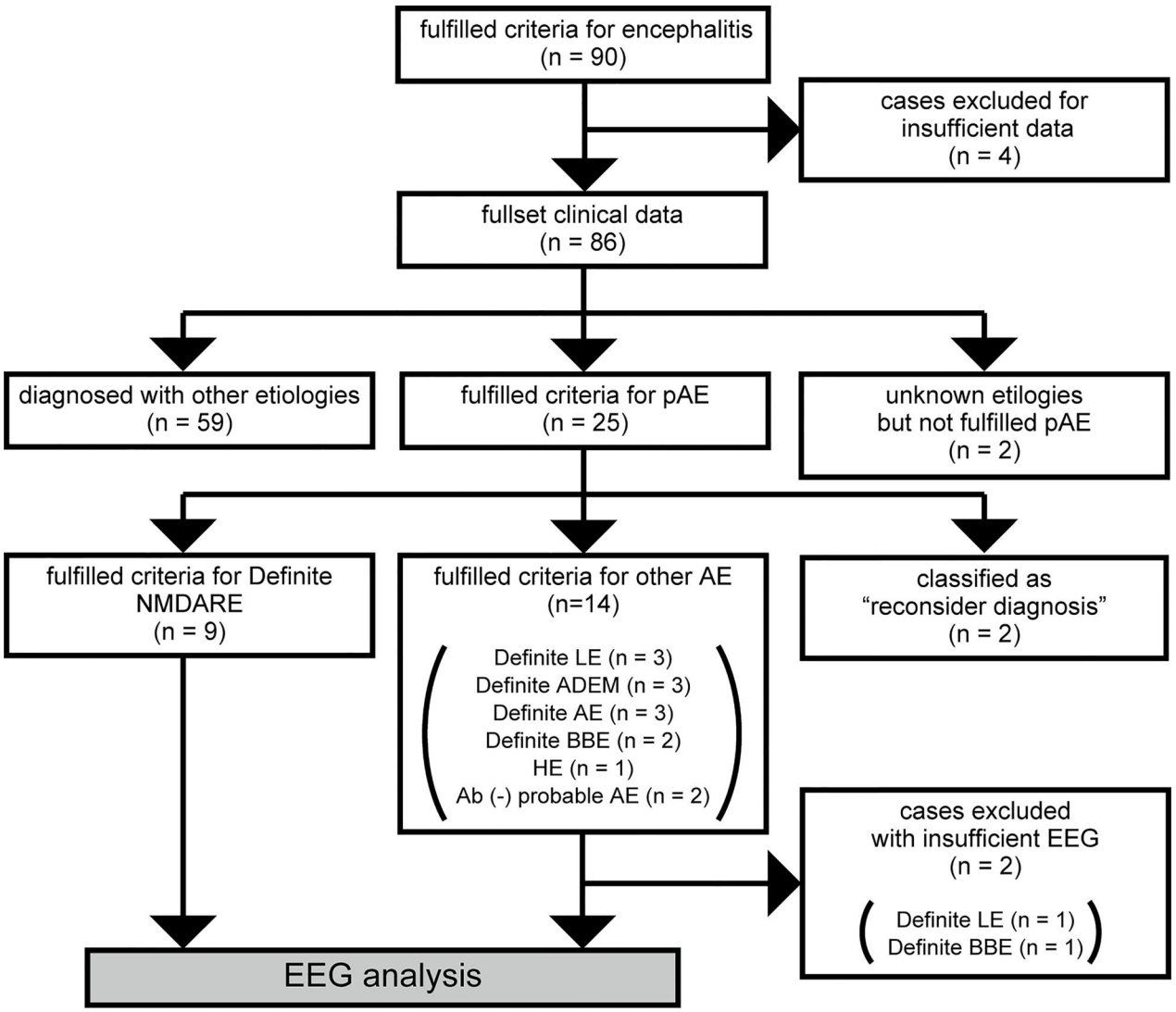

FIGURE 1 | Flowchart of patient selection and classification. Out of 90 cases that fulfilled diagnostic criteria for acute encephalitis, four were excluded because of insufficient clinical data. Out of the other 86 cases, 59 were diagnosed with encephalitis of etiologies other than autoimmunity such as infection, vasculitis, or connective tissue disorder. We could not determine an etiology of encephalitis for two cases. Twenty-five cases fulfilled criteria for pAE, which were classified using the Graus diagnostic algorithm for AE (4): 9 cases diagnosed with definite NMDARE, 14 cases diagnosed with other AE, and 2 cases classified as "reconsider diagnosis." Two out of the 14 cases with other AE were excluded from the following EEG analysis because of insufficient EEG data. Eventually, we analyzed EEGs from 21 cases, including 9 cases with NMDARE and 12 cases with other AEs. ADEM, acute disseminated encephalomyelitis; AE, autoimmune encephalitis; BBE, Bickerstaff's brainstem encephalitis; EEG, electroencephalogram; HE, Hashimoto's encephalopathy; LE, limbic encephalitis; NMDARE, anti-N-methyl-d-aspartate receptor encephalitis; pAE, possible autoimmune encephalitis.

(ROC) curve analyses were implemented to determine specificity and sensitivity of an appropriate threshold value in discriminating NMDARE from other AEs. A threshold p-value of 0.05 indicated statistical significance in all cases.

RESULTS

This study included 21 patients with AE, whose clinical records and complementary tests including EEG could be fully accessed. The patients were classified into 9 with NMDARE and 12 with other AEs, who were also classified into six categories of AE according to Graus criteria (4) (Figure 1).

Comparison of the Clinical Features of Patients With NMDARE and Other AEs

Table 1 shows a summary of demographics, main symptoms, complementary tests, treatments, and outcomes of the patients with NMDARE (n = 9) and other AEs (n = 12); detailed clinical courses of seven representative cases can be found in

TABLE 1 | Comparison of the clinical features between NMDARE and other AEs.

	NMDARE (n = 9)	Other AEs (n = 12)	p-value
Sex, female	8	6	0.159
Age, years, median (range)	21 (16–50)	38 (17–71)	0.056
Hospitalization, day, median (range)	74 (37–210)	44 (19–197)	0.164
Follow up period, months, median (range)	23 (8–81)	14.5 (4–64)	0.474
Symptoms			
Prodrome	7	9	1.000
Abnormal behaviour or cognitive dysfunction	9	11	1.000
Speech dysfunction	9	4	0.005**
Seizures	6	4	0.198
Movement disorder, dyskinesias, or rigidity/abnormal postures	6	1	0.016*
Decreased level of consciousness	6	10	0.610
Autonomic dysfunction or central hypoventilation	4	9	0.203
CSF with pleocytosis (cell >5/㎖)	8	9	0.603
MRI abnormality	2	9	0.030*
EEG			
Range from onset, day, median (range)	8 (2–23)	11.5 (1–32)	0.452
EEG findings			
Focal/diffuse slowing	9	12	1.000
Beta activity[1]	5	1	0.046*
Epileptiform activity	1	1	1.000
Extreme Delta Brush	1	0	0.429
Rhythmic Delta Activity[2]	3	7	0.387
Lateralized Periodic Discharge	0	1	1.000
Intractable epilepsy (AEDs≧3)	3	1	0.272
Sedative drug required	4	5	1.000
Immunotherapies			
IVMP	9	12	1.000
IVIg	8	6	0.159
Plasma exchange	1	1	1.000
Second line immunotherapies	5	0	0.006**
Modified Rankin Scale			
Peak (range)	5 (1–5)	5 (2–5)	0.603
Current (range)	3 (0–4)	3 (0–4)	0.555

NMDARE, anti-N-methyl-d-aspartate receptor encephalitis; AEs, autoimmune encephalitis; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; AEDs, antiepileptic drugs; IVMP, intravenous methylprednisolone; IVIg, intravenous immunoglobulins; mRS, modified Rankin scale.

[1]Beta activity included diffuse or focal beta activity and excessive beta activity.

[2]RDA included focal or generalized and intermittent or continuous RDA.

**p < 0.05, ** p < 0.01.*

Supplementary Results. Demographic data revealed that all but one NMDARE were female, while six with other AEs were female. The median age was 21 (16−50) years and 37.5 (17−53) years. Prodrome emerged in seven and nine patients with NMDARE and other AEs, respectively. Speech dysfunction (9/9 vs. 4/12, p = 0.005) and movement disorders (6/9 vs. 1/12, p = 0.016) were significantly more frequent in the patients with NMDARE than in those with other AEs. The frequencies of other symptoms that included abnormal behavior or cognitive dysfunction, decreased level of consciousness, seizures, and autonomic dysfunction/central hypoventilation were not significantly different between the groups.

Complementary tests detected CSF pleocytosis in 8 and 10 patients, respectively, in the NMDARE and other AEs groups. EEGs were recorded at 8 (2−23) days and 12 (1−32) days in the NMDARE and other AEs groups, respectively; representative EEG findings from each group are shown in Figure 2. Focal/diffuse slow activity was observed in all 21 patients. Diffuse beta activity occurred more frequently in the NMDARE group than in other AEs (5/9 vs. 1/12, p= 0.046). EDB was observed in one patient with NMDARE but in no patients with the other AEs. One patient with other AEs showed periodic lateralized epileptiform discharges, though the frequency of rhythmic delta activity was similar between the groups. Cranial MRI showed specific lesions in two patients with NMDARE, and MRI-specific lesions were more frequent in the other AEs group (2/9 vs. 9/12, p = 0.030), which included demyelinating lesions in ADEM and limbic lesions in autoimmune LE.

All patients were treated with the first-line immunotherapies that included intravenous methyl prednisolone pulse, intravenous immunoglobulins, and plasmapheresis. Five with NMDARE were resistant to first-line immunotherapies, and all were treated with several cycles of intravenous cyclophosphamide pulse therapies. One-third of NMDARE patients and one out of twelve patients with other AEs had intractable epilepsy. Four and five patients, respectively, received sedative drugs to control the confused non-reassuring condition.

Median hospitalization period was 74 (37−210) and 44 (19−197) days in NMDARE and for other AEs, respectively (p = 0.164). Outcomes evaluated with modified Rankin scale (mRS) in the peak and current status were not significantly different between the groups.

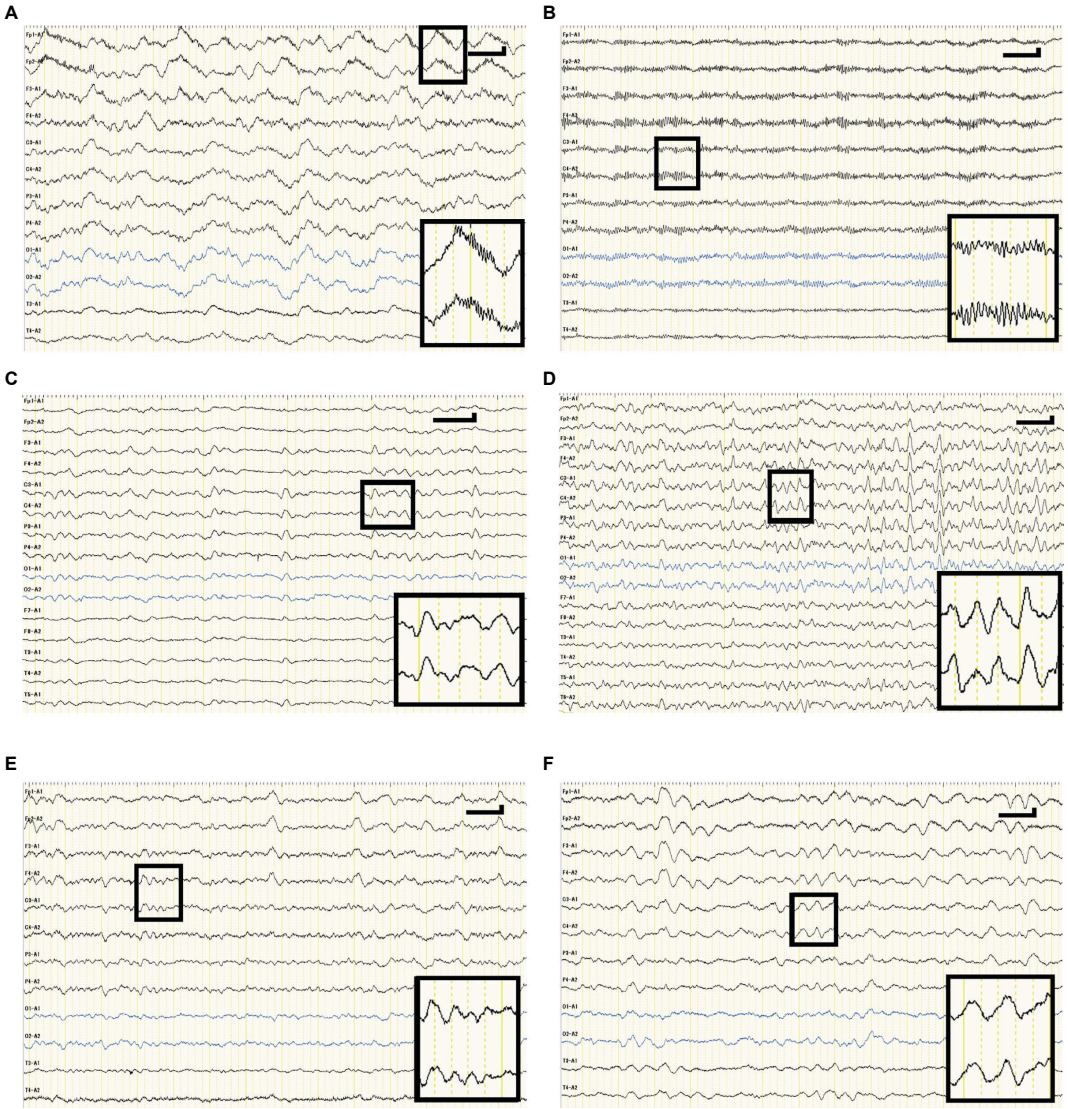

FIGURE 2 | Representative EEG waveforms of cases with NMDARE (A, B), definite autoimmune encephalitis (C, D), ADEM (E), and LE (F). (A) shows extreme delta brush consisting of rhythmic beta activity upon rhythmic delta activity—a waveform specific to patients with severe NMDARE—observed in case 1 in NMDARE group. (B) shows excessive beta activity observed in case 6 in the NMDARE group. (C) shows background slowing and intermittent rhythmic delta activity observed in case 5 of the other AEs group. (D) shows background slowing and generalized rhythmic delta activity observed in case 6 of the other AEs group. (E) shows background slowing whose frequency was 3–5 Hz observed in case 7 of the other AEs group. (F) shows frontal intermittent rhythmic delta activity observed in case 9 of the other AEs group. Vertical and horizontal bars in each panel indicate 50 μV and 1 s, respectively. ADEM, acute disseminated encephalomyelitis; LE, limbic encephalitis.

Novel qEEG Parameter FSR and ROC Curve Analyses

FSR, or the PV ratio between fast and slow EEG components, was compared across groups (Figure 3). The median FSR was significantly higher in the NMDARE group than the other AEs (0.139 vs. 0.029, $p = 0.004$) (Figure 3A). The FSR in sedative-free patients was also greater (0.283 vs. 0.040, $p = 0.018$) in NMDARE ($n = 5$) patients than in other AEs ($n = 7$) (Figure 3B).

We performed ROC curve analysis to distinguish NMDARE from other AEs using FSR, where the ROC curve area was 0.861 (95% CI 0.698–1.000), and the FSR cutoff value of 0.047 yielded a specificity of 0.75 and a sensitivity of 1.00 when indicating NMDARE (Figure 4).

Comparative Analyses of Well-Characterized Clinical Indicator and FSR for the Distinction of NMDARE From Other AEs

We evaluated the diagnostic usefulness of the novel qEEG index FSR compared with proNMDARE criteria (4). Results of qEEG

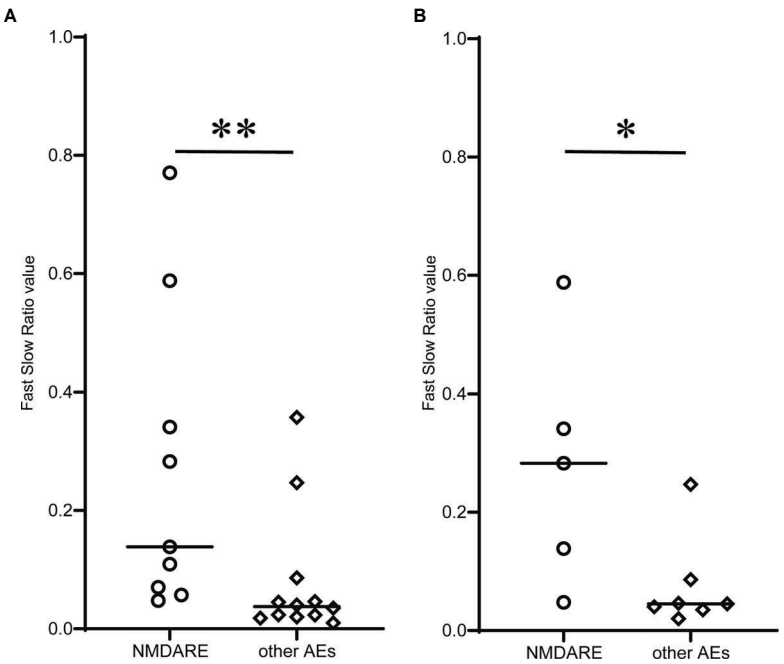

FIGURE 3 | Comparison of novel qEEG parameter Fast Slow Ratio (FSR) between NMDARE and other AEs groups. (A) shows FSR of all patients, and (B) shows FSR of sedative-free population in each group. Circles and rhombuses indicate FSR of individual cases of NMDARE and other AEs groups, respectively, and horizontal bars indicate the median of each group. Significantly higher FSR in the NMDARE group than other AEs group was observed both when all patients were included and when only the sedative-free population was included. The statistical significance was tested using Mann–Whitney *U* test. $^{*}p < 0.05$, $^{**}p < 0.01$.

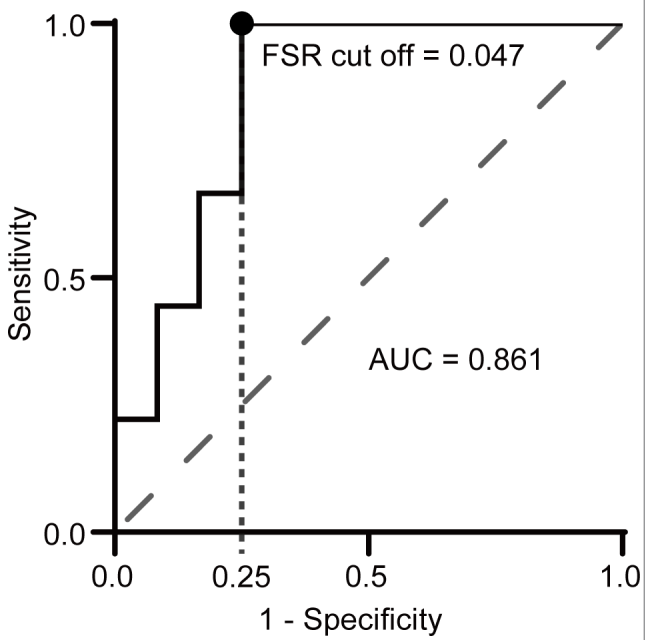

FIGURE 4 | Receiver operating characteristic (ROC) curve analysis of FSR for discriminating NMDARE from other AEs. A circle indicates the point closest to the upper left corner. The AUC was 0.86 (95% CI 0.70–1.00), and the FSR cutoff value of 0.047 yielded a sensitivity of 1.00 and a specificity of 0.75.

analyses for all 21 individuals are shown in Supplementary Table 1. Comparative analyses of the proNMDARE criteria and the FSR are shown in Table 2. Two-thirds of patients with definite NMDARE while only five of twelve with other AEs fulfilled proNMDARE criteria. The sensitivity and specificity for the diagnosis of NMDARE according to proNMDARE criteria were 0.67 (6/9) and 0.58 (7/12), respectively. Comparatively, all nine patients with definite NMDARE had higher FSR values than the cutoff of 0.047—this was the case for only three of twelve patients with other AEs. The sensitivity and specificity for the diagnosis of NMDARE using the FSR cutoff value are 1.00 (9/9) and 0.75 (3/12), respectively. Thus, the positive likelihood ratio for the diagnosis of NMDAR with the FSR above cutoff was greater than that of proNMDARE criteria (4.00 vs. 1.60). In addition, the positive predictive value for the diagnosis of NMDARE with proNMDARE criteria and FSR above cutoff is 0.55 (6/11), and 0.75 (9/12), while negative predictive value was 0.70 (7/10) and 1.00 (9/9), respectively.

DISCUSSION

We reviewed the clinical records of 90 patients who fulfilled the diagnostic criteria for encephalitis and encephalopathy (9) and extracted 25 patients who met the pAE criteria. Twenty-three were classified into 9 patients with NMDARE and 12 patients with other AEs according to the criteria (4); two patients were eventually excluded for classifying as "reconsider diagnosis".

TABLE 2 | Number and frequency of patients who met criteria of probable NMDARE (proNMDARE) and patients whose FSR was higher than our cutoff value.

		Higher FSR than cutoff		
		Yes, *n* (%)	No, *n* (%)	Total, *n* (%)
NMDARE group (*n* = 9)				
proNMDARE	Yes, *n* (%)	6 (67)	0 (0)	6 (67)
	No, *n* (%)	3 (33)	0 (0)	3 (33)
	Total, *n* (%)	9 (100)	0 (0)	9 (100)
other AEs group (*n* = 12)				
proNMDARE	Yes, *n* (%)	1 (8)	4 (33)	5 (42)
	No, *n* (%)	2 (17)	5 (42)	7 (58)
	Total, *n* (%)	3 (25)	9 (75)	12 (100)

The clinical features of all 21 patients diagnosed with AE were evaluated, and initial qEEG indices were compared between the NMDARE and other AEs groups. Our study revealed significantly more frequent speech dysfunction and movement disorders among the NMDARE patients. A novel qEEG index—FSR, which was defined as the PV ratio of beta and slow frequency bands—distinguished the NMDARE from other AEs with reasonable specificity and sensitivity.

Antibodies that flock to neuronal surface antigens trigger both paraneoplastic and non-paraneoplastic AE, which includes a variety of inflammatory brain disorders (2), accounting for 21%–39% of acute encephalitis (10–12). Since Graus et al. (4) developed an algorithm for the diagnosis of AE, which consisted of syndrome-based approach and antibody testing, several studies have been reported that classified the encephalitis cohort into specific conditions of autoimmune etiology by the criteria (5, 12–14). Given that the AE defined by the criteria is not a single disease entity, it is no wonder the proportion of each specific condition is varied among the studies. For instance, the proportion of NMDARE accounted for 17%–67% of AE and was on average 48% (43/90) across three studies (5, 12, 13). Our study agrees with others in that the proportion of AE encephalitis was 26% (23/90), of which NMDARE accounted for 39% (9/23) of AE. Recent studies also recommend diagnosing AE by immunolabeling with the rat brain tissue (tissue-based assay: TBA) and/or culturing live primary neurons to screen a series of neuronal surface antibodies (NSAs) in patients' CSF and serum (15, 16). Accordingly, we analyzed all 90 paired samples (both CSF and serum) by using in-house screening assays; 11 positive patients, whose samples produced neuropil immunostaining on TBA and detected immunofluorolabeled neurons on Live-neuron assay, were then classified into nine NMDARE, of whom two (cases 5 and 6 in other AEs) screened positives without detection of the 7 types of commercially available antigens on the cell-based assay (Supplementary Table 1).

Previous studies reported that speech dysfunction and movement disorders were more frequent in NMDARE than other AEs (17, 18) (Table 1). Consistently, we also identified speech dysfunction (100% vs. 33%, $p = 0.005$) and movement disorders (67% vs. 8%, $p = 0.016$) as the characteristic symptoms of NMDARE when compared to other AEs, though the cohort size was relatively small. We found highly frequent CSF pleocytosis in NMDARE cases (89%), which agrees with a previous large cohort study (3), but found no significant difference between the groups. We also found that the specific abnormality on cranial MRI was less frequent in patients with NMDARE than that on patients with other AEs (22% vs. 75%).

We analyzed qEEGs by comparing PVs in each frequency band between groups; this method was theoretically established for diagnosing other neuropsychiatric disorders (19–22). The findings in EEGs from AE patients have found a fast component (beta activity) in 25%–50% of those with NMDARE (7, 23–25) but not other AEs (26, 27). Actually, the present study revealed that diffuse beta activity occurred more frequently in initial EEGs from NMDARE patients (5 vs. 1 patient, $p = 0.046$) than those with other AEs. On the other hand, a recent study more commonly detected a slow component, such as delta activity, in patients with AEs (28–33): 51% in total AEs, 56% in NMDARE, and 40% in other AEs (33). In addition, GRDA with fast activity is more common in NMDARE than in other AEs (34). These findings suggest that comparing the power ratios of fast and slow components can extract NMDARE from patients with AE.

Foff et al. (19) focused on beta and delta activity (beta/delta power ratio: BDPR) in the qEEGs from patients with NMDARE. Their EEG PV analyses distinguished NMDARE from other neurological disorders (specificity 0.60, sensitivity 0.71), although they excluded the AE from the non-NMDARE control group. Meanwhile, the present study exactly focused on definite NMDARE with other AEs according to Graus criteria (4), where FSR distinguished NMDARE from other AEs (FSR: cutoff value 0.047, specificity 0.75, sensitivity 1.00), even in patients who were not administered sedative drugs. These results suggest that FSR derived from qEEG is a promising diagnostic marker when combined with specific syndrome criteria.

This study sought not to clarify the neurophysiological features of FSR but rather to show how the FSR can be used to diagnose NMDARE. The sensitivity of the proNMDARE criteria (4) was 0.67 in our cohort, as three of nine patients with NMDARE were false negatives. This value was consistent with that of other cohort studies (approximately 0.70) (5, 12–14). However, the method using an FSR cutoff value salvaged the three patients who did not meet proNMDARE criteria, thereby achieving a sensitivity of 1.00 (Table 2). Focusing on patients who did not meet the proNMDARE criteria ($n = 10$), the pretest probability from NMDAR antibody test was only 0.30 (3/10). When we further focused on patients with higher FSR than the cutoff ($n = 5$), the pretest probability increased to 0.60 (3/5). These results suggest that the diagnostic approach for NMDARE using FSR adding to

proNMDARE criteria can contribute to prevent the undervaluation of the candidates who require the antibody tests.

This study also explored the early distinction of NMDARE patients from those who only meet the pAE criteria, which only require the syndrome, cranial MRI, CSF study, and EEG (4). Thus, the pAE criteria can include the patients eventually classified as "reconsider diagnosis," as was the case for two patients in the present study. We also analyzed how FSR contributed to early distinction of NMDARE from the patients who only fulfilled the pAE criteria despite the small cohort size (n = 23, 9 NMDARE vs. 14 other pAEs) (Figure 1). The FSR value of NMDARE patients was significantly higher than that of other pAEs in both all-inclusive and sedative-free groups (Supplementary Figure 3), and ROC analyses of proNMDARE and FSR revealed that using the FSR cutoff value was both specific and sensitive (0.72 and 1.00, respectively) (Supplementary Table 2). Indeed, FSR is a promising qEEG marker for distinguishing NMDARE from the wider range of AE in early stages of disease. Yet, further investigations with larger pAE cohorts are required to confirm its usefulness.

Regarding the EEG findings of NMDARE in the recovery phase, Raja et al. reported that EEG abnormalities remained in 75% of the patients 8 months after onset, although some patients' EEG findings had returned to normal1 year after onset (35). In our study, follow-up EEG recordings in the recovery phase were available in 14 patients (7 with NMDARE and 7 with other AEs), and the median period from onset was 29 (range 12−58) and 10 (range 3−65) months in those with NMDARE and other AEs, respectively (p = 0.434) (Supplementary Table 1). We additionally implemented comparative PV analyses with qEEG in the recovery phase (described in Supplementary Methods and Results). Notably, all 14 patients had an increase in the proportion of PV in the alpha band but a decrease in the delta band (Supplementary Figure 4A). The individual FSR value in the recovery phase was higher than that in the acute phase (Supplementary Figures 4B, C), and the median FSR value did not differ between the NMDARE and other AEs groups (0.270 vs. 0.355, p = 0.805). These additional analyses revealed that the FSR derived from qEEG in the recovery phase does not seem suitable for distinguishing NMDARE from other cases of autoimmune encephalitis.

The present study had some limitations, as it was retrospective and had a relatively small cohort of AEs (n = 21). No patients with specific NSAs other than NMDAR antibodies (e.g., antibodies against leucine-rich glioma-inactivated 1, contactin-associated protein-like 2, and dipeptidyl-peptidase-like protein 6) were included, though two screening tests of different techniques were used for all patients' CSF and serum. Moreover, the cohort size classified into other AEs (n = 21) was too small to establish the characteristics of the syndromes and complementary results that included qEEG analyses in each autoimmune condition.

CONCLUSIONS

Comparisons between NMDARE and other AEs revealed that the speech dysfunction and movement disorders were more prominent in the NMDARE group. A novel qEEG indicator, FSR, which was defined as the PV ratio of beta and slow frequency bands, distinguished the NMDARE patients from other AEs with a reasonable specificity and sensitivity despite the small cohort size. The FSR derived from qEEG analyses combined with the proNMDARE criteria is a promising early diagnostic marker in patients with NMDAR but should be confirmed in a larger cohort study.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Nihon University Itabashi Hospital, Clinical Research Judging Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. The animal study was reviewed and approved by Nihon University Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

The study was designed by TM and MH. Data were collected by TM, MH, and SH. Data were analyzed by TM. The manuscript was mainly drafted by TM, and SH provided assistance to this work. The manuscript was revised by MH and HN. The study was supervised by MH and HN. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported in part by MHLW Grant Number 19 HA1 002 and JSPS KAKENHI Grant Number JP20K07875 (MH).

ACKNOWLEDGMENTS

The authors are grateful to Professor Seiichi Udagawa, Division of Natural Sciences, Nihon University School of Medicine, Tokyo, Japan, for providing advice regarding statistical analyses.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.845272/full#supplementary-material

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Supplementary Material

1 Supplementary methods

1.1 In-house assays for screening of NSAs and onconeural antibodies

A series of NSAs (e.g., antibodies against *N*-methyl-d-aspartate receptor [NMDAR], leucine-rich glioma-inactivated 1 [LGI1], contactin-associated protein-like 2 [Caspr2], dipeptidyl-peptidase-like protein 6 [DPPX], and immunoglobulin-like cell adhesion molecule 5 [IgLON5]), and onconeural antibodies (e.g., ANNA1, Yo, Ri, Ma, and CV2) for all 90 patients' CSF and serum samples were screened using the following two techniques: tissue-based assay (TBA) with rat brain sections and immunocytochemistry with rat primary cultured neurons (Live-neuron assay).

1.1.1 In-house TBA

TBA, which involved immunohistochemical analyses of rat brain tissue, was implemented as reported (1). Briefly, adult female Wistar rats were sacrificed without perfusion, and the brain was removed and fixed in 4% paraformaldehyde for 1 h at 4°C, cryoprotected in 40% sucrose for 48 h, embedded in freezing compound media, and snap frozen in isopentane chilled with liquid nitrogen. Thereafter, 6-µm-thick tissue sections were sequentially incubated with 0.3% H_2O_2 for 15 min, 5% goat serum for 1 h, and patients and control CSF (1:2) or serum (1:200) at 4°C overnight. After incubating with biotinylated secondary antibodies against human IgG (1:2000, BA-3000, Vector), the reactivity was developed using the avidin-biotin-peroxidase method. The results of the assay were independently evaluated by two experts (MH and HN) familiar with the immunohistochemical technique, who then classified the samples into "positive (neuropil pattern, astrocytic pattern, white matter pattern, and intracellular pattern)," "negative," or "dubious." The samples categorized into "dubious" required retesting to determine the final TBA results. The samples deemed "positive" were subsequently examined with the confirmation tests described below to determine the specific neuronal antigens.

1.1.2 In-house Live-neuron assay

Rat hippocampal neuronal cultures were prepared as reported (1). Briefly, matured live neurons grown on coverslips were incubated for 1 h at 4°C with patient or control CSF (1:2) or serum (1:80). After removing the media and extensive washing with PBS, neurons were fixed with 4% paraformaldehyde and immunolabeled with Alexa Fluor® 488 goat anti-human IgG (1:1000, A11013, Invitrogen). The results were photographed using a fluorescent microscope (BZ-X810, KEYENCE, Osaka, Japan). The results of the assay were evaluated by an expert (MH) familiar with the indirect immunofluorescence assay, who then classified the samples into "positive" or "negative." The samples classified as "positive" were subsequently examined with the confirmation tests below to determine the specific neuronal surface antigens.

1.2 Confirmation tests of NSAs and onconeural antibodies with commercially available tests

For patients with a positive result during in-house TBA and/or Live-neuron assay, subsequent confirmation tests using commercially available cell-based assay (CBA) for 7 neuronal surface antigens (NMDAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, LGI1, Caspr2,

gamma-aminobutyric acid receptor type B, DPPX, and IgLON5) (BIOCHIP, Euroimmun, performed by Labor Berlin) and/or commercially available line blot assays for 12 onconeural antigens (EUROLINE, Euroimmun, Lübeck, Germany) were performed.

1.3 Detection of other types of autoantibodies associated with autoimmune encephalitis

Antibodies against aquaporin-4 and myelin oligodendrocyte glycoprotein in the serum were screened using CBA (Cosmic Corporation Co., Ltd., Tokyo, Japan) for all 25 patients who fulfilled the diagnostic criteria for possible AE (2). Similarly, antibodies against thyroid peroxidase, thyroglobulin, and GQ1b were tested for the serum samples of the 25 patients.

1.4 EEG power value analysis

EEG power value (PV) analyses, which have been employed for the purpose of evaluating various neuropsychiatric disorders (3-5), were conducted using the following procedure (Supplementary Figure 2). First, after EEG recording on admission, we randomly selected 10 regions of 13-second artifact-free/seizure-free areas (3, 6). We then calculated the PVs for each frequency for each of the 10 selected regions from channels C3-C4 via fast Fourier transform (FFT) with the nonoverlapping Hanning bins, using EMSE® version 5.5 (Cortech Solutions, Inc., NC, USA) software. The PVs at each frequency were summed up across the 10 selected regions, and then summed up according to four frequency bands of alpha (8.0–13.0 Hz), beta (13.1–30.0 Hz), theta (4.0–7.9 Hz), or delta (0.5–3.9 Hz) bands. The calculated PVs of the three frequency bands (beta, theta, delta) (Supplementary Figure 2) were used to produce our novel qEEG parameter, which we termed the "Fast Slow Ratio" (FSR; PV of beta band/PV of theta and delta bands) for each patient. Finally, we compared the FSR values between the NMDARE and other AEs groups, and further conducted receiver operating characteristic (ROC) curve analysis for discriminating NMDARE from other AEs.

1.5 Comparative PV analyses between NMDARE and other AEs in acute and recovery phase

We comparatively analyzed the EEG power value (PV) in the NMDARE and other AEs groups between the acute and recovery phases. On EEG recording, the acute phase was defined as that initially recorded upon admission, whereas the recovery phase depended on the patients' status 3 months or more after onset. PV analyses were implemented using the aforementioned procedures. Given that most of the patients in the recovery phase could follow instructions involving eye opening and closing, we extracted the 10 selected regions recorded during the eyes-closed resting-state conditions. The FSR values were compared between the NMDARE and other AEs group in the acute and recovery phase, and statistical differences between the groups were tested using the Mann–Whitney *U* test, with a threshold *p* value of 0.05 indicating statistical significance.

2 Supplementary Results

2.1 Clinical course of the representative cases

Here, we present the detailed clinical courses of seven representative cases: one from the anti-*N*-methyl-d-aspartate receptor encephalitis (NMDARE) group and six from the other autoimmune encephalitis (other AEs) group.

2.1.1 Example 1 (case 1 in NMDARE group in Supplementary Table 1)

A 24-year-old woman developed symptoms of NMDARE that started with cognitive dysfunction and psychosis, followed by speech disorder, seizures, involuntary movements, decreased level of consciousness, and hypoventilation. The CSF test indicated pleocytosis. The tissue-based assays (TBAs), including indirect immunolabeling with rat frozen brain sections and live primary hippocampal neurons1, revealed a positive result, and anti-NMDAR antibody was detected in the CSF using cell-based assays. Cranial MRI revealed non-specific lesions. The initial EEG showed background slowing, intermittent generalized beta activity, and extreme delta brush (EDB) (Figure 2A). Consequently, she fulfilled the diagnostic criteria for definite NMDARE (2). During 210 days of hospitalization, she required sedative drugs, three or more antiepileptic drugs, and mechanical ventilation. She received intravenous corticosteroids, plasma exchange, and immunoglobulins as first-line immunotherapies, and further received multiple cycles of intravenous cyclophosphamide as a second-line immunotherapy. Her poorest mRS status was 5, which improved to 3 at the time of discharge. No relapse occurred and her seizures were well controlled during 81 months of follow-up after discharge.

2.1.2 Example 2 (case 6 in other AEs group in Supplementary Table 1)

An 18-year-old woman developed symptoms of AE that started with pyrexia, headache, and cognitive dysfunction, followed by speech dysfunction, decreased level of consciousness, seizure, and hypoventilation. The CSF test indicated pleocytosis. TBAs revealed a positive result in the CSF. Cranial MRI revealed no lesions. The initial EEG showed background slowing and generalized rhythmic delta activity (Figure 2D). Consequently, she fulfilled the diagnostic criteria for definite AE (2). During 33 days of hospitalization, she required more of three antiepileptic drugs and mechanical ventilation. She received intravenous corticosteroids as first-line immunotherapies. Her poorest mRS status was 5, which improved to 2 at the time of discharge. No relapse occurred during 25 months of follow-up after discharge.

2.1.3 Example 3 (case 7 in other AEs group in Supplementary Table 1)

A 36-year-old woman developed symptoms of acute disseminated encephalomyelitis (ADEM) that started with pyrexia, headache, dysfunction of the bladder and bowel, and cognitive dysfunction, followed by a decreased level of consciousness. The CSF test indicated pleocytosis. TBAs revealed a negative result in the CSF. Cranial MRI revealed hyperintensity of the bilateral thalami and basal ganglia in the T2-weighted image. The initial EEG showed background slowing and frontal intermittent rhythmic delta activity (FIRDA) (Figure 2E). Consequently, she fulfilled the diagnostic criteria for definite ADEM (2). During 33 days of hospitalization, she required mechanical ventilation and received intravenous corticosteroids as first-line immunotherapies. Her poorest mRS status was 4, which improved to 1 at the time of discharge. No relapse occurred during 12 months of follow-up after discharge.

2.1.4 Example 4 (case 9 in other AEs group in Supplementary Table 1)

A 46-year-old woman developed symptoms of limbic encephalitis (LE) that started with pyrexia, abnormal behavior, and cognitive dysfunction, followed by speech dysfunction, decreased level of consciousness, hypoventilation, and urinary retention. The CSF test indicated pleocytosis. TBAs revealed negative results in both the serum and CSF. Cranial MRI revealed hyperintensities in the bilateral temporal regions in the diffusion-weighted image and the T2-weighted image. The initial EEG showed background slowing and FIRDA (Figure 2F). Consequently, she fulfilled the diagnostic criteria for definite acute autoimmune LE (2). During 197 days of hospitalization, she required

sedative drugs and mechanical ventilation, and received intravenous corticosteroids and immunoglobulins as first-line immunotherapies. Her poorest mRS status was 5, which improved to 4 at the time of discharge. She showed no relapse during the 6 months of follow-up after discharge, but required assistance in daily life because of severe sequelae.

2.1.5 Example 5 (case 3 in other AEs group in Supplementary Table 1)

A 31-year-old man developed symptoms of Bickerstaff's brainstem encephalitis (BBE) that started with cognitive dysfunction, dysarthria followed by bilateral external ophthalmoplegia, muscle weakness, decreased level of consciousness, ataxia, and hypoventilation. The CSF test indicated pleocytosis, and anti-GQ1b antibody was detected in the serum. Cranial MRI revealed no lesions. The initial EEG showed mild background slowing. Consequently, he fulfilled the diagnostic criteria for definite BBE (2). During 37 days of hospitalization, he required mechanical ventilation, and received intravenous corticosteroids and immunoglobulins as first-line immunotherapies. His poorest mRS status was 5, which improved to 2 at the time of discharge. No relapse occurred during 26 months of follow-up after discharge.

2.1.6 Example 6 (case 4 in other AEs group in Supplementary Table 1)

A 49-year-old woman developed symptoms of Hashimoto's encephalopathy (HE) that started with abnormal behavior, pyrexia, hallucinations followed by seizure, myoclonus, and decreased level of consciousness. The CSF test was normal. TBAs revealed a negative result in the CSF. Both anti-thyroid peroxidase antibody and anti-thyroglobulin antibody were detected in the serum, which is associated with mild hyperthyroidism. Cranial MRI revealed non-specific lesions in the white matter. The initial EEG showed background slowing and low-voltage generalized beta activity. Consequently, she fulfilled the diagnostic criteria for HE (2). During 54 days of hospitalization, she required sedative drugs and received intravenous corticosteroids as first-line immunotherapies. Her poorest mRS status was 5, which improved to 3 at the time of discharge. No relapse occurred and seizures were well controlled during 64 months of follow-up after discharge.

2.1.7 Example 7 (case 12 in other AEs group in Supplementary Table 1)

A 45-year-old man developed symptoms of AE that started with pyrexia, followed by decreased level of consciousness and hypoventilation. The CSF test indicated pleocytosis. TBA revealed negative results both in the serum and CSF. Cranial MRI revealed hyperintensity of the left thalamus and pons in the T2-weighted image. The initial EEG showed background slowing and FIRDA. Consequently, he fulfilled the diagnostic criteria for autoantibody-negative but probable autoimmune encephalitis (2). During 42 days of hospitalization, he required sedative drugs and mechanical ventilation, and received intravenous corticosteroids as first-line immunotherapies. His poorest mRS status was 5, which improved to 4 at the time of discharge. No relapse occurred during 4 months of follow-up after discharge.

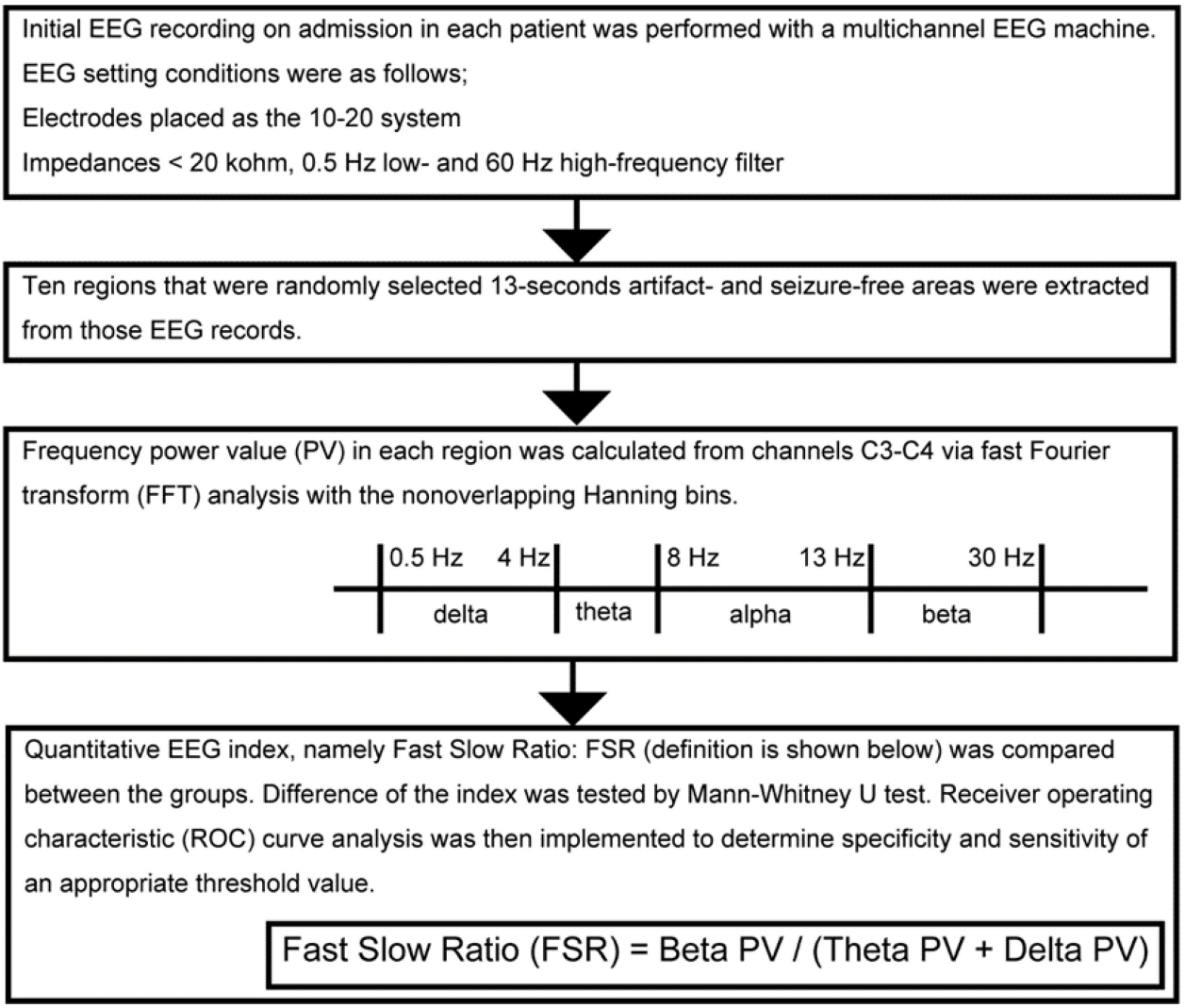
2.2 Comparative PV analyses between NMDARE and other AEs in acute and recovery phase

Follow-up EEG data in the recovery phase were available for 14 patients (7 with NMDARE and 7 with other AEs). The median period from onset was 29 (range 12–58) and 10 (range 3–65) months in those with NMDARE and other AEs, respectively (p=0.434) (Supplementary Table 1). The proportion of PV in each frequency band is shown in Supplementary Figure 4A. In the recovery phase, all 14 patients showed an increase in the proportion of PV in the alpha band but a decrease in the delta band. The individual FSR value in the recovery phase was higher than that in the acute

phase in both groups (Supplementary Table 1, Supplementary Figure 4B and 4C). The median FSR in the recovery phase did not differ between the NMDARE and other AEs groups (0.270 vs. 0.355, p=0.805).

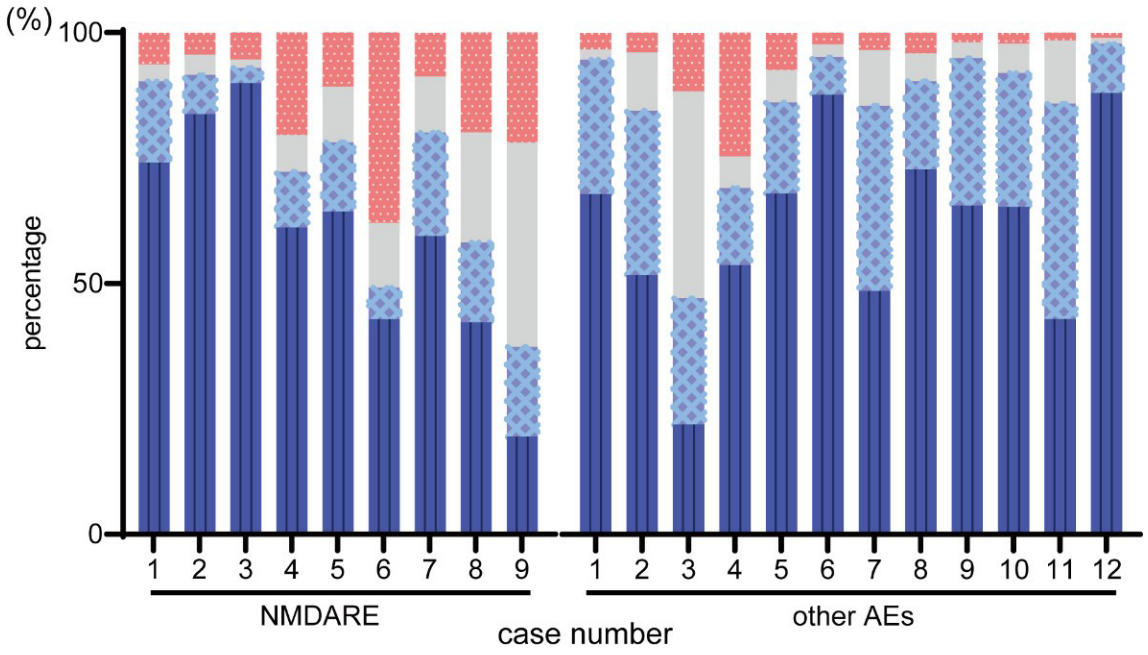
3 Supplementary Figures

3.1 Supplementary Figure 1. Flowchart of EEG recording and analysis



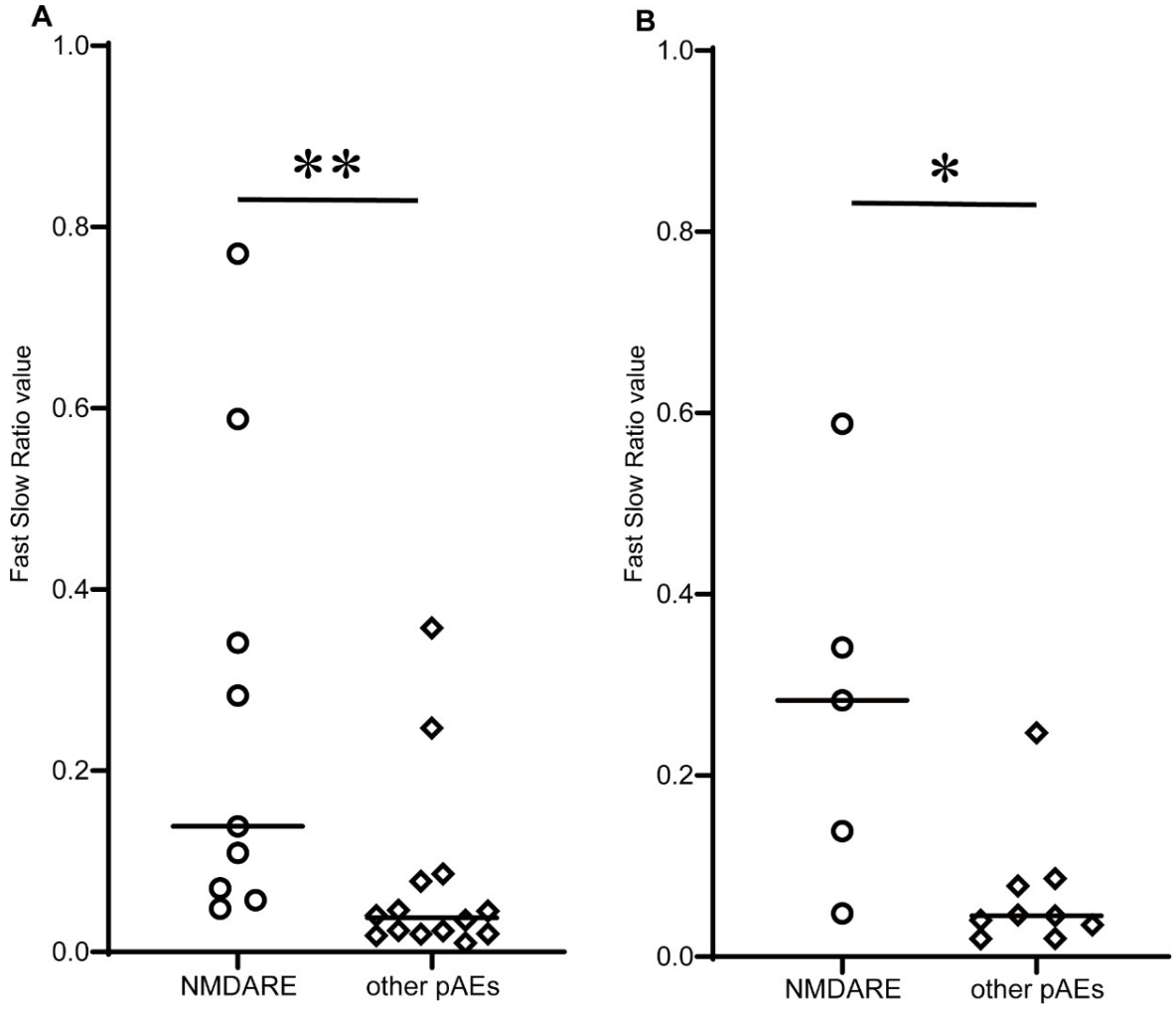
After EEG recording on admission, we randomly selected 10 regions of 13-second artifact-free/seizure-free areas, and conducted fast Fourier transform (FFT) to yield the total power value (PV) for each frequency for each of the 10 selected regions. The PVs were summed up according to the frequency bands such as alpha (8.0–13.0 Hz), beta (13.1–30.0 Hz), theta (4.0–7.9 Hz), or delta (0.5–3.9 Hz) bands. The PVs of each frequency bands were used to product Fast Slow Ratio (FSR) for each patient. We compared the FSR values between NMDARE and other AE groups, and further conducted receiver operating characteristic (ROC) curve analysis for discriminating NMDARE from other AEs.

3.2 Supplementary Figure 2. Power values (PVs) of each frequency bands for each individual case



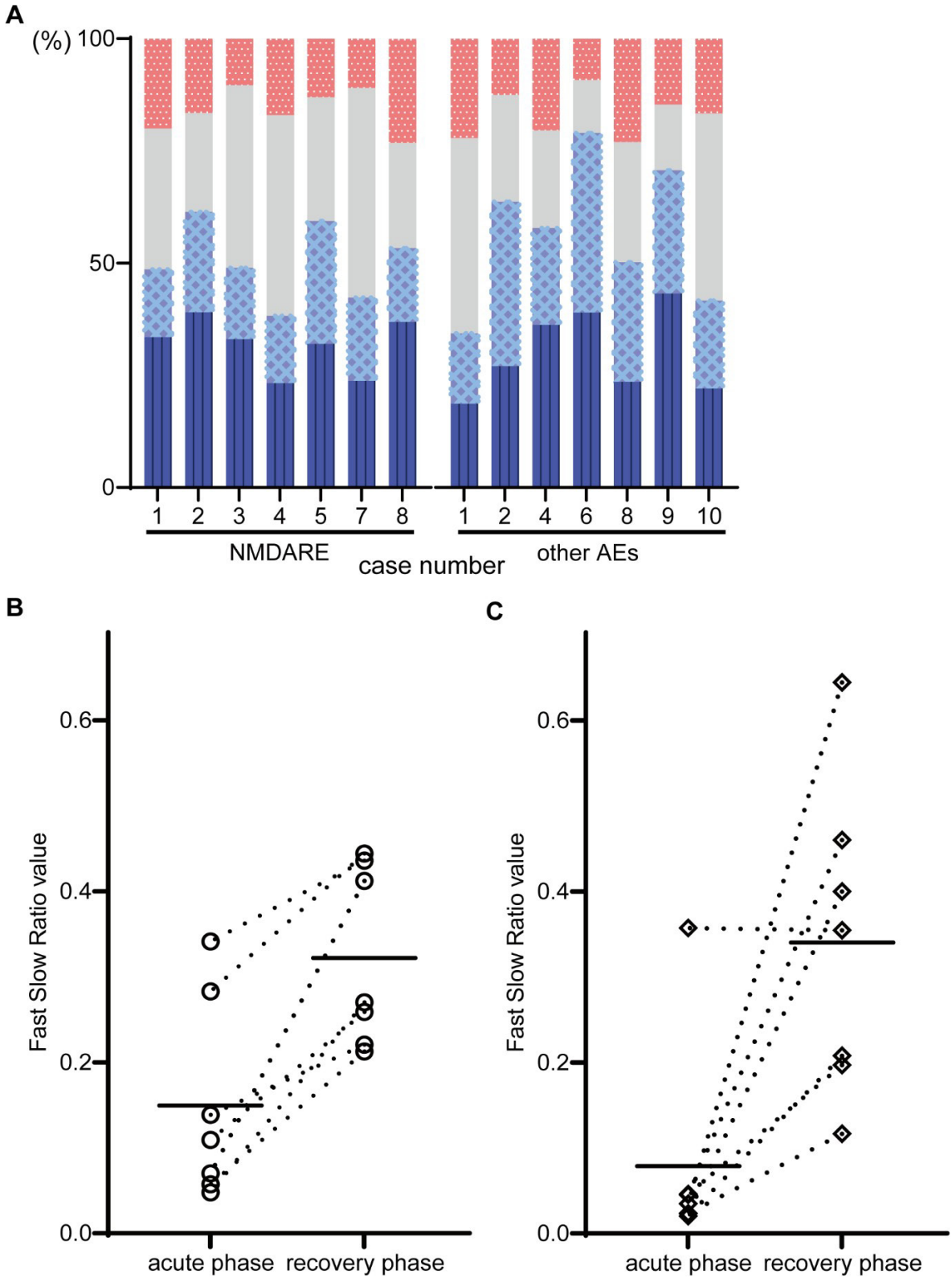
The left 9 bars and the right 12 bars indicate the PV of individual cases in the NMDARE group and other AEs group, respectively. In each bar, the four colors represent the percentage of PV for each frequency band: blue represents the delta band; light blue represents the theta band; light gray represents the alpha band; and red represents the beta band. NMDARE: anti-*N*-methyl-d-aspartate receptor encephalitis, other AEs: other types of autoimmune encephalitis.

3.3 Supplementary Figure 3. Comparison of novel qEEG parameter Fast Slow Ratio (FSR) between NMDARE group and possible autoimmune encephalitis other than NMDARE (other pAEs) group



Comparison of novel qEEG parameter Fast Slow Ratio (FSR) between NMDARE group and possible autoimmune encephalitis other than NMDARE (other pAEs) group. NMDARE group contained 9 patients who fulfilled criteria for definite NMDARE, and other pAEs group contained 14 patients who fulfilled criteria for possible AE but not fulfilled criteria for definite NMDARE. Panel A shows FSR of all patients, and panel B shows FSR of sedative free population in each group. Circles and rhombuses indicate FSR of individual cases of NMDARE and other pAEs groups respectively, and horizontal bars indicate median of each group. Significantly higher FSR in NMDARE group than other pAEs group was observed both when all patients were included and when only sedative free population was included. The statistical significance was tested using Mann–Whitney *U* test. *$p < 0.05$, **$p < 0.01$

3.4 Supplementary Figure 4. Power values (PVs) of each frequency bands in the recovery phase, and comparison of FSR between the acute and recovery phases



Follow-up EEG in the recovery phase were available for 14 patients (7 with NMDARE and 7 with other AEs). The left 7 bars and the right 7 bars on panel A indicate the PV of individual cases in the recovery phase in the NMDARE and other AEs groups, respectively. In each bar, the four colors represent the percentage of PV for each frequency band: blue represents the delta band; light blue represents the theta band; light gray represents the alpha band; and red represents the beta band. In the recovery phase, all 14 patients showed an increase in the proportion of PV in the alpha band but a decrease in the delta band. Panel B shows the FSR of the NMDARE group, and panel C shows the FSR of the other AEs group. The circles in panel B (NMDARE) or rhombuses in panel C (other AEs) connected by dashed lines represent the FSR in the acute (left) or recovery phase (right), and each horizontal bar indicates the median FSR. The individual FSR value in the recovery phase was higher than that in the acute phase in both groups (4B and 4C). NMDARE: anti-*N*-methyl-d-aspartate receptor encephalitis, other AEs: other types of autoimmune encephalitis, FSR: Fast Slow Ratio.

4 Supplementary Tables

4.1 Supplementary Table 1. Demographic and clinical data of 21 patients with autoimmune encephalitis

case	diagnosis	sex	age (years)	hospitalization (days)	Follow up period (months)
NMDARE					
1	NMDARE	F	24	210	81
2	NMDARE	M	18	129	23
3	NMDARE	F	21	103	20
4	NMDARE	F	19	108	36
5	NMDARE	F	19	55	54
6	NMDARE	F	16	74	9
7	NMDARE	F	26	46	8
8	NMDARE	F	31	37	77
9	NMDARE	F	50	51	9
other AEs					
1	ADEM	F	22	44	12
2	Def AE	M	17	19	17
3	BBE	M	31	37	26
4	HE	F	49	54	64
5	Def AE	M	34	44	6
6	Def AE	F	18	33	25
7	ADEM	F	36	38	12
8	ADEM	M	71	108	26
9	LE	F	46	197	7
10	LE	F	53	59	54
11	ProAE	M	40	186	41
12	ProAE	M	45	42	4

ADEM: acute disseminated encephalomyelitis, BBE: Bickerstaff's brainstem encephalitis, Def AE: definite autoimmune encephalitis, HE: Hashimoto's encephalopathy, LE: limbic encephalitis, NMDARE: anti-N-methyl-d-aspartate receptor encephalitis, other AEs: other types of autoimmune encephalitis, ProAE: autoantibody-negative but probable autoimmune encephalitis

Supplementary Table 1. (continued)

case	prodrome	Abnormal behavior or cognitive dysfunction	speech dysfunction	seizure	Movement disorder	decreased level of consciousness	Autonomic dysfunction or central hypoventilation	fulfilled criteria for probable NMDARE
NMDARE								
1	+	+	+	+	+	+	+	+
2	-	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	-	+
5	-	+	+	+	-	-	-	-
6	+	+	+	-	+	+	+	+
7	+	+	+	-	-	+	-	-
8	+	+	+	-	-	-	-	-
9	+	+	+	+	+	-	-	+
other AEs								
1	+	+	-	+	-	+	+	+
2	+	+	-	+	-	-	-	-
3	-	+	-	-	-	-	+	-
4	+	+	+	+	-	+	-	+
5	+	+	-	-	-	+	+	-
6	+	+	+	-	-	+	+	+
7	+	+	-	-	-	+	+	-
8	-	+	-	-	-	+	-	-
9	+	+	+	-	+	+	+	+
10	-	+	+	+	-	+	+	+
11	+	+	-	-	-	+	+	-
12	+	-	-	-	-	+	+	-

NMDARE: anti-*N*-methyl-d-aspartate receptor-r encephalitis, other AEs: other types of autoimmune encephalitis, +: yes, -: no

Supplementary Table 1. (continued)

case	pleocytosis (cell >5 / μL)	antibodies				MRI abnormality
		In-house screening assays		confirmed antigen	others	
		TBA (pattern)	Live-neuron assay			
NMDARE						
1	+	Positive (neuropil)	Positive	NMDAR		+
2	+	Positive (neuropil)	Positive	NMDAR		-
3	+	Positive (neuropil)	Positive	NMDAR		-
4	+	Positive (neuropil and intracellular)	Positive	NMDAR, SOX1		-
5	-	Positive (neuropil)	Positive	NMDAR		-
6	+	Positive (neuropil)	Positive	NMDAR		-
7	+	Positive (neuropil)	Positive	NMDAR		-
8	+	Positive (neuropil)	Positive	NMDAR		-
9	+	Positive (neuropil)	Positive	NMDAR		+
other AEs						
1	+	Positive (astrocytic)	Negative	none		+
2	+	Positive (white matter)	Negative	MOG		+
3	+	Negative	Negative	none	GQ1b	-
4	-	Negative	Negative	none	TPO, Tg	+
5	+	Positive (neuropil)	Positive	none		-
6	+	Positive (neuropil)	Positive	none		-
7	+	Negative	Negative	none		+
8	-	Negative	Negative	none		+
9	+	Negative	Negative	none		+
10	-	Negative	Negative	none		+
11	+	Negative	Negative	none		+
12	+	Negative	Negative	none		+

CSF: cerebrospinal fluid, MOG: myelin oligodendrocyte glycoprotein, MRI: magnetic resonance imaging, NMDAR: *N*-methyl-d-aspartate receptor, NMDARE: anti-N-methyl-d-aspartate receptor encephalitis, other AEs: other types of autoimmune encephalitis, SOX1: SRY-Related HMG-Box Gene 1, TBA: Tissue-based assay, Tg: thyroglobulin, TPO: Thyroid peroxidase, +: yes, -: no

Supplementary Table 1. (continued)

case	EEG recording from onset (days)	EEG findings					
		Focal or diffuse slowing	beta activity[1]	epileptiform activity	EDB	RDA[2]	LPD
NMDARE							
1	7	+	+	-	+	-	-
2	5	+	+	-	-	+	-
3	9	+	+	-	-	+	-
4	8	+	-	-	-	+	-
5	2	+	-	+	-	-	-
6	23	+	+	-	-	-	-
7	10	+	-	-	-	-	-
8	12	+	-	-	-	-	-
9	2	+	+	-	-	-	-
other AEs							
1	21	+	-	+	-	+	-
2	1	+	-	-	-	+	-
3	15	+	-	-	-	-	-
4	2	+	+	-	-	-	-
5	21	+	-	-	-	+	-
6	4	+	-	-	-	+	-
7	17	+	-	-	-	+	-
8	10	+	-	-	-	-	-
9	32	+	-	-	-	+	-
10	1	+	-	-	-	-	+
11	10	+	-	-	-	-	-
12	13	+	-	-	-	+	-

EDB: extreme delta brush, EEG: electroencephalogram, LPD: lateralized periodic discharges, NMDARE: anti-*N*-methyl-d-aspartate receptor encephalitis; other AEs: other types of autoimmune encephalitis, RDA: rhythmic delta activity, +: detected, -: not detected

[1] Beta activity included diffuse or focal beta activity and excessive beta activity

[2] RDA included focal or generalized and intermittent or continuous RDA

Supplementary Table 1. (continued)

case	FSR at onset	FSR >cut off	FSR in the recovery phase	EEG recording in the recovery phase (months)
NMDARE				
1	0.070	+	0.412	58
2	0.048	+	0.270	37
3	0.057	+	0.213	29
4	0.283	+	0.444	31
5	0.139	+	0.221	28
6	0.771	+	N/A	N/A
7	0.109	+	0.259	18
8	0.341	+	0.436	12
9	0.588	+	N/A	N/A
other AEs				
1	0.035	-	0.644	6
2	0.046	-	0.197	10
3	0.247	+	N/A	N/A
4	0.357	+	0.355	34
5	0.086	+	N/A	N/A
6	0.024	-	0.117	39
7	0.040	-	N/A	N/A
8	0.045	-	0.460	3
9	0.020	-	0.208	6
10	0.023	-	0.400	65
11	0.018	-	N/A	N/A
12	0.010	-	N/A	N/A

EEG: electroencephalogram, FSR: fast slow ratio, NMDARE: anti-*N*-methyl-d-aspartate receptor encephalitis, N/A: not available; other AEs, other types of autoimmune encephalitis, +: yes, -: no

Supplementary Table 1. (continued)

case	Intractable epilepsy (AEDs≧3)	Sedative drug required	Immunotherapies				mRS	
			IVMP	IVIg	Plasma exchange	Second line immunotherapies	worst	latest
NMDARE								
1	+	+	+	+		+	5	3
2			+	+		+	5	3
3	+	+	+			+	5	3
4			+	+			5	4
5			+	+			5	0
6		+	+	+		+	5	2
7		+	+	+		+	5	4
8			+	+			5	1
9	+		+	+	+		1	1
other AEs								
1			+	+			5	1
2			+	+			2	0
3			+	+			5	2
4		+	+				5	3
5			+				4	3
6	+	+	+				5	2
7			+	+			4	1
8			+	+			5	4
9			+	+	+		5	4
10		+	+				5	4
11		+	+				5	4
12		+	+				5	4

AEDs: anti-epileptic drugs, IVMP: intravenous methylprednisolone, IVIg: intravenous immunoglobulins, mRS: modified Rankin scale, NMDARE: anti-*N*-methyl-d-aspartate receptor encephalitis, other AEs: other types of autoimmune encephalitis

4.2 Supplementary Table 2. Number and frequency of patients who met criteria of probable NMDARE and patients whose FSR was higher than our cutoff value

		higher FSR than cutoff		
		yes, n (%)	no, n (%)	total, n (%)
NMDARE group (n = 9)				
proNMDARE	yes, n (%)	6 (67)	0 (0)	6 (67)
	no, n (%)	3 (33)	0 (0)	3 (33)
	total, n (%)	9 (100)	0 (0)	9 (100)
other pAEs group (n = 14)				
proNMDARE	yes, n (%)	1 (7)	5 (36)	6 (43)
	no, n (%)	3 (21)	5 (36)	8 (57)
	total, n (%)	4 (28)	10 (72)	14 (100)

NMDARE: anti-N-methyl-d-aspartate receptor encephalitis, other pAEs: possible autoimmune encephalitis other than NMDARE, proNMDARE: probable NMDARE, FSR: fast slow ratio

5 Supplementary references

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背景と目的

脳炎は、意識障害と痙攣など局所神経症状を主症状とし、髄液細胞増多や頭部 MRI 異常を認める脳実質の炎症性疾患である。原因は多様だが、近年、神経細胞表面のシナプス関連蛋白に対する自己抗体が関与する自己免疫性脳炎（autoimmune encephalitis: AE）が明らかになり、脳炎の重要な原因となっている。抗 *N*-methyl-d-aspartate 受容体脳炎（anti-*N*-methyl-d-aspartate receptor encephalitis: NMDARE）は、興奮性シナプスの NMDA 受容体を標的とする抗 NMDA 受容体抗体（NMDAR 抗体）の産生により引き起こされる AE で、2007 年 Dalmau らにより提唱され、脳生検例では広範なミクログリア増殖、神経細胞変性、軽微な炎症細胞浸潤を認める。NMDARE は精神症状、痙攣や多彩な不随意運動を呈し、しばしば昏睡となるため、症状極期は ICU 管理を要するが、積極的免疫療法により良好な転帰が得られる。しかし、現時点では NMDARE を含む AE の診療ガイドラインがなく、NMDAR 抗体の検索体制が普及しておらず、臨床現場では早期診断・治療導入に苦慮している。

現在、本邦における AE の診断には 2016 年に Graus らが提唱した AE の診断指針（Graus 指針）が頻用されており、本指針では症候と通常診療で可能な検査所見に基づいて AE 疑い例（possible AE）を判別したのち、自己免疫性辺縁系脳炎、急性散在性脳脊髄炎（acute disseminated encephalomyelitis: ADEM）、NMDARE、Bickerstaff 脳幹脳炎、橋本脳症、definite AE、自己抗体陰性 probable AE へとアルゴリズムに沿って分類する。この Graus 指針では NMDARE の definite と probable の診断基準が提唱され、definite NMDARE には NMDAR 抗体の証明が必須となる。一方、probable NMDARE（proNMDARE）は症候と標準的な検査所見から診断することが可能であるが、発症 2 週間以内の診断感度は十分ではなく、NMDARE の早期診断に必要な臨床指標を確立することが強く求められてきた。

脳炎患者の脳波所見として、一般的に背景の徐波化と高振幅徐波の出現が知られている。NMDARE においては高振幅徐波（δ 成分）に速波（β 成分）が重畳した extreme delta brush（EDB）が特異的な脳波所見として報告され、診断に有用な所見として注目されたが、EDB の感度は本症の 30 %程度に留まることが問題点であった。しかし、EDB の報告以降も、NMDARE の脳波では速波成分の出現が複数報告で指摘されてきた。そこで私は AE 患者の定量脳波（quantitative EEG：qEEG）解析により、速波成分の割合を数値化することで NMDARE を他の AE（other AE）から弁別するために有用な脳波指標を確立できると考えた。

本研究では、自験 AE 患者の入院時 qEEG を用いて周波数毎の振幅から求まるパワー値を周波数帯ごとに算出し、徐波成分と速波成分の比を新規脳波指標として定義し、NMDARE と他の AE 群で比較することで、この脳波指標は両者の早期判別に有用であるかを明らかにすることを目的とした。

対象と方法

本研究は後ろ向き症例対照研究で、2014 年 1 月から 2020 年 10 月の期間に当科で入院加療を受けた急性脳炎 90 例の患者から検査不十分例 4 例、その他の原因例(感染性 30 例、代謝性 7 例、腫瘍 7 例、クロイツフェルト・ヤコブ病 3 例、てんかん 3 例、膠原病 1 例、ミトコンドリア病 1 例)及び Possible AE の診断基準を満たさないが非自己免疫機序が証明されなかった原因不明 2 例を除外し、Possible AE の診断基準を満たす 25 例の患者を抽出した。このうち 2 例は臨床データが不十分であったため除外した。23 例について、AE の Graus 指針を用いて NMDARE 及び、other AE (自己免疫性辺縁系脳炎、ADEM、Bickerstaff 脳幹脳炎、橋本脳症、definite AE、抗体陰性 probableAE) の 2 群に分類した。患者選択のフローチャートを Figure 1 に示す。

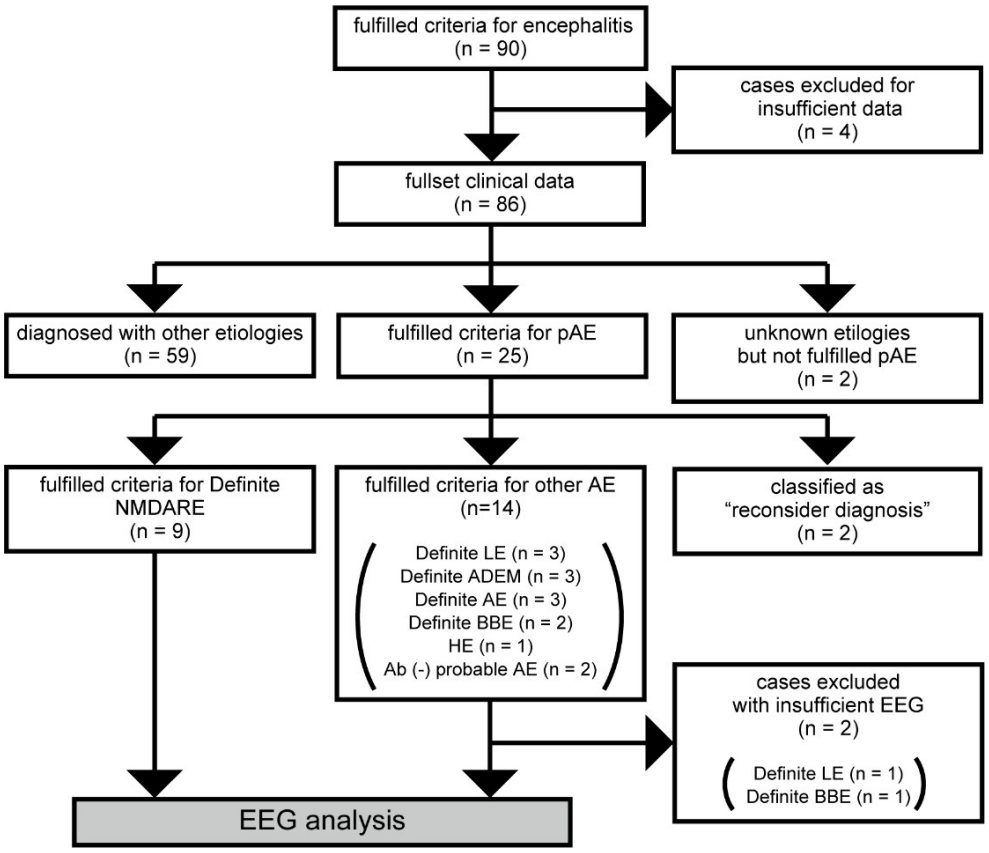

Figure 1　患者選択のフローチャート

23 例のうち 9 例が抗 NMDA 受容体抗体陽性を認め、definite NMDARE の診断基準を満たす NMDARE 群、12 例が other AE の診断基準を満たす other AE 群に分類され、2 例はいずれの診断基準にも該当せず対象から除外した。全 AE21 例の背景、主要症候、検査所見、治療、予後に関する臨床情報を集積し、入院後初回の脳波を用いて qEEG 解

析を施行した。解析では記録脳波から 13 秒間のエリアを 10 か所サンプリングした。サンプリングは、無作為にサンプリング部を選択し、アーチファクト・てんかん性放電を含んでいた際には同サンプリング部を除外し、同様の手順を合計 10 か所のサンプリングが終了するまで繰り返しておこなった。波形を解析では例えば 5.0Hz の脳波パワー値は 0.47、5.1Hz の脳波パワー値は 0.32 というように周波数 0.1Hz 刻みで周波数毎の振幅から求まる脳波パワー値が算出される。α 帯域（8.0-13.0Hz）、β 帯域（13.1-30.0Hz）、θ 帯域（4.0-7.9Hz）、δ 帯域（0.5-3.9Hz）の 4 つの各周波数帯域に属する脳波パワー値をそれぞれで合計して、各周波数帯の総パワー値とした。この総パワー値を用いて、脳炎に共通する脳波所見である徐波成分（θ 帯域、δ 帯域）を分母に速波成分（β 帯域）の比率を算出して得られる qEEG 指標（β / (θ+δ)パワー比）を Fast Slow Ratio（FSR）と定義し、NMDAR と other AE 両群で FSR 値を比較した。またミダゾラムやプロポフォールを含む鎮静薬は脳波活動に影響する可能性が指摘されており、鎮静薬投与を受けなかった患者に限定した両群間の FSR 値も比較した。

加えて本研究は NMDAR 抗体検査が判明する前の早期診断に有用な臨床的指標を探索することを目的としていることから、従来の proNMDARE の診断基準と FSR における NMDARE の判別精度を評価するため、NMDARE 群と other AE 群について FSR 及び proNMDARE の感度・特異度を算出した。

統計解析について、連続変数はMann-Whitney *U* 検定、名義変数は Fisher の正確確率検定を用いた。FSR 値の適正カットオフ値の算出に ROC 曲線を用いた。全ての検定で有意水準 α=0.05 未満を有意とした。

結果

患者背景、主要症候、検査所見、治療、予後に関して NMDARE 群と other AE 群を比較した結果をTable 1 に示す。症候では言語障害（9/9 vs 4/12, p＝0.005）と不随意運動（6/9 vs 1/12, p＝0.016）は有意に NMDARE 群で高頻度に認めたが、他の症候に関しては両群に差を認めなかった。脳波所見に関して、局所性またはびまん性徐波化を両群全例に認めた。速波は NMDARE 群で有意に多く認めた（5/9 vs 1/12, p＝0.046）。EDB は NMDARE 群で 1 例のみにみられたが、other AE 群にはみられなかった。

FSR の中央値は NMDARE 群で有意に高値であった（0.283 vs 0.040, p=0.018）（Figure 2 A）。鎮静薬投与を受けてなかった患者（5/9 vs 7/12）の比較においても FSR は NMDARE 群で有意に高値であった（0.283 vs 0.040, p=0.018）（Figure 2 B）。

ROC 曲線は、曲線下面積 0.861（95%信頼区間 0.698-1.000）で FSR のカットオフ値を 0.047 とした際、感度 1.00、特異度 0.75 で other AE から NMDARE を弁別した（Figure 3）。

また FSR と ProNMDARE 診断基準について感度・特異度を比較した結果を Table 2

に示す。NMDARE 群の 6/9 例、other AE 群の 5/12 例が proNMDARE の診断基準を満たし、感度・特異度は感度 0.67、特異度 0.58 であった。対して、definite NMDARE の患者 9 例すべての FSR 値はカットオフ以上であり、other AE 群では 3/12 例で FSR 値がカットオフ以上を示したことから、感度・特異度はそれぞれ 1.00、0.75 であった。このため陽性尤度比は、FSR > 0.047 であることが proNMDARE 診断基準を満たすことよりも大きく（4.00 vs 1.60）、また陽性適中度は 0.75 vs 0.55、陰性的中度は 1.00 vs 0.70 であった。

Table 1　NMDARE 群と otherAE 群の臨床所見の比較

	NMDARE 群 (n=9)	other AE 群 (n=12)	*P* value
性別（女性）	8	6	0.159
年齢, 歳,中央値	21 (16-50)	38 (17-71)	0.056
入院期間,日,中央値	74 (37-210)	44 (19-197)	0.164
フォローアップ期間, 月, 中央値	23 (8-81)	14.5 (4-64)	0.474
症状			
前駆症状	7	9	1.000
異常行動または認知機能障害	9	11	1.000
言語障害	9	4	0.005**
けいれん	6	4	0.198
不随意運動	6	1	0.016*
意識障害	6	10	0.610
自律神経障害または中枢性低換気	4	9	0.203
髄液細胞増多 (cell >5 / μL)	8	9	0.603
MRI 異常	2	9	0.030*
脳波			
発症日から記録日までの期間, 日,中央値	8 (2-23)	11.5 (1-32)	0.452
脳波所見			
局所性またはびまん性徐化	9	12	1.000
β 活動	5	1	0.046*
てんかん性活動	1	1	1.000
Extreme Delta Brush	1	0	0.429
律動性徐波活動	3	7	0.387
片側性周期性放電(LPD)	0	1	1.000
難治性けいれん (AEDs≧3)	3	1	0.272
鎮静薬投与	4	5	1.000
免疫治療			
ステロイドパルス療法	9	12	1.000
免疫グロブリン大量静注療法	8	6	0.159
血液浄化療法	1	1	1.000
セカンドライン免疫治療	5	0	0.006**
modified Rankin Scale			
最重症期,中央値	5 (1-5)	5 (2-5)	0.603
現在,中央値	3 (0-4)	3 (0-4)	0.555

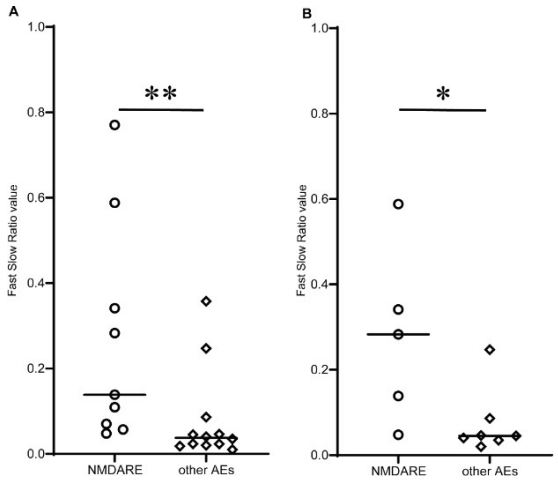

Figure 2 NMDARE 群とother AE 群のFast Slow ratio (FSR)値の比較（*$p < 0.05$, **$p < 0.01$）

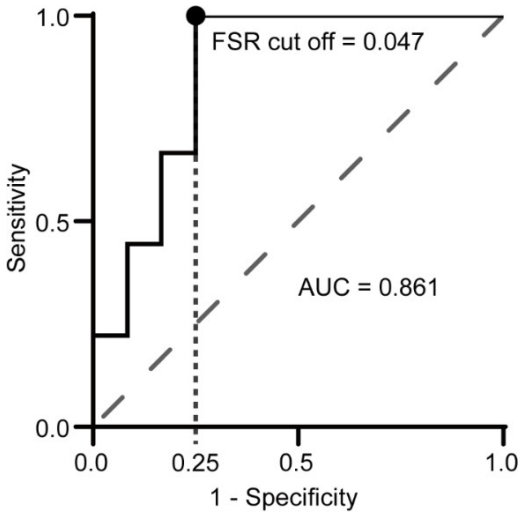

Figure 3 FSR 値の ROC 曲線

Table 2 FSR と ProNMDARE 診断基準の感度・特異度に関する比較

		FSR カットオフ値以上		
		yes, n (%)	no, n (%)	total, n (%)
NMDARE 群 (n = 9)				
proNMDARE	yes, n (%)	6 (67)	0 (0)	6 (67)
	no, n (%)	3 (33)	0 (0)	3 (33)
	total, n (%)	9 (100)	0 (0)	9 (100)
other AE 群 (n = 12)				
proNMDARE	yes, n (%)	1 (8)	4 (33)	5 (42)
	no, n (%)	2 (17)	5 (42)	7 (58)
	total, n (%)	3 (25)	9 (75)	12 (100)

考察

本研究では自験 AE 患者を NMDARE 群と other AE 群に分け、入院時 qEEG 解析により得られた qEEG 指標（β / (θ+δ)パワー比）を Fast Slow Ratio（FSR）と定義して比較検討した。NMDARE において EDB という律動性徐波に速波が重畳した脳波所見が報告されている。また速波が NMDARE で出現するという報告が散見されることから β 帯

域の成分が NMDARE に特徴的である可能性が指摘されている。既報において β 帯域と δ 帯域のパワー値の比較が報告されていたが疾患弁別精度が低く、この原因として、定性脳波において比較的程度の軽い脳症変化では δ 帯域をあまり含まず θ 帯域が主体であったことから β 帯域と θ 帯域を含む徐波（θ＋δ 帯域）のパワー値で定義した FSR に注目し検討をおこなった。

FSR の中央値は NMDARE 群で有意に高く、ROC 解析で FSR のカットオフ値を 0.047 とした際、感度 1.00、特異度 0.75 で other AE から NMDARE を弁別することができた。また、AE の臨床症候について、言語障害と不随意運動が other AE よりも NMDARE に頻度が高いことが報告されており、本研究でも other AE と比較した場合、言語障害と不随意運動が NMDARE の特徴的症候として同定された。言語障害や不随意運動の出現機序に関して、NMDA 受容体数が減少することにより、シナプス後膜における NMDA 受容体と AMPA 受容体の不均衡、GABA 作動性介在ニューロンの GABA 放出低下によるグルタミン酸作動性ニューロンの脱抑制が生じ、また側坐核や線条体に投射しているドパミン作動性ニューロンの制御逸脱状態を起こし、精神症状や不随意運動の発現に関与しているとされている。

AE 患者の脳波では、NMDARE の 25-50%に速波を認める一方で、other AE では認めないとの報告がある。本研究においても、NMDARE 群において速波成分を高頻度に認めた。EDB はグルタミン酸作動性神経伝達の障害から視床皮質求心路遮断・遅延を生じることで引き起こされると考えられている。EDB ではない速波に関しては、鎮静薬の使用と関連している可能性があるとする報告やNMDARE の発症早期に出現するとの報告があるものの、発生機序に関する定まった見解はない。

NMDARE の qEEG 解析について、Foff らは NMDARE 患者の β および δ 活動のパワー比 (β / δ パワー比: BDPR) に注目し、BDPR が NMDARE を他の神経障害と区別するのに有用(特異度 0.60、感度 0.71)であると報告したが、比較対象から AE 群を除外して評価している。一方、本研究は Graus 指針に従って、NMDARE 群と対照 AE 群を明確に区別して定義した。さらに鎮静薬非投与群のみに限定しても、FSR が NMDARE と other AE を区別できることを示した。

proNMDARE 基準の感度は、本研究では 0.67 で他の AE コホート研究 (約 0.70)の結果と概ね合致していた。しかし、カットオフ値以上の FSR では、proNMDARE 基準を満たさなかった 3 人の NMDARE 患者を検出することが可能であった。さらに proNMDARE 基準 (n=10)を満たさなかった患者群では、抗 NMDAR 抗体の検査前確率はわずか 0.30 であったが、カットオフよりも高い FSR を有する患者(n = 5) に限定すると、検査前確率は 0.60 (3/5)に上昇した。これらから、AE のコホートサイズが小さいことが本研究の限界であるものの、従来の proNMDARE 診断基準に FSR を併用して評価することにより、NMDAR 抗体検査を必要とする患者の過小評価を防ぐことに寄与する可能性が示唆された。

本研究では急性期に加え、回復過程における FSR の変化についても解析した。結果は、回復期では両群間での FSR 値に有意差はなく、両群とも FSR 値は急性期と比較して上昇していた。各周波数帯域のパワー値の割合をみるといずれの例でも α 帯域のパワー値が増加し、δ 帯域のパワー値が低下しており、回復期に徐波が減少したことで FSR値が上昇したと考えられる。

さらにサブ解析としてpossible AE 基準を満たしreconsider diagnosis に分類された 2 例を追加して FSR の検討を行った。この 2 例を加えた比較においても FSR 値が NMDARE で有意に高値(中央値 0.139 vs 0.038、p=0.003)であることが示された。

結語

AE 患者の入院時qEEG 解析から確立した新規脳波指標である FSR は、カットオフ値以上で NMDARE を other AE と精度良く判別することが可能であり、早期の非侵襲的臨床指標としての有用性が示された。さらに AE では ProNMDARE 基準と FSR を併せて診断することで、効率的な NMDAR 抗体診断につながる可能性が示唆された。