European Journal of Paediatric Neurology 54 (2025) 50–57

Contents lists available at ScienceDirect

European Journal of Paediatric Neurology

journal homepage: www.journals.elsevier.com/european-journal-of-paediatric-neurology

Original article

Evidence-based diagnostic prediction score for pediatric NMDA receptor encephalitis

Shimpei Matsuda [a,b], Takayuki Mori [a], Mariko Kasai [a], Kuniko Kohyama [a], Hiroya Nishida [c], Shimpei Abe [b], Ichiro Kuki [d], Satoko Kumada [e], Hirokazu Kurahashi [f], Sahoko Miyama [g], Motomasa Suzuki [h], Jun-ichi Takanashi [i], Satoshi Usami [j], Satoshi Yamaguchi [k], Syudo Yamasaki [k], Atsushi Nishida [k], Hiroshi Sakuma [a,*]

[a] Department of Brain & Neurosciences, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
[b] Department of Pediatrics, Juntendo University School of Medicine, Tokyo, Japan
[c] Kids Neuroscience Centre, The Children's Hospital at Westmead, Faculty of Medicine and Health, University of Sydney, Westmead, Australia
[d] Department of Pediatric Neurology, Children's Medical Center, Osaka City General Hospital, Osaka, Japan
[e] Department of Neuropediatrics, Tokyo Metropolitan Neurological Hospital, Tokyo, Japan
[f] Department of Pediatrics, Aichi Medical University, Aichi, Japan
[g] Department of Neurology, Tokyo Metropolitan Children's Medical Center, 2-8-29 Musashidai, Fuchu, Tokyo, Japan
[h] Department of Pediatric Neurology, Aichi Children's Health and Medical Center, Aichi, Japan
[i] Department of Pediatrics and Pediatric Neurology, Tokyo Women's Medical University Yachiyo Medical Center, Chiba, Japan
[j] Graduate School of Education, The University of Tokyo, Tokyo, Japan
[k] Unit for Mental Health Promotion, Research Center for Social Science & Medicine, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

A B S T R A C T

Objective: Early diagnosis and treatment of anti-N-methyl-D-aspartate receptor encephalitis (NMDARE) are crucial for a favorable prognosis. Detecting the causative autoantibodies can be challenging. Probable diagnostic criteria are useful in adults less so in children. We aimed to develop a novel diagnostic score for pediatric NMDARE using cohort data.

Methods: We retrospectively analyzed pediatric participants (0–18 years) with suspected autoimmune encephalitis who underwent cerebrospinal fluid analysis for antineuronal antibodies (Abs) between January 2015 and March 2023. Clinical data, including symptoms and laboratory findings, were analyzed. Symptoms were selected through univariate analysis and then analyzed with multivariate logistic regression model. Resulting odds ratios were used to calculate scores. Scoring systems were developed and evaluated with five-fold validation and univariate logistic regression. One scoring system was selected to create a diagnostic prediction score for pediatric NMDARE.

Results: Of the 504 patients, 264 met the inclusion criteria, and 39 tested positive for NMDAR Abs. Comparing clinical symptoms between cohorts and identified 15 variables significantly different ($p < 0.05$) to create a pediatric NMDARE prediction score. This score showed 82.1 % sensitivity and 82.2 % specificity, with an 8-point cutoff. The area under the curve was 0.888 (95 % confidence interval: 0.838–0.939). A five-fold cross-validation showed a sensitivity of 95.6 %, specificity of 71.4 %, and kappa coefficient of 0.670.

Conclusion: We developed a novel evidence-based diagnostic prediction score for pediatric NMDARE that incorporates specific clinical features and laboratory findings. This score may improve diagnostic accuracy and guide early therapy in children with suspected autoimmune encephalitis.

1. Introduction

N-methyl-D-aspartate receptor (NMDAR) encephalitis (NMDARE) is an autoimmune encephalitis characterized by a unique clinical course, including psychosis, dyskinesia, and the production of autoantibodies against the NMDAR [1]. NMDARE is the most common autoimmune encephalitis in childhood, with 37%–42.8 % of cases developing before the age of 18 [2,3]. NMDAR antibodies (Abs) are measured in the cerebrospinal fluid (CSF) via a cell-based assay, and their detection is essential for the diagnosis of definite NMDARE [4]. NMDARE is a treatable condition, and antibody testing should be performed in all suspected or presumed cases to guide treatment decisions. However, cell-based assays require the use of antigen-transfected cultured cells and largely remain research-based methodologies, making it difficult to apply widely in clinical practice. Therefore, the diagnosis of NMDARE is generally time-consuming, and delays in treatment initiation are

* Corresponding author.
E-mail address: sakuma-hs@igakuken.or.jp (H. Sakuma).

https://doi.org/10.1016/j.ejpn.2024.12.004
Received 20 September 2024; Received in revised form 27 November 2024; Accepted 16 December 2024
Available online 17 December 2024

problematic because early treatment correlates with a better prognosis [5].

As such, the diagnosis of NMDARE, which does not rely on Ab tests, is essential for making early therapeutic decisions in clinical practice. Previous studies have shown that probable diagnostic criteria, consisting of clinical symptoms and laboratory findings for NMDARE in adults, are clinically useful [6]. However, we previously examined whether these criteria are applicable to children and found that the existing criteria had 81.2 % sensitivity and 76.9 % specificity but only 31.7 % positive predictive value in a pediatric cohort. These results suggest that meeting these criteria does not necessarily ensure a diagnosis of NMDARE in children, and thus, deciding whether to progress to second-line therapy based solely on these criteria remains challenging [7].

Although a standard clinical approach for autoimmune encephalitis in children has been developed [8], a specific diagnostic strategy for pediatric NMDARE has not yet been established. Therefore, in this study, we aimed to develop and validate a diagnostic prediction score for NMDARE in children. To achieve this goal, we applied logistic regression analysis and k-fold cross-validation based on data from a pediatric cohort.

2. Methods

2.1. Study design/participants

N-methyl-D-aspartate receptor (NMDAR) encephalitis (NMDARE) is an autoimmune encephalitis characterized by a unique clinical course, including psychosis, dyskinesia, and the production of autoantibodies against the NMDAR.1 NMDARE is the most common autoimmune encephalitis in childhood, with 37%–42.8 % of cases developing before the age of 18.2, 3 NMDAR antibodies (Abs) are measured in the cerebrospinal fluid (CSF) via a cell-based assay, and their detection is essential for the diagnosis of definite NMDARE.4 However, cell-based assays require the use of antigen-transfected cultured cells and largely remain research-based methodologies, making it difficult to apply widely in clinical practice. Therefore, the diagnosis of NMDARE is generally time-consuming, and delays in treatment initiation are problematic because early treatment correlates with a better prognosis.5.

As such, the diagnosis of NMDARE, which does not rely on Ab tests, is essential for making early therapeutic decisions in clinical practice. Previous studies have shown that probable diagnostic criteria, consisting of clinical symptoms and laboratory findings for NMDARE in adults, are clinically useful.6 However, we previously examined whether these criteria are applicable to children and found that the existing criteria had 81.2 % sensitivity and 76.9 % specificity but only 31.7 % positive predictive value in a pediatric cohort. These results suggest that meeting these criteria does not necessarily ensure a diagnosis of NMDARE in children, and thus, deciding whether to progress to second-line therapy based solely on these criteria remains challenging.7.

Although a standard clinical approach for autoimmune encephalitis in children has been developed,8 a specific diagnostic strategy for pediatric NMDARE has not yet been established. Therefore, in this study, we aimed to develop and validate a diagnostic prediction score for NMDARE in children. To achieve this goal, we applied logistic regression analysis and k-fold cross-validation based on data from a pediatric cohort.

This was a retrospective analysis of pediatric patients (aged 0–18 years) who underwent CSF analysis for NMDAR Abs in our laboratory between January 1, 2015, and March 31, 2023. These patients were suspected by their attending physicians of having an autoimmune pathological background in the central nervous system. All eligible patients who underwent NMDAR Ab testing during the study period were included. Patients were excluded if they were older than 18 years, if their samples were collected more than 180 days after disease onset, if there were no analyses of NMDAR-Abs, or if no clinical data were available. Among these eligible participants, only those who fulfilled the probable criteria for pediatric autoimmune encephalitis were included [8]. In brief, a diagnosis of probable autoimmune encephalitis was made in previously healthy children who presented with acute or subacute (<3 months) onset of two or more of the following specific features suggesting clinical evidence of neurologic dysfunction: altered mental status/level of consciousness or electroencephalogram (EEG) with slowing or epileptiform activity (focal or generalized), focal neurologic deficits, cognitive difficulties, acute developmental regression, movement disorder (except tics), psychiatric symptoms, seizures not explained by a previously known seizure disorder or other condition; one or more paraclinical evidence of neuroinflammation: CSF inflammatory changes (leukocytosis >5 cells/mm^3 and/or oligoclonal banding), magnetic resonance imaging (MRI) features of encephalitis, brain biopsy showing inflammatory infiltrates, and in whom other etiologies were reasonably excluded. The patients were divided into NMDAR Ab–negative and –positive cohorts.

Clinical data were collected using a standardized form completed by each attending physician (Table 2), which included: (1) period from onset to sample collection, age, sex, past history, and antecedent infection; (2) presenting with relevant clinical signs, including disturbance of consciousness, seizures, movement disorders, psychiatric symptoms, behavioral disorders, aphasia/speech disorder, apraxia/loss of purposeful movement, agnosia, autonomic symptoms, intellectual regression, memory impairment, sleeping disorder, stereotypy, central apnea, headache, motor paralysis, sensory disturbance, cerebellar ataxia, visual symptom, eye movement disorder, ovarian teratoma; and (3) laboratory and radiographic findings including abnormal blood test results; pancytopenia, disseminated intravascular coagulation, elevated C-reactive protein (>10 mg/dL), hepatic dysfunction (aspartate transaminase and alanine aminotransferase >100 IU/L), renal dysfunction (Creatinine >2.0 mg/dL), increased CSF cell count, positive oligoclonal band, abnormal EEG findings, abnormal MRI findings, and myelin-oligodendrocyte glycoprotein Ab positivity. These characteristics facilitate a comprehensive assessment of clinical neurological findings.

2.2. Indirect immunocytochemistry

Anti-NMDAR Abs were tested using a cell-based indirect immunofluorescence assay (Autoimmune Encephalitis Mosaic1, Euroimmun Lübeck, Germany), in accordance with the manufacturer's instructions. Briefly, BIOCHIP-containing HEK cells transfected with the relevant antigen were serially incubated with undiluted CSF and Alexa Fluor 488 goat anti-human immunoglobulin G (IgG) (1:1000; Jackson ImmunoResearch, West Grove, PA, USA). Fluorescent images were obtained using

Table 1
Diagnoses of 225 patients in the NMDAR Ab–negative cohort.

Diagnosis	n
Probable Ab-negative autoimmune encephalitis/limbic encephalitis	87
Febrile infection-related epilepsy syndrome	23
Myelin oligodendrocyte glycoprotein Ab-associated disease	22
Acute encephalopathy of unknown cause	12
Epilepsy/epileptic encephalopathy, acute disseminated encephalomyelitis	8 each
Basal ganglia encephalitis	6
Probable autoimmune cerebellar ataxia, mild encephalitis/encephalopathy with reversible splenial lesion	4 each
PANDAS, brainstem encephalitis	3 each
Mycoplasma encephalitis, Sydenham chorea, Hashimoto encephalopathy, Kawasaki disease, demyelinating lesions, acute cortical encephalitis, multiple sclerosis	2 each
Others	1 each
Unknown	4

Abbreviations: PANDAS = pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections; HHV-6 = human herpesvirus 6; NMOSD; neuromyelitis optica spectrum disorders; Ab = antibody.

Table 2
Clinical and laboratory characteristics of participants.

Variables			NMDAR Ab –positive cohort (n = 32)	NMDAR Ab –negative cohort (n = 180)	p value
Median Period from onset to sample collection (range)	days	[b]	28 (0–153)	27 (0–180)	0.792
Median age (range)	years	[b]	7 (1–17)	8 (0–17)	0.876
Female sex	%	[a]	71.8	44.4	0.002
History					
Febrile seizure	%	[a]	0.0	8.0	0.084
Epilepsy	%	[a]	0.0	6.7	0.138
Developmental delay	%	[a]	0.0	7.6	0.085
Developmental disorders	%	[a]	2.6	6.2	0.706
Autoimmune disease	%	[a]	0.0	2.7	0.596
Antecedent infection	%	[a]	41.0	53.3	0.169
Disturbance of consciousness	%	[a]	74.4	78.7	0.535
Seizure	%	[a]	51.3	59.6	0.38
Generalized seizure	%	[a]	17.9	28.0	0.24
Generalized tonic-clonic seizure	%	[a]	17.9	29.8	0.176
Myoclonic seizure	%	[a]	0.0	1.8	1
Absence seizure	%	[a]	2.6	0.0	0.148
Focal seizure	%	[a]	28.2	36.4	0.368
Ocular deviation	%	[a]	25.6	27.6	1
Facial clonus	%	[a]	15.4	11.6	0.594
Unilateral extremity-clonic seizure	%	[a]	17.9	13.3	0.455
Tonic seizure	%	[a]	10.3	12.9	0.797
Impaired awareness seizure	%	[a]	12.8	8.9	0.388
Apnea	%	[a]	5.1	8.0	0.747
Movement disorders	%	[a]	59.0	32.4	0.002
Myoclonus	%	[a]	7.7	13.8	0.437
Chorea	%	[a]	20.5	3.6	<0.001
Athetosis	%	[a]	5.1	2.7	0.336
Ballism	%	[a]	10.3	1.3	0.01
Dystonia	%	[a]	10.3	5.8	0.291
Oral dyskinesia	%	[a]	53.8	15.1	<0.001
Psychiatric symptom	%	[a]	79.5	51.6	0.001
Hallucination	%	[a]	12.8	16.0	0.811
Agitation	%	[a]	64.1	27.6	<0.001
Aggression	%	[a]	20.5	8.4	0.039
Depression	%	[a]	5.1	2.7	0.336
Personality change	%	[a]	30.8	19.6	0.137
Emotional variability	%	[a]	43.6	24.4	0.019
Behavioral disorder	%	[a]	30.8	11.1	0.004
Hyperactivity	%	[a]	25.6	8.4	0.004
Autism	%	[a]	7.7	2.7	0.133
Sexually abnormal behavior	%	[a]	5.1	0.4	0.058
Aphasia/speech disorder	%	[a]	41.0	14.7	<0.001
Apraxia/loss of purposeful movement	%	[a]	17.9	3.1	0.001
Agnosia	%	[a]	7.7	5.8	0.714
Autonomic symptom	%	[a]	33.3	24.4	0.24
Blood pressure fluctuation	%	[a]	12.8	7.6	0.341
Tachycardia/ bradycardia	%	[a]	17.9	14.2	0.624
Bladder rectal disorder	%	[a]	17.9	9.3	0.153
Intellectual regression	%	[a]	41.0	11.1	<0.001
Memory impairment	%	[a]	30.8	20.4	0.207
Sleeping disorder	%	[a]	28.2	14.2	0.036
Stereotypy	%	[a]	28.2	5.3	<0.001
Central apnea	%	[a]	15.4	6.7	0.01
Headache	%	[a]	20.5	23.6	0.837
Motor paralysis	%	[a]	15.4	16.9	1
Sensory disturbance	%	[a]	7.7	7.6	1
Cerebellar ataxia	%	[a]	10.3	11.1	1
Visual symptom	%	[a]	2.6	7.1	0.482
Eye movement disorder	%	[a]	0.0	9.3	0.052
Ovarian teratoma	%	[a]	7.7	1.3	0.044
Abnormal blood test	%	[a]	10.3	16.4	0.472
Increased cerebrospinal fluid cell count	%	[a]	87.2	55.6	<0.001
Oligoclonal band positive	%	[a]	25.6	10.2	0.029
EEG abnormal finding	%	[a]	74.4	67.6	0.459
MRI abnormal finding	%	[a]	48.7	54.2	0.486
MOG antibody positive	%	[a]	0.0	0.4	1

EEG, electroencephalogram; MRI, magnetic resonance imaging; MOG, myelin-oligodendrocyte glycoprotein.
[a] the Fisher exact test.
[b] Mann–Whitney test.

a FV3000 laser-scanning confocal microscope (Olympus, Tokyo, Japan). Images were blindly reviewed by two independent investigators (K.K. and H.S.), and any discrepancies were resolved by consensus.

2.3. Tissue-based assay using frozen rat brain sections

Indirect immunohistochemistry using 8-μm frozen rat brain tissue sections that included the hippocampus was performed to screen anti-neuronal Abs. Sections were fixed with ice-cold acetone for 5 min at 4 °C and were then serially incubated with patient CSF (1:10) for 2 h and Alexa Fluor 488 anti-human IgG (1:500, Jackson ImmunoResearch, West Grove, PA) for 1 h. Images were blindly reviewed by two independent investigators (K.K. and H.S.), and any discrepancies were resolved by consensus.

2.4. Statistical analysis

1. Selection of variables for the prediction score

First, the characteristics of the two cohorts were compared using univariate analysis. Clinical and laboratory characteristics were compared between the NMDAR Ab–negative and –positive groups using the Fisher's exact test or Mann–Whitney test. For significant characteristics ($p < 0.05$), the correlations among the selected characteristics were calculated using the phi coefficient. We considered a Phi coefficient with an absolute value of ≥ 0.5 to indicate a strong correlation, which was excluded from further analyses.

2. Weighting variables and setting cutoff values for prediction scores

Second, a multivariate logistic regression model was used to weigh the selected variables. This approach allows for quantitative evaluation of the impact of each predictive variable on the overall predictive score to establish accurate diagnostic criteria. Several potential scoring models were developed based on the calculated (ORs). Cutoff values for each score were determined at the point where the sum of the sensitivity and specificity was maximized using the roc package in R.

3. Evaluation of the prediction models

Finally, each score was evaluated using a univariate logistic regression. The receiving operating characteristics (ROC) curve analysis was performed for each prediction model, and the area under the curve

(AUC) was calculated and compared. In addition, we assessed the predictive performance of each scoring system using a five-fold cross-validation method facilitated by the caret package in R [9]. For cross-validation, the population was randomly divided into five exclusive and exhaustive partitions, each accounting for 20 % of the entire dataset. For each validation cycle, one subset was used as the validation set for model testing, and the remaining 80 % formed the training set. This process was repeated five times to ensure that each subset was used as validation data exactly once. Results from these iterations were then averaged to provide a single estimate of the model performance. This cross-validation approach effectively reduces the risk of overfitting while enhancing the generalizability and robustness of the evaluation. Each scoring system was assessed using these procedures, and the optimal scoring system was determined.

The significance level was set at $\alpha = 0.05$, and all statistical analyses were performed using the R software (version 3.3.0).

2.5. Ethics

This study was approved by the Review Board of the Tokyo Metropolitan Institute of Medical Science (No. 15-3, 18-3 and 21-2). Informed consent was obtained from all participants or their guardians before enrollment in the study.

This study adhered to the Standards for Reporting Diagnostic Accuracy Studies (STARD) for Prognostic Studies.

2.6. Data availability

Anonymous raw data from the study cohort (n = 325) are available upon request.

3. Results

3.1. Study participants

A total of 504 participants were enrolled in the study. Participants who did not meet the following inclusion criteria were excluded: age >18 (n = 24), sample collection >180 days from onset (n = 105), or insufficient clinical information (n = 50). Among 325 eligible participants, 264 fulfilled the probability criteria for pediatric autoimmune encephalitis and were included in this study. In total, 39 patients were positive for NMDAR Abs (Fig. 1). The diagnoses of the 225 participants in the NMDAR Ab–negative cohort are shown in Table 1. The diagnosis of each condition was based on specific diagnostic criteria [4,8,10–12].

3.2. Result of statistical analysis

1. Selection of the variables for prediction score

Univariate analysis of the NMDAR Ab–positive and –negative cohorts confirmed that females were more likely to be diagnosed with NMDARE ($p = 0.002$). Moreover, the incidences of movement disorders ($p = 0.002$), chorea ($p < 0.001$), ballism ($p = 0.01$), oral dyskinesia ($p = < 0.001$), psychiatric symptoms ($p = 0.001$), agitation ($p < 0.001$), aggression ($p = 0.039$), emotional variability ($p = 0.019$), behavioral disorders ($p = 0.004$), hyperactivity ($p = 0.003$), aphasia/speech disorder ($p < 0.001$), apraxia/loss of purposeful movement ($p = 0.001$), intellectual regression ($p < 0.001$), sleeping disorder ($p = 0.036$), stereotypy ($p < 0.001$), ovarian teratoma ($p = 0.044$), increased CSF cell count ($p < 0.001$), and positive oligoclonal band ($p = 0.029$) were significantly higher in the NMDAR Ab-positive cohort than in the NMDAR Ab-negative cohort. Conversely, no significant differences were observed in the presence of seizures, autonomic symptoms, abnormal EEG findings, or abnormal MRI findings between the cohorts with or without NMDARE (Table 2). OCB was excluded as a candidate for score generation because 58.3 % (n = 154) of the patients did not undergo OCB. Inclusive variables such as movement disorders, psychiatric symptoms, and behavioral disorders were excluded to avoid duplicating symptoms in the subcategories.

None of the 15 characteristics showed a strong association ($\varphi < 0.5$) (Table 3). To ensure that the results were applicable to a wider range of cases, we included all 15 characteristics in the subsequent analysis.

2. Weighting variables and setting cutoff values for prediction scores

Results of a multivariate logistic regression analysis performed using the selected 15 characteristics are presented in Table 4. Chorea and increased CSF cell counts demonstrated high ORs, exceeding 10. Conversely, emotional variability, sleeping disorders, and stereotypy exhibited ORs below 1.

Next, we used univariate logistic regression to identify useful predictors of NMDARE. Four scoring models were created to generate a predictive score (Fig. 2). Scores of 1, 2, and 4 were based on 15 characteristics, whereas 3 characteristics with ORs <1 (emotional variability, sleeping disorder, and stereotypy) were excluded from Score 3 (Fig. 2). Score 1 did not use any weighting, whereas Scores 2 and 4 used different weights for the variables. (Fig. 2).

For Score 1, the cutoff was determined as four points, with an AUC of 0.868 (95 % CI: 0.814–0.922). For Score 2, the cutoff was five points,

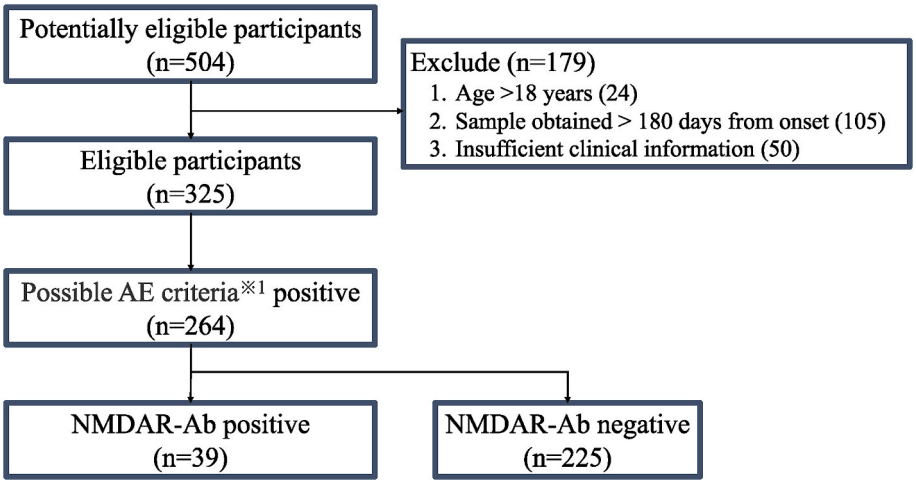

Fig. 1. Study cohort and inclusion/exclusion criteria.
Possible Autoimmune encephalitis(AE) Criteria: Cases presenting with at least two focal or diffuse neurological deficits, cognitive deficits, motor abnormalities, psychiatric symptoms, or seizures.

Table 3
Interrelationship among the 15 variables. (Phi coefficient).

	Female sex	Chorea	Ballism	Oral dyskinesia	Agitation	Aggression	Emotional variability	Hyperactivity	Aphasia/ speech disorder	Apraxia/loss of purposeful movement	Intellectual regression	Sleeping disorder	Stereotypy	Ovarian teratoma	Increased CSF cell count
Female sex		0.07	−0.11	0.16	0.01	−0.05	0.14	0.00	0.10	0.14	0.07	0.13	0.18	0.16	−0.05
Chorea	0.07		0.06	0.22	0.02	−0.09	0.06	0.11	0.04	0.22	0.11	0.10	0.26	0.07	−0.09
Ballism	−0.11	0.06		0.21	0.14	0.02	0.06	0.24	0.04	0.07	0.25	0.12	0.12	−0.03	0.04
Oral dyskinesia	0.16	0.22	0.21		0.20	0.07	0.17	0.12	0.11	0.13	0.32	0.10	0.17	0.17	0.09
Agitation	0.01	0.02	0.14	0.20		0.40	0.29	0.19	0.06	0.05	0.17	0.17	0.21	0.16	−0.01
Aggression	−0.05	−0.09	0.02	0.07	0.40		0.30	0.12	−0.03	−0.02	0.13	0.16	0.03	−0.05	0.04
Emotional variability	0.14	0.06	0.06	0.17	0.29	0.30		0.08	0.12	0.04	0.18	0.24	0.20	0.08	0.01
Hyperactivity	0.00	0.11	0.24	0.12	0.19	0.12	0.08		0.05	−0.03	0.22	0.30	0.41	0.03	0.01
Aphasia/speech disorder	0.10	0.04	0.04	0.11	0.06	−0.03	0.12	0.05		0.32	0.23	0.11	0.30	−0.01	0.03
Apraxia/loss of purposeful movement	0.14	0.22	0.07	0.13	0.05	−0.02	0.04	−0.03	0.32		0.32	0.12	0.29	−0.04	0.05
Intellectual regression	0.07	0.11	0.25	0.32	0.17	0.13	0.18	0.22	0.23	0.32		0.15	0.24	0.00	−0.01
Sleeping disorder	0.13	0.10	0.12	0.10	0.17	0.16	0.24	0.30	0.11	0.12	0.15		0.30	0.00	−0.02
Stereotypy	0.18	0.26	0.12	0.17	0.21	0.03	0.20	0.41	0.30	0.29	0.24	0.30		0.13	0.11
Ovarian teratoma	0.16	0.07	−0.03	0.17	0.16	−0.05	0.08	0.03	−0.01	−0.04	0.00	0.00	0.13		0.07
Increased CSF cell count	−0.05	−0.09	0.04	0.09	−0.01	0.04	0.01	0.01	0.03	0.05	−0.01	−0.02	0.11	0.07	

CSF, cerebrospinal fluid.

with an AUC of 0.877 (95 % CI: 0.821–0.932). For a Score of 3, the cutoff was three points, with an AUC of 0.892 (95 % CI, 0.845–0.938). For a Score of 4, the cutoff was eight points, with an AUC of 0.888 (95 % CI, 0.838–0.939).

3. Evaluation of weighting models

ROC curve analysis revealed no significant difference in AUC values between Scores 1 and 2 ($p = 0.296$). Further, Score 4 had a higher AUC value than Score 2 (0.888 vs. 0.877, respectively; $p = 0.025$). The AUC values did not significantly differ between Scores 3 and 4 (0.892 and 0.888, respectively; $p = 0.635$) (Fig. 3).

Finally, using data from a cohort of 264 patients with possible autoimmune encephalitis, we randomly divided the patients into training ($n = 212$) and test sets ($n = 52$). We conducted a five-fold cross-validation by incorporating a 20 % test set to account for potential overfitting. The results for Score 1, with a cutoff value of four points, showed a kappa value of 0.561, sensitivity of 95.6 %, and specificity of 57.1 %. For Score 2, with a cutoff value of five points, the kappa value, sensitivity, and specificity were 0.626, 97.8 %, and 57.1 %, respectively. For Score 3, using a cutoff value of three points, the kappa value, sensitivity, and specificity were 0.611, 93.3 %, and 71.4 %, respectively. For Score 4, using a cutoff value of eight points, we achieved a kappa value of 0.670, sensitivity of 95.6 %, and specificity of 71.4 %.

Even when different random seeds were used, cross-validation yielded similar results, indicating the robustness of the findings. Since the kappa value for Score 4 was superior and reducing variables (in Score 3) did not improve the kappa value, Score 4 was finally selected as the pediatric NMDAR encephalitis prediction score (PedNEP score).

4. Discussion

The clinical manifestations of pediatric NMDARE reportedly differ from those in adults [3]. For example, speech and movement disorders are more common in children, whereas memory impairment and central hypoventilation are less frequent [3]. These age-dependent differences in clinical features underpins the need for a diagnostic score specific to the pediatric population.

Our study found that female sex, apraxia/loss of purposeful movement, and sleeping disorders were all significantly more prevalent in pediatric NMDARE cases than in non-NMDARE cases, which were not included in the current probable diagnostic criteria for NMDARE [4]. In the adolescent population, NMDARE was significantly more common in females [12,13], whereas no female predominance was observed in younger children. Seizures and impaired consciousness, which are included in the adult criteria, do not predict an NMDARE diagnosis in children. This may be because these symptoms are often observed in other inflammatory neurological diseases in childhood [14–16]. Our newly developed score is expected to help predict NMDARE more accurately in children with suspected autoimmune encephalitis.

One unique feature of the proposed score is the more-detailed definition of clinical symptoms. For example, the existing probable diagnostic criteria describe movement disorders as major symptoms, including oral dyskinesia, rigidity, and postural abnormalities [4], whereas the proposed PedNEP score includes chorea, ballism, and oral dyskinesia. These results are consistent with reported findings that dystonia, chorea, and stereotypies are the principal dominant movement disorders of NMDARE [17]. Furthermore, among psychiatric symptoms, agitation was found to be more closely associated with NMDARE. Dyskinesia and other psychiatric symptoms in children can be described in greater detail, which may further improve the diagnostic accuracy for NMDARE.

Previous probable diagnostic criteria for autoimmune encephalitis mostly comprised empirical expert opinions and were not based on real-world data [4]. As such, to our knowledge, our prediction score is the first to be determined using statistical methods. However, the primary

Table 4
Multivariate logistic regression model of the 15 selected variables.

	Odds ratio	95 % CI	Estimate	Std. error	p value	Z value
Female	4.73	1.58–14.2	1.554	0.561	0.006	2.770
Chorea	17.4	2.2–137	2.856	1.054	0.007	2.709
Ballism	4.89	0.434–55.2	1.588	1.237	0.199	1.284
Oral dyskinesia	1.85	0.645–5.29	0.613	0.537	0.253	1.143
Aggression	2.91	0.783–10.8	1.068	0.670	0.111	1.595
Agitation	4.24	1.43–12.6	1.446	0.555	0.009	2.605
Emotional variability	0.927	0.344–2.5	−0.075	0.507	0.882	−0.149
Hyperactivity	2.98	0.804–11.1	1.093	0.669	0.102	1.634
Aphasia/speech disorder	4.56	1.51–13.8	1.518	0.566	0.007	2.683
Apraxia/loss of purposeful movement	1.43	0.231–8.81	0.355	0.929	0.703	0.382
Intellectual regression	1.98	0.562–6.97	0.683	0.642	0.288	1.062
Sleeping disorder	0.748	0.214–2.62	−0.290	0.638	0.650	−0.454
Stereotypy	0.538	0.131–2.22	−0.620	0.722	0.391	−0.858
Ovarian teratoma	1.77	0.199–15.8	0.571	1.116	0.609	0.511
Increased CSF cell count	12.3	3.33–45.4	2.509	0.666	<0.001	3.766

CI, confidence interval; Std, standard; CSF, cerebrospinal fluid.

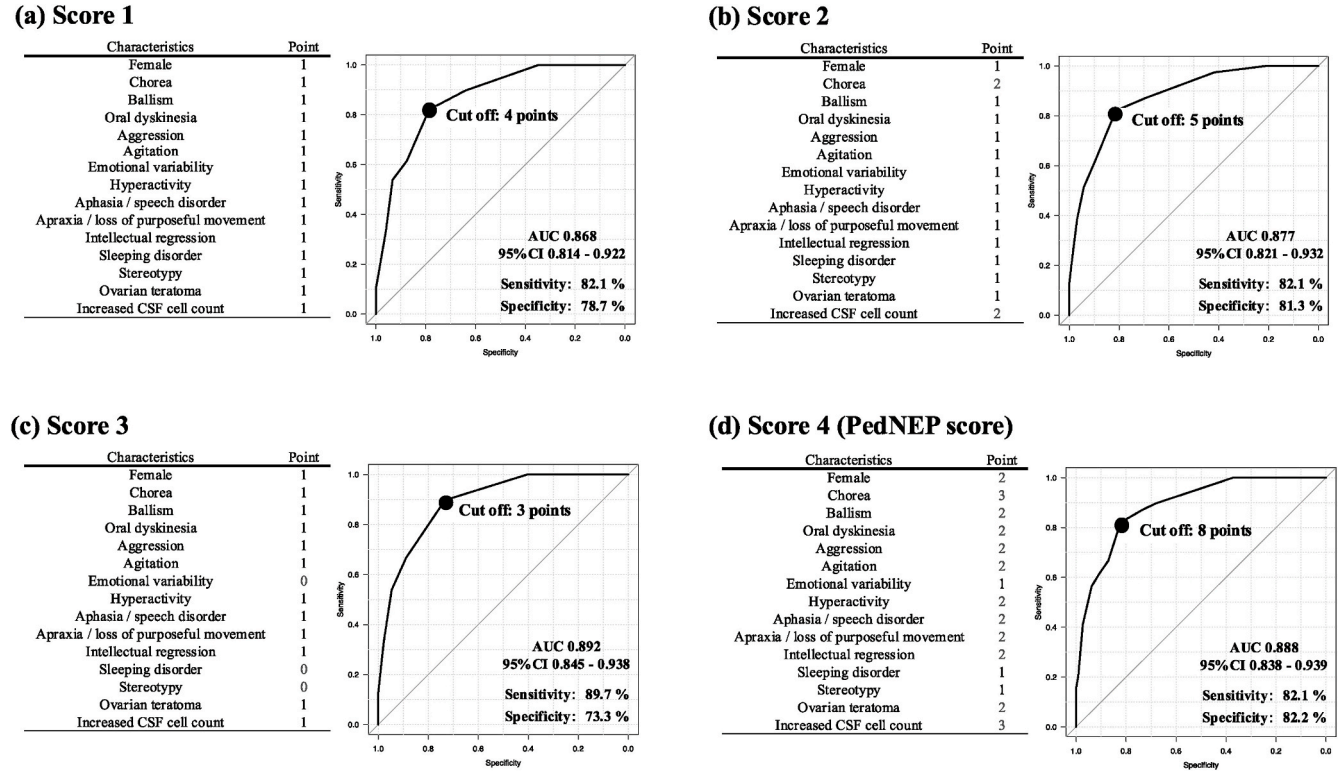

Fig. 2. Validity of the four candidate scores for probable pediatric anti-NMDAR encephalitis prediction score.
(a) Score 1 is the unweighted score. The cutoff was four points, with an AUC of 0.868 (95 % CI: 0.814–0.922).
(b) Score 2 is weighted by the magnitude of the OR. The two characteristics with particularly high ORs (chorea and increased CSF cell count) were assigned two points each. The cutoff was five points, with an AUC of 0.877 (95 % CI: 0.821–0.932).
(c) Score 3 is weighted by the magnitude of the OR. the three characteristics with low ORs (motional variability, sleeping disorder, and stereotypy) were assigned zero points. The cutoff was three points, with an AUC of 0.892 (95 % CI: 0.845–0.938).
(d) Score 4 (PedNEP score) is weighted by the magnitude of the OR. The two characteristics with particularly high ORs were assigned three points, the three characteristics with low ORs were assigned one point, and the remaining items were assigned two points each. The cutoff was eight points, with an AUC of 0.888 (95 % CI: 0.838–0.939).
AUC, area under the curve, CI, confidence interval; CSF, cerebrospinal fluid; OR, odds ratio; PedNEP, pediatric anti-N-methyl-D-aspartate receptor encephalitis prediction.

weakness of this study was the small cohort size; considering the number of variables analyzed in the multivariate analysis, this sample size was insufficient. To eliminate the uncertainty in the results caused by the small cohort, we performed five-fold cross-validation and demonstrated that this scoring system could predict pediatric NMDARE. Nevertheless, the kappa value exhibited a degree of limitation, potentially stemming from an inadequate number of cases and variability in the symptoms within each case. Thus, the PedNEP scores should be validated in a larger cohort of children. Furthermore, apraxia or loss of purposeful movement may be difficult to confirm in infants [18], and whether these parameters predict the diagnosis of NMDARE in younger children remains to be determined. Moreover, because of the small cohort size, perform statistical examinations of age-specific clinical symptoms was difficult.

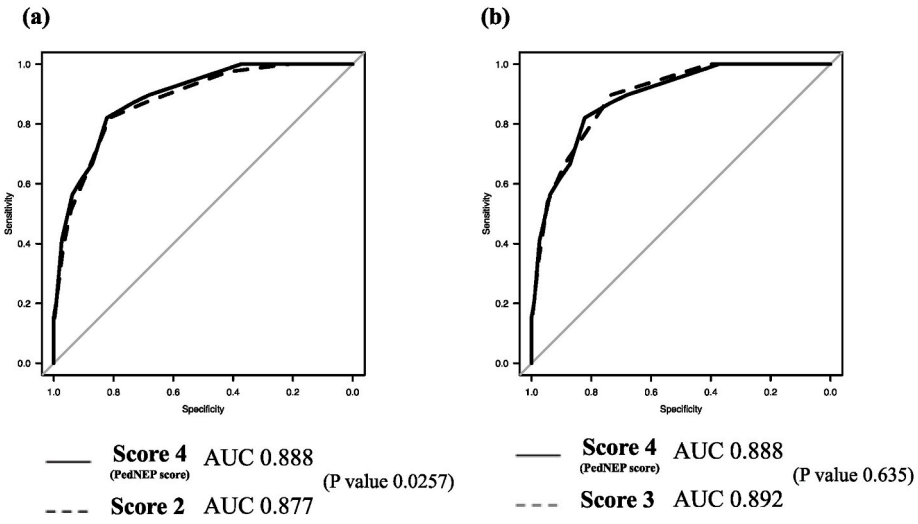

Fig. 3. Comparison of scores using ROC curves.
(a) Score 4 demonstrated superiority with an AUC of 0.888, compared to Score 2, which had an AUC of 0.877 ($p = 0.0257$).
(b) Score 4 had an AUC of 0.888 while Score 3 had an AUC of 0.892; the two AUC values did not significantly differ ($p = 0.635$)
AUC, area under the curve; ROC, receiver operating characteristics.

Another limitation of this study is that we did not consider the temporal progression of symptoms. For example, movement disorders, including chorea, which are characteristic of NMDARE, may appear late and thus may not be useful for early diagnosis. Furthermore, patients with NMDARE may not have high PedNEP scores during the early stages of the disease. There has been no established method for objective assessments of patients who are not responding adequately to first-line immunotherapy and require an escalation to second-line treatments, and developing a score to predict treatment response in paediatric NMDARE in a larger cohort would be of great clinical significance.

5. Conclusion

Using data from a cohort of 264 patients with possible autoimmune encephalitis, we extracted the clinical parameters characteristic of pediatric NMDARE using univariate analysis, and subsequently developed a "PedNEP" score to predict NMDARE in children using logistic regression analysis. The newly developed score has sufficient sensitivity and specificity and is expected to serve as a criterion for improving diagnosis and treatment before Ab identification.

Funding/support

A. Grant-in-Aid for Research on Measures for Intractable Diseases [grant number 21FC1005] B. A grant for Research on Emerging and Re-emerging Infectious Diseases and Immunization [grant number 22HA1003] from the Japanese Ministry of Health, Labour and Welfare C. JSPS Kakenhi Grant [grant number 22K07831] from Japan Society for the Promotion of Science.

Conflict of interest declaration

The authors declare no conflicts of interest related to this manuscript.

Acknowledgments

The authors thank Akimitsu Watanabe, Shin-ichiro Hamano, Jun Natsume, Tomoyuki Akiyama, and Noboru Yoshida for providing clinical information for a patient with anti-NMDAR encephalitis, or for 2 or more patients with NMDAR encephalitis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpn.2024.12.004.

References

[1] J. Dalmau, NMDA receptor encephalitis and other antibody-mediated disorders of the synapse: the 2016 Cotzias Lecture, Neurology 87 (23) (2016) 2471–2482.
[2] J. Xu, N. Zhao, H. Guan, J.H. Walline, H. Zhu, X. Yu, Anti-N-methyl-D-aspartate receptor encephalitis: characteristics and rapid diagnostic approach in the emergency department, BMC Neurol. 22 (1) (2022) 224.
[3] M.J. Titulaer, L. McCracken, I. Gabilondo, T. Armangue, C. Glaser, T. Iizuka, et al., Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study, Lancet Neurol. 12 (2) (2013) 157–165.
[4] F. Graus, M.J. Titulaer, R. Balu, S. Benseler, C.G. Bien, T. Cellucci, et al., A clinical approach to diagnosis of autoimmune encephalitis, Lancet Neurol. 15 (4) (2016) 391–404.
[5] M. Nosadini, T. Thomas, M. Eyre, B. Anlar, T. Armangue, S.M. Benseler, et al., International consensus recommendations for the treatment of pediatric NMDAR antibody encephalitis, Neurol Neuroimmunol Neuroinflamm 8 (5) (2021).
[6] L. Li, L. Sun, R. Du, Y. Zheng, F. Dai, Q. Ma, et al., Application of the 2016 diagnostic approach for autoimmune encephalitis from Lancet Neurology to Chinese patients, BMC Neurol. 17 (1) (2017) 195.
[7] H. Nishida, K. Kohyama, S. Kumada, J.I. Takanashi, A. Okumura, A. Horino, et al., Evaluation of the diagnostic criteria for anti-NMDA receptor encephalitis in Japanese children, Neurology 96 (16) (2021) e2070–e2077.
[8] T. Cellucci, H. Van Mater, F. Graus, E. Muscal, W. Gallentine, M.S. Klein-Gitelman, et al., Clinical approach to the diagnosis of autoimmune encephalitis in the pediatric patient, Neurol Neuroimmunol Neuroinflamm 7 (2) (2020).
[9] T.M. Dutschmann, L. Kinzel, A. Ter Laak, K. Baumann, Large-scale evaluation of k-fold cross-validation ensembles for uncertainty estimation, J. Cheminf. 15 (1) (2023) 49.
[10] L.B. Krupp, M. Tardieu, M.P. Amato, B. Banwell, T. Chitnis, R.C. Dale, et al., International Pediatric Multiple Sclerosis Study Group criteria for pediatric multiple sclerosis and immune-mediated central nervous system demyelinating disorders: revisions to the 2007 definitions, Mult. Scler. 19 (10) (2013) 1261–1267.
[11] B. Banwell, J.L. Bennett, R. Marignier, H.J. Kim, F. Brilot, E.P. Flanagan, et al., Diagnosis of myelin oligodendrocyte glycoprotein antibody-associated disease: international MOGAD Panel proposed criteria, Lancet Neurol. 22 (3) (2023) 268–282.
[12] J. Dalmau, E. Lancaster, E. Martinez-Hernandez, M.R. Rosenfeld, R. Balice-Gordon, Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis, Lancet Neurol. 10 (1) (2011) 63–74.
[13] J. Dalmau, E. Tuzun, H.Y. Wu, J. Masjuan, J.E. Rossi, A. Voloschin, et al., Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma, Ann. Neurol. 61 (1) (2007) 25–36.
[14] K.S. Fisher, A. Illner, V. Kannan, Pediatric neuroinflammatory diseases in the intensive care unit, Semin. Pediatr. Neurol. 49 (2024) 101118.

[15] H. Sakuma, J.I. Takanashi, K. Muramatsu, H. Kondo, T. Shiihara, M. Suzuki, et al., Severe pediatric acute encephalopathy syndromes related to SARS-CoV-2, Front. Neurosci. 17 (2023) 1085082.
[16] J.I. Takanashi, H. Uetani, Neuroimaging in acute infection-triggered encephalopathy syndromes, Front. Neurosci. 17 (2023) 1235364.
[17] J.A. Varley, A.J.S. Webb, B. Balint, V.S.C. Fung, K.D. Sethi, M.A.J. Tijssen, et al., The Movement disorder associated with NMDAR antibody-encephalitis is complex and characteristic: an expert video-rating study, J. Neurol. Neurosurg. Psychiatry 90 (6) (2019) 724–726.
[18] R. Brandsma, M.E. van Egmond, M.A.J. Tijssen, C. Groningen Movement Disorder Expertise, Diagnostic approach to paediatric movement disorders: a clinical practice guide, Dev. Med. Child Neurol. 63 (3) (2021) 252–258.