Application of the APE2-CHN and RITE2-CHN scores for autoimmune epilepsy in Chinese patients: a retrospective study

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Abstract

Background : Autoimmune epilepsy is recognized as a distinct entity of epilepsy with underestimated incidence. Our previous study reported that prompt diagnosis and early-initiated immunotherapy led to better outcome. We proposed to assess the feasibility and reasonability of the Antibody Prevalence in Chinese Patients with Epilepsy and Encephalopathy (APE 2 -CHN) and Response to Immunotherapy in Chinese Patients with Epilepsy and Encephalopathy (RITE 2 -CHN) scores in predicting Chinese patients with autoimmune epilepsy.

Methods : We conducted a retrospective study of consecutive patients from Xiangya Hospital, Central South University (01/01/2017-02/28/2019) whose serum and/or cerebrospinal fluid (CSF) samples were examined for autoimmune encephalitis antibodies. Of these, patients with new-onset epilepsy or established epilepsy of unknown etiology were selected in our study. An APE 2 -CHN score was assigned to each patient and a RITE 2 -CHN score was calculated for each patient who received immunotherapy.

Results : 191 patients meeting the diagnostic criteria for epilepsy were enrolled in our study. 36 were subsequently identified with specific etiologies. The rest of the 155 patients had an unknown etiology. Central nervous system-specific antibodies were detected in 76 (49.0%) of them, after excluding solely thyroid peroxidase antibody or glutamic acid decarboxylase antibody. N-methyl-D-aspartate receptor antibody (48.7%, 37/76) was the most common antibody specificity, followed by γ-aminobutyric acid type B receptor antibody (14.5%, 11/76). Certain clinical features such as new-onset epilepsy, autonomic dysfunction, viral prodrome, facio-brachial dystonic seizures/oral dyskinesia, inflammatory CSF profile, and mesial temporal magnetic resonance imaging abnormalities correlated with positive antibody results. Sensitivity and specificity of an APE 2 -CHN score ≥ 5 to predict the presence of specific neural auto-antibodies in our study were 85.5% and 58.9%, respectively. In the subset of patients who received immunotherapy (n = 112), sensitivity and specificity of a RITE 2 -CHN score ≥ 8 to predict favorable seizure outcome were 98.6% and 63.2% respectively.

Conclusion : The APE 2 -CHN and RITE 2 -CHN scores were preferable tools in predicting positive serologic findings and prognosis of autoimmune epilepsy in Chinese patients with epilepsy.

Background

Epilepsy is a debilitating neurological disorder. Although structural, metabolic, genetic and infectious factors are commonly identified as contributors to the cause of epilepsy, in a substantial number of patients, the etiology remains unclear [1]. In recent years, increasing data reveal an autoimmune nature in patients with cryptogenic epilepsies [2–6]. The concept of “autoimmune epilepsy” was first proposed in February 2002 at the International Congress of Autoimmunity in Geneva, Switzerland. Now autoimmune epilepsy is recognized as a distinct entity by the International League Against Epilepsy (ILAE) in their latest Epilepsy Classification (2017) [1]. The discovery of autoimmune antibodies targeting proteins in the brain has been applied to the diagnosis of autoimmune encephalitis, which frequently has seizures as its

prominent feature [7]. Most of these patients have antibodies directly react with neuronal synaptic and cell membrane antigens, including synaptic neurotransmitter receptors, ion channels, or other related proteins. Currently known antibodies include ones against N-methyl-D-aspartate receptor (NMDAR), voltage-gated potassium channel (VGKC) complex with leucine-rich glioma inactivated 1 (LGI1) and contactin-associated protein-like 2 (CASPR2) specificities, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR), γ-aminobutyric acid type B and A receptor (GABABR and GABAAR), dipeptidyl-peptidase-like protein 6 (DPPX), and IgLON family member 5 (IgLON5). Another group of antibodies are specific for intraneuronal nuclear or cytoplasmic antigens, which includes antineuronal nuclear type 1 (ANNA-1/Hu), antineuronal nuclear type 2 (ANNA-2/Ri), purkinje cell autoantibody (PCA-1/Yo), glutamic acid decarboxylase antibody (GAD-Ab), amphiphysin, collapsin response mediator protein 5 (CRMP-5/CV2), and Ma/Ta antibodies) [2, 8–13].

Identification of patients with an underlying autoimmune origin is central because early diagnosis and induction of immunotherapy in addition to conventional antiseizure medications is associated with better seizure outcomes [7, 14–17]. Since the introduction and establishment of the diagnostic test for NMDAR antibody in China in 2010 [18], a growing body of autoimmune encephalitis cases have been diagnosed and treated, broadening the spectrum of epilepsy etiologies [19]. Although detection of autoantibodies in the cerebrospinal fluid (CSF) and serum is particularly important for the identification of autoimmune epilepsy, timely diagnosis is usually hindered or missed by the lack of laboratory resources and clinical experience in some areas of China. Our previous study reported that admission diagnosis for patients with autoimmune epilepsy was associated with duration of symptom onset to Ab detection and immunotherapy, and more importantly, with seizure outcome after immunotherapy [20]. These findings indicated that early diagnosis of AE and prompt initiation of immunosuppressive treatment were crucial to increase the likelihood of achieving favorable seizure outcome.

Application of clinical tools predicting autoimmune etiology to Chinese patients with epilepsy has not been reported to date. In this study, we designed and validated scoring systems as predictive models of neural specific antibody positivity and response to immunotherapy in epilepsy cases. Serial clinical and laboratory data were retrospectively acquired from Xiangya Hospital, one of the largest hospitals in central China, by searching for those whose serum and/or CSF were evaluated for specific neural antibodies. Our aim was to confirm the feasibility, sensitivity and specificity of these clinical scoring systems in Chinese patients.

Patients And Methods

Patients

We conducted a retrospective single-center study in Xiangya Hospital, Central South University between Jan 1st, 2017 to Feb 28th, 2019. Adult patients who had autoimmune encephalopathy evaluations of serum and/or CSF were collected. Of these, we further identified patients with new-onset epilepsy (the presence of two unprovoked seizures at least 24 h apart or one unprovoked seizure with additional

clinical features suggesting a high probability of recurrence defined by the ILAE [21]) or a history of epilepsy of unknown etiology. Patients with proven or suspected seizure etiology (e.g., genetic, metabolic, neoplastic, or structural causes) were excluded from the study. Patients with mesial temporal sclerosis were not excluded. The patients' demographic information, clinical characteristics, admission/discharge diagnosis, clinical course, signs and symptoms, autoantibody specificity and titer, CSF characteristics, electroencephalography (EEG) results and imaging findings were recorded in detail.

Design Of Predictive Models

The predictive models used in this study were designed based on Antibody-Prevalence in Epilepsy and Encephalopathy (APE^2) and Responsive to immunotherapy in Epilepsy and Encephalopathy ($RITE^2$) scores [22]. The APE^2 score is a composite of ten items including clinical, brain magnetic resonance imaging (MRI), and CSF parameters that are differentially weighted to a maximum score of 18. The $RITE^2$ score included variables from the APE^2 score and two additional items: (1) immunotherapy initiated within six month of symptom onset, (2) detection of neural plasma membrane autoantibody. According to the diagnostic criteria for anti-NMDAR encephalitis published in Lancet Neurology [23], we added new variables to the scoring system and allocated points to them, creating the APE^2-Chinese (APE^2-CHN) and $RITE^2$-Chinese ($RITE^2$-CHN) scores. These include: cognitive dysfunction (+ 1), speech dysfunction (+ 1), movement dysfunction (+ 2) and decreased level of consciousness (+ 2) (Table 1). APE^2 and APE^2-CHN scores were evaluated for each patient. $RITE^2$ and $RITE^2$-CHN scores were calculated for each patient who received immunotherapy. Favorable seizure outcome was defined as > 50% reduction of seizure frequency at the first follow-up after completion of immunotherapy trial.

Table 1

Components of the APE^2-CHN score (1A) and RITE^2-CHN score (1B).

1A: Antibody prevalence in epilepsy and encephalopathy in Chinese patients (APE^2-CHN)	Value	1B: Response to immunotherapy in epilepsy and encephalopathy in Chinese patients (RITE^2-CHN)	Value
New new onset seizures activity (within one year of evaluation)	(+ 1)	New onset, rapidly progressive mental status changes that developed over 1–6 weeks or new onset seizures activity (within one year of evaluation)	(+ 1)
Neuropsychiatric changes: agitation, aggressiveness, emotional disability	(+ 1)	Neuropsychiatric changes; agitation, aggressiveness, emotional disability	(+ 1)
Cognitive dysfunction*[a]	(+ 1)	Cognitive dysfunction*[a]	(+ 1)
Speech dysfunction*[b]	(+ 1)	Speech disorder or silence*	(+ 1)
Autonomic dysfunction: sustained atrial tachycardia or bradycardia, orthostatic hyotension (≥ 20 mmHg fall in systolic pressure or ≥ 10 mmHg fall in diastolic pressure within three minutes of quiet standing), hyperhidrosis, persistently labile blood pressure, ventricular tachycardia, cardiac asystole, gastrointestinal dysmotility][c]	(+ 1)	Autonomic dysfunction: sustained atrial tachycardia or bradycardia, orthostatic hyotension (≥ 20 mmHg fall in systolic pressure or ≥ 10 mmHg fall in diastolic pressure within three minutes of quiet standing), hyperhidrosis, persistently labile blood pressure, ventricular tachycardia, cardiac asystole, gastrointestinal dysmotility][a]	(+ 1)
Viral prodrome (rhinorrhea, sore throat, low grade fever) to be scored in the absence of underlying systemic malignancy within 5 years of neurological symptom onset	(+ 2)	Viral prodrome (rhinorrhea, sore throat, low grade fever) to be scored in the absence of underlying systemic malignancy within 5 years of neurological symptom onset	(+ 2)
Faciobrachial dystonic seizures	(+ 3)	Faciobrachial dystonic seizures	(+ 3)
Movement disorder*[d]	(+ 2)	Movement disorder*[d]	(+ 2)
Decreased level of consciousness*	(+ 2)	Decreased level of consciousness*	(+ 2)
Seizure refractory to at least two antiseizure medications	(+ 2)	Seizure refractory to at least two antiseizure medications	(+ 2)
CSF findings consistent with inflammation[e] (elevated CSF protein > 50 mg/dL and/or lymphocytic pleocytosis > 5 cells/mCL, if the total number of CSF RBC < 1000 cells/mCL)	(+ 2)	CSF findings consistent with inflammation[e] (elevated CSF protein > 50 mg/dL and/or lymphocytic pleocytosis > 5 cells/mCL, if the total number of CSF RBC < 1000 cells/mCL)	(+ 2)

1A: Antibody prevalence in epilepsy and encephalopathy in Chinese patients (APE^2-CHN)	Value	1B: Response to immunotherapy in epilepsy and encephalopathy in Chinese patients ($RITE^2$-CHN)	Value
Brain MRI suggesting encephalitis[e] (T2/FLAIR hyperintensity restricted to one or both medial temporal lobes, or multifocal in grey matter, white matter, or both compatible with demyelination or inflammation)	(+ 2)	Brain MRI suggesting encephalitis[e] (T2/ FLAIR hyperintensity restricted to one or both medial temporal lobes, or multifocal in grey matter, white matter, or both compatible with demyelination or inflammation)	(+ 2)
Systemic cancer diagnosed within 5 years of neurological symptom onset (excluding cutaneous squamous cell carcinoma, basal cell carcinoma, brain tumor, cancer with brain metastasis)	(+ 2)	Systemic cancer diagnosed within 5 years of neurological symptom onset (excluding cutaneous squamous cell carcinoma, basal cell carcinoma, brain tunor, cancer with brain metastasis)	(+ 2)
	Total (max: 22)	Immunotherapy initiated within 6 months of symptom onset	(+ 2)
		Neural plasma membrane autoantibody detected (NMDAR, $GABA_AR$, $GABA_BR$, AMPAR, DPPX, mGluR1, mGluR2, mGluR5, LGI1, IgLON5, CASPRA2 or MOG)	(+ 2)
			Total (max: 26)

$RITE^2$-CHN score included all the components of APE^2-CHN score and two additional variables: initiation of immunotherapy within 6 months of symptom onset and plasma membrane-specific autoantibody detected (1B)/The assigned APE^2-CHN and $RITE^2$-CHN score are the sum of values of all components.

AMPAR: amino-3-hydroxy-5-methyl-4-isoxazolepropionic; CASPR-2: Contactin Associated Protein 2; CRMP5: Collapsin response-mediator protein-5; DPPX: dipeptidyl-peptidase-like protein 6; FLAIR: fluid attenuated inversion recovery; $GABA_BR$: γ-aminobutyric acid-B receptor; LGI1: leucine-rich glioma-inactivated protein-1; mGluR: metabotropic glutamate receptor; MOG: myelin oligodendrocyte glycoprotein; NMDAR: N-methyl D-Aspartate Receptor.

a Memory disorder, disorientation and dyscalculia.

b Pressured speech, verbal reduction, mutism.

c Scored only if no history of autonomic dysfunction prior to onset of suspected autoimmune syndrome and the autonomic dysfunction not attributable to medications, hypovolemia, plasmapheresis or infection.

d Ataxia, dyskinesia, rigidity/abnormal postures or involuntary movement; facial dyskinesias to be scored in the absence of faciobrachial systonic seizures.

e Patients scored zero if MRI brain or CSF analysis not performed.

1A: Antibody prevalence in epilepsy and encephalopathy in Chinese patients (APE^2-CHN)	Value	1B: Response to immunotherapy in epilepsy and encephalopathy in Chinese patients (RITE^2-CHN)	Value

* Modifications for the -CHN versions of the APE^2 and RITE^2 scores.

Antibody Testing

Serum and/or CSF samples were evaluated by: (1) standardized indirect immunofluorescence assays (IFAs) for immunoglobulin G (IgG) against the following antigens: NMDAR, AMPA1R, AMPA2R, GABABR, LGI1, CASPR2, DPPX, IgLON5, myelin oligodendrocyte (MOG), GAD 65 isoform (GAD-65) (Euroimmun, Germany); combined cell-based assays (CBAs) using human embryonic kidney 293 cells and tissue-based assays (TBAs) using rat cerebellum and hippocampus tissue were utilized for antibody detection; both positive and negative controls were provided; (2) IFAs and linear immunoblotting for IgG against the following antigens: Hu, Ri, Yo, amphiphysin, CRMP5/CV2 (Euroimmun, Germany). Samples were collected prior to any immunotherapy in most cases.

Statistical analysis

Univariate analyses of nominal and interval variables were performed using chi-square and Mann-Whitney U-tests, respectively. Receiver-operating curve (ROC) analyses were used to evaluate predictive scoring models.

Sensitivity: the probability of detecting the disease among patients who have the disease (TP/[TP + FN])

Specificity: the probability of detecting no disease among patients who do not have the disease (TN/[TN + FP]).

Results

Clinical and demographic findings

Serum and/or CSF from 1250 patients were tested for autoimmune encephalopathy antibodies in the Xiangya Pediatric Laboratory or Oumeng between Jan 1st, 2017 and Feb 28th, 2019 (Fig. 1). 192 of these patients had an admission/discharge diagnosis of epilepsy, although one of them was eventually diagnosed with stiff-person syndrome (SPS). In 191 patients with epilepsy, serum only was analyzed for three patients, CSF only for ten patients, and in 178 patients both specimen types were analyzed concurrently. 36 patients with epilepsy caused by other etiologies were subsequently excluded: 23 had intracranial infection, eight had intracranial tumor, one had meningeal carcinomatosis, two had cerebral hemorrhage, one had ischemic hypoxic encephalopathy, and one had structural brain malformation. Antibody was detected in only one of these cases (2.8%). The remaining 155 were patients with new-

onset epilepsy or established epilepsy of unknown etiology. The mean age (± standard deviation) was 34.7 ± 18.7 years in the total population, 37.4 ± 19.9 years among males and 32.0 ± 17.2 years among females ($p = 0.069$). Due to the uncertain role of TPO-Ab in the diagnosis of autoimmune epilepsy, 4 patients with anti-TPO as the only antibody marker were excluded from the antibody-positive group for subsequent analysis. Similarly, two patients with low titer GAD-65 (< 20 nmol/L) were excluded since approximately 8% of the general population can present low-titer GAD-Ab. After exclusion, 76 patients (49.0%) were left with neural-specific antibodies strongly suggesting an autoimmune cause of epilepsy (Fig. 1). Patients with antibody only detected in the serum (antibody negative in the CSF) were not counted as positive cases. Multiple antibodies were detected in 12 patients (15.8%, 12/76): six (7.9%, 6/76) had anti-NMDAR and MOG, two (2.6%, 2/76) of whom had anti-NMDAR and TPO, two (2.6%, 2/76) had anti-NMDAR and GAD-65, one (1.3%, 1/76) had anti-GABABR and TPO, and one (1.3%, 1/76) had anti-GABABR and Hu. A single antibody was found in 64 patients (84.2%, 64/76): 37 had anti-NMDAR (48.7%, 37/76), 11 had anti-GABABR (14.5%, 11/76), four had anti-MOG (5.3%, 4/76), three had anti-CASPR2 (3.9%, 3/76), two had anti-Yo (2.6%, 2/76), two had anti-CRMP5 (2.6%, 2/76), one had anti-LGI1 (1.3%, 1/77), two had anti-Ma2/Ta (1.3%, 1/76), one had anti-AMPA2 (1.3%, 1/76), and one had anti-Hu (1.3%, 1/76). None of the following antibodies investigated was detected: anti-AMPA1, anti-DPPX, anti-IgLON5, anti-Ri, and anti-Amphiphysin (Fig. 1). Among the seropositive patients, the predominant neurological antibodies detected were NMDAR (61.8%, 47/76), GABABR (17.1%, 13/76) and MOG (13.2%, 10/76).

Clinical and demographic findings were compared between antibody-positive and antibody-negative cases (Table 2). There was a higher prevalence of new-onset epilepsy in antibody-positive cases than in antibody-negative cases (98.7% vs. 78.1%, $\chi2 = 15.636$, $p < 0.001$). Additionally, other clinical characteristics were found more commonly in patients with positive serologic findings than in patients with negative serologic findings: neuropsychiatric changes (48.7% vs. 19.2%, $\chi2 = 14.400$, $p < 0.001$), speech disorder (46.1% vs. 12.3%, $\chi2 = 6.462$, $p < 0.05$) and movement disorder (19.7% vs. 4.1%, $\chi2 = 8.561$, $p < 0.05$). Interestingly, brain MRI suggesting encephalitis (43.4% vs. 39.7%, $\chi2 = 0.209$, $p = 0.647$) was statistically comparable between the two groups, while inflammatory CSF was more common in antibody-positive cases than in antibody-negative cases (44.7% vs. 24.7%, $\chi2 = 6.608$, $p < 0.05$).

Table 2

Comparison of antibody-positive and antibody-negative cases.

Variables	Antibody-positive cases (n = 76)	Antibody-negative cases (n = 73)	χ^2/Z	p-Value
Median age (range)[a]	26.5 (10.0–68.0)	41.0 (1.0–80.0)	-3.379	0.001
Female (%)[b]	41 (53.9%)	34 (46.6%)	0.809	0.368
Median APE^2 score (range)[a]	4.0 (0.0–9.0)	3.0 (0.0–11.0)	-2.755	0.006
Median APE^2-CHN score (range)[a]	6.5 (0.0–12.0)	4.0 (0.0–13.0)	-3.991	< 0.001
$APE^2 \geq 4$	49 (64.5%)	26 (35.6%)	12.403	< 0.001
APE^2-CHN ≥ 5	65 (85.5%)	30 (41.1%)	31.810	< 0.001
New-onset seizures (%)[b]	75 (98.7%)	57 (78.1%)	15.636	< 0.001
Neuropsychiatric changes (%)[b]	37 (48.7%)	14 (19.2%)	14.400	< 0.001
Recent memory disorder [b]	11 (14.5%)	5 (6.8%)	2.258	0.133
Speech disorder or silence [b]	35 (46.1%)	19 (12.3%)	6.462	0.011
Autonomic dysfunction (%)[b]	10 (13.2%)	10 (13.7%)	0.009	0.923
Viral prodrome (%)[b]	10 (13.2%)	12 (16.4%)	0.318	0.573
Faciobrachial dystonic seizures or Facial dyskinesias (%)[b]	4 (5.3%)	0 (0%)	2.190	0.139
Ataxia, dyskinesia or involuntary movement, dystonia[b]	16 (21.1%)	3 (4.1%)	9.607	0.002
Disorder of consciousness or coma[b]	31 (40.8%)	26 (35.6%)	0.422	0.516
Refractory seizure (%)[b]	11 (14.5%)	15 (20.1%)	0.954	0.329
CSF findings consistent with inflammation[b]	34 (44.7%)	18 (24.7%)	6.608	0.010
Brain MRI suggesting encephalitis[b]	32 (42.1%)	30 (41.1%)	0.016	0.901

[a]Mann-Whitney U-TEST; [b]chi-square or Fisher's exact test.

Variables	Antibody-positive cases (n = 76)	Antibody-negative cases (n = 73)	χ^2/Z	p-Value
Systemic malignancy detected (%)[b]	4 (5.3%)	2 (2.7%)	0.134	0.710

[a]Mann-Whitney U-TEST; [b]chi-square or Fisher's exact test.

Performance Of APE^2-CHN Score In Predicting Neural Ab Positivity

A significantly higher proportion of CNS-specific antibody-positive patients were found in APE^2 score ≥ 4 (Fig. 1, 64.5% vs. 35.6%, $\chi2 = 12.403$, $p < 0.001$) and APE^2-CHN score ≥ 5 (Fig. 2, 85.5% vs. 15.1%, $\chi2 = 73.968$, $p < 0.001$) groups. Conversely, antibody-positive patients had significantly higher median APE^2 score (4 [range 0–9] versus 3 [range 0–11], $\chi2 = -2.713$, $p < 0.01$) and median APE^2-CHN score (7 [range 0–13] versus 3 [range 0–13], $\chi2 = -5.367$, $p < 0.001$) (Table 2). Based on ROC analysis with a cutoff of 4, the sensitivity and specificity of the APE^2 score in predicting the presence of CNS-specific auto-antibodies in cases of epilepsy of unknown etiology (149) were 64.5% and 64.4% respectively, with an area under the curve (AUC) of 0.630 (see Additional file). Based on ROC analysis with a cutoff of 5, the sensitivity and specificity of the APE^2-CHN score were 85.5% and 58.9% respectively, with an AUC of 0.688 (see Additional file 1).

Performance of APE ^{2} -CHN and RITE ^{2} -CHN scores in predicting favorable immunotherapy outcome

In the subset of patients who received immunotherapy (Fig. 3, n = 112), factors related to favorable seizure outcome included (Table 3): neural antibody positivity (77.0% vs. 28.9%, $p < 0.001$), plasma membrane protein antibody positivity (68.9% vs. 18.4%, $p < 0.001$), neuropsychiatric changes (51.4% vs. 21.1%, $p < 0.01$), disorder of consciousness or coma (54.1% vs. 34.2%, $p < 0.05$), CSF findings consistent with inflammation (50.0% vs. 26.3%, $p < 0.05$), early initiation of immunotherapy (98.6% vs. 84.2%, $p < 0.01$). An APE^2 score of ≥ 4 recognized a higher proportion of patients responsive to immunotherapy than non-responders (75.7% vs. 44.7%, $p < 0.001$) and so did an APE^2-CHN score of ≥ 5 (98.6% vs. 50.0%, $p < 0.001$). The interval between immunotherapy initiation and follow-up did not differ significantly between responders (median 47.5 days, range 30 to 210 days) and non-responders (median 52 days, range 28 to 210 days). There was no significant difference in symptom onset to autoimmune epilepsy diagnosis, symptom onset to immunotherapy and therapeutic regimen ($p > 0.05$). Additionally, RITE^2-CHN score ≥ 8 had a slight advantage (sensitivity 98.6%; specificity 63.2%; AUC = 0.856) than RITE^2 score ≥ 6 (sensitivity 95.9%; specificity 50.0%; AUC = 0.772) in predicting favorable immunotherapy outcome. (Fig. 3 and Additional file 2).

Table 3

Comparison of responders and non-responders following immunotherapy.

Variables	Responders (n = 74)	Non-responders (n = 38)	χ^2/Z	p-Value
Median age (range)[a]	29 (10–80)	29 (11–66)	-0.086	0.931
Female (%)[b]	38 (51.4%)	14 (36.8)	2.125	0.145
Neural autoantibody detected (%)[b]	57 (77.0%)	11 (28.9%)	24.333	< 0.001
Neural antibody of plasma membrane specificity (%)[b]	51 (68.9%)	7 (18.4%)	25.642	< 0.001
Neural antibody of intracellular specificity (%)[b]	5 (6.8%)	1 (2.6%)	0.225	0.635
Median APE^2 score (range)[a]	8.0 (5.0–12.0)	5.5 (1.0–15.0)	-4.572	< 0.001
Median APE^2-CHN score (range)[a]	11.0 (7.0–15.0)	7.0 (1.0–17.0)	-6.210	< 0.001
$APE^2 \geq 4$	56 (75.7%)	17 (44.7%)	10.598	< 0.001
APE^2-CHN ≥ 5	73 (98.6%)	19 (50.0%)	40.510	< 0.001
New-onset seizures (%)[b]	74 (100%)	35 (92.1%)	3.356	0.067
Neuropsychiatric changes (%)[b]	38 (51.4%)	8 (21.1%)	9.523	0.002
Autonomic dysfunction (%)[b]	10 (13.5%)	7 (18.4%)	0.470	0.493
Viral prodrome (%)[b]	14 (18.9%)	5 (13.2%)	0.592	0.442
Faciobrachial dystonic seizures or Facial dyskinesias (%)[b]	3 (4.1%)	1 (2.6%)	0.000	1.000
Ataxia, dyskinesia or involuntary movement, dystonia[b]	16 (21.6%)	3 (7.9%)	3.358	0.067
Disorder of consciousness or coma[b]	40 (54.1%)	13 (34.2%)	3.966	0.046
Refractory seizure (%)[b]	14 (18.9%)	9 (23.7%)	0.349	0.554
CSF findings consistent with inflammation[b]	37 (50.0%)	10 (26.3%)	5.783	0.016

[a]Mann-Whitney U-TEST; [b]chi-square or Fisher's exact test; [c]rituximab or cyclophosphamide; AE: autoimmune epilepsy. Responders are defined as > 50% deduction in seizure frequency.

Variables	Responders (n = 74)	Non-responders (n = 38)	χ^2/Z	p-Value
Brain MRI suggesting encephalitis[b]	37 (50.0%)	15 (39.5%)	1.118	0.290
Systemic malignancy detected (%)[b]	5 (6.8%)	1 (2.6%)	0.225	0.635
Early initiation of immunotherapy (< 6 months from symptom onset)	73 (98.6%)	32 (84.2%)	6.638	0.010
Symptom onset to AE diagnosis, day (range)[a]	14 (0-1100)	15 (3-7400)	-1.033	0.301
Symptom onset to immunotherapy, day (range)[a]	19 (0-1100)	18 (0-7400)	-0.162	0.871
IVMP (%)[b]	55 (74.3%)	26 (68.4%)	0.437	0.509
IVIG (%)[b]	63 (85.1%)	29 (76.3%)	1.331	0.249
PLEX (%)[b]	3 (4.1%)	0 (0%)	0.410	0.522
Second-line agent[c] (%)[b]	14 (18.9%)	3 (7.9%)	2.370	0.124

[a]Mann-Whitney U-TEST; [b]chi-square or Fisher's exact test; [c]rituximab or cyclophosphamide; AE: autoimmune epilepsy. Responders are defined as > 50% deduction in seizure frequency.

Discussion

Studies on autoimmune epilepsy have implicated autoimmune factors in the etiology of 15–35% patients with epilepsy of unknown cause [2–5]. In this consecutive retrospective series of patients with cryptogenic epilepsy, a higher rate of patients (49.0%) had detectable serum neurological antibody levels suggesting an autoimmune etiology. Multiple antibodies were detected in 12/76 (15.8%) patients, also higher than that reported in the literature (2.6–6.3%) [4, 6]. The apparent increasing detection rate of autoimmune epilepsy is partly due to increased frequency of neural autoantibody evaluations. On the other hand, the difference in detection rates may also be due to different diagnostic definitions of "epilepsy of unknown cause." In our study, amongst epilepsy patients with other etiologies (n = 36), 23 were diagnosed with intracranial infections (18 viral encephalitis, three neurosyphilis, one tuberculous encephalitis, one suppurative encephalitis) on discharge. In our hospital, viruses including Herpes simplex 1 (HSV-1) IgM, respiratory syncytial virus (RSV) IgM, adenovirus IgM, Epstein-Barr virus (EBV), Coxsackievirus IgM and cytomegalovirus (CMV) are tested using serum and CSF serology, and in many cases viral encephalitis is a clinical diagnosis based on symptoms, signs and routine CSF analysis without viral serology. In contrast, in the United States (for example the Mayo Clinic), real-time PCR for a viral panel including HSV1/2, EBV, CMV, Enterovirus, human herpes virus-6, Japanese encephalitis virus, varicella zoster virus and West Nile virus is available as part of the viral encephalitis workup, which plays an important role in the diagnosis and treatment of viral encephalitis [24]. The difference in diagnostic

algorithms and available tests may result in different proportion of patients being diagnosed with “epilepsy of unknown cause”, and therefore is responsible for the difference in detection rates of autoimmune epilepsy in our study.

Most of the variables in the APE^2 and $RITE^2$ scores were associated with auto-antibody positivity and favorable immunotherapy outcome respectively [22]. Furthermore, as composite scores, APE^2 and $RITE^2$ scales along with their previous versions-APE and RITE scales have been validated as efficient predictive models in the diagnosis and treatment of autoimmune epilepsy and cognitive dysfunction [4, 5, 22]. While prospectively assigned to patients prior to antibody testing, an APE score of ≥ 4 had a sensitivity from 82.6–100%, with a reasonably high specificity (79.4–82.0%) [4]. A RITE score of ≥ 7 had 87.5% sensitivity and 83.8% specificity on predicting better outcomes [5]. Surprisingly, when implemented in Chinese patients, the performance of APE^2 and $RITE^2$ scores was only marginally satisfactory. 18 patients with anti-NMDAR encephalitis had low APE^2 scores, which usually score very high on these predictive models. It is unlikely attributed to a false positive rate because patients with only serum NMDAR-Ab positivity (without CSF NMDAR-Ab detection) were not included. On the other hand, APE^2-CHN enhanced screening for anti-NMDAR encephalitis by supplementary variables covering other representative symptoms including cognitive dysfunction, speech dysfunction, movement disorder and decreased level of consciousness. Only eight patients with anti-NMDAR detection had APE^2-CHN score < 5, all of whom were with antibody weakly positive in the CSF, with or without serum antibody detection. The difference in clinical application of the scores could be largely attributed to the difference of antibody spectrum in different regions. In Asian and some European countries, anti-NMDAR encephalitis is the most common cause of autoimmune encephalitis, followed by anti-LGI1 encephalitis [19, 25–27]. In the United States, however, the more frequently identified antibodies associated with autoimmune epilepsy are directed against the VGKC complex (specifically LGI1 and CASPR2), GAD-65 and NMDAR antigens [4, 5, 7]. Notably, one patient with weakly NMDAR antibody positivity (1:3.2) was eventually diagnosed with tuberculous meningoencephalitis, and this case was considered as false positivity.

Our study showed higher accuracy of APE^2-CHN than the APE^2 score in predicting the existence of neural-specific autoantibodies. Several variables including new-onset epilepsy, neuropsychiatric changes, speech disorder, movement disorder and inflammatory CSF correlated significantly with auto-antibody positivity. Therefore, an APE^2-CHN score of ≥ 5 (sensitivity: 85.5%, specificity: 58.9%) may be a preferable tool in predicting neural-specific antibody positivity in Asian countries. Detection of antibodies was also associated with better seizure outcomes after immunotherapy. For these seropositive patients, the decision for a trial of immunotherapy is relatively straightforward. However, the neural antibody evaluation could also be negative, delayed or even not available in some primary hospitals. In such scenarios, with a sensitivity of 98.6%, the $RITE^2$-CHN score could provide an evidence-based approach to guide therapeutic decision making. Additionally, $RITE^2$-CHN score < 8 is suggestive of refractoriness to initial immunotherapy. In these circumstances, early consideration of second-line agents such as rituximab and cyclophosphamide or a more comprehensive search for underlying malignancy, if not already detected, should be undertaken [22]. In all, APE^2-CHN and $RITE^2$-CHN score may work together to

judiciously stratify patients according to their likelihood of autoimmune epilepsy in order to select individuals for neural autoantibody tests and immunotherapy in Chinese hospitals where healthcare resources are relatively scarce.

An autoimmune etiology makes up a significant proportion of epilepsy causes. Seizures could also present as the most common disease manifestation in autoimmune encephalitis. However, autoimmune epilepsy has rarely been considered as an admission diagnosis in epilepsy patients [28]. Because early-initiated immunotherapy leads to better outcome for seropositive patients [20] and misdiagnosis of autoimmune epilepsy may delay diagnosis beyond the window of reversibility (6–12 months) with devastating consequences for the patient and family [29], it is essential that an autoimmune etiology be considered in the initial differential diagnosis of new onset epilepsy. Currently, a definite diagnosis of autoimmune encephalitis relies heavily on the detection of autoimmune antibodies. Various guidelines have been suggested for identifying cases with potential autoimmune etiology, but none of them are as objective as a scoring system [8, 14, 30]. In addition, most of these guidelines are based on expert consensus rather than evaluated using an outcome variable such as antibody positivity or immunotherapy response [8, 30]. For example, in a paper published in Lancet Neurology (2016) [23], the proposed flowchart when applied to Chinese patients as a whole has an acceptable accuracy [25]. However, for autoimmune limbic encephalitis and anti-NMDAR encephalitis, the sensitivity of the flowchart is relatively low. In this regard, APE^2-CHN and RITE^2-CHN scores have considerably higher sensitivity and comparable specificity to the diagnostic flowchart.

Our study had limitations. More than a dozen autoantibodies have been found in the spectrum of autoimmune epilepsy; therefore, broad autoantibody testing of serum-CSF pairs offers the best diagnostic yield. However, resources for identification of certain autoantigens are limited in our hospital (such as GABAAR-Ab for all patients, DPPX-Ab and IgLON5-Ab for most patients). Some patients with clinical features strongly supporting a diagnosis of autoimmune epilepsy lack any currently identifiable antibody biomarker. Ideally, information on all variables should be available while computing these scoring systems. Few patients (15%) did not have an MRI and/or CSF analysis at the time of evaluation and received a score of 0 for these variables. Unlike Dubey's retrospective study, only a small proportion (6.8%) of patients did not have paired serum and CSF specimens for autoimmune antibody testing in our study. The retrospective design of this study is a limitation and confounders such as irregular medications before antibody testing could not be controlled for. However, despite these limitations which may have affected sensitivity, our predictive models had a high accuracy nonetheless.

Conclusion

In this retrospective study, 49.0% patients with epilepsy of unknown etiology had detectable CSF neurological antibody levels, suggesting the incidence of autoimmune epilepsy had been largely underestimated. Unlike the United States, the most common neurological antibody detected in our study was NMDAR, followed by GABABR, rather than LGI1. This could partially explain the difference in the performance of APE^2 and RITE^2 scores in American and Chinese patients. In this regards, APE^2-CHN and

RITE^2-CHN scores were preferable in predicting positive serologic findings and prognosis of autoimmune epilepsy in Chinese patients.

Abbreviations

APE2-CHN: Antibody Prevalence in Chinese Patients with Epilepsy and Encephalopathy; RITE2-CHN: Response to Immunotherapy in Chinese Patients with Epilepsy and Encephalopathy; APE2 : Antibody-Prevalence in Epilepsy and Encephalopathy; RITE2: Responsive to immunotherapy in Epilepsy and Encephalopathy; CSF: cerebrospinal fluid; MRI: magnetic resonance imaging; ILAE: International League Against Epilepsy; NMDAR: N-methyl-D-aspartate receptor; VGKC: voltage-gated potassium channel; LGI1: leucine-rich glioma inactivated 1; CASPR2: contactin-associated protein-like 2; AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; GABABR: γ-aminobutyric acid type B receptor; GABAAR: γ-aminobutyric acid type A receptor; DPPX: dipeptidyl-peptidase-like protein 6; IgLON5: IgLON family member 5; ANNA-1/Hu: antineuronal nuclear type 1; ANNA-2/Ri: antineuronal nuclear type 2; PCA-1/Yo: purkinje cell autoantibody; GAD-Ab: glutamic acid decarboxylase antibody; GAD-65: GAD 65 isoform; CRMP-5/CV2: amphiphysin, collapsin response mediator protein 5; EEG: electroencephalography; ROC: receiver-operating curve; SPS, stiff-person syndrome; HSV-1: herpes simplex 1; RSV: respiratory syncytial virus; EBV: Epstein-Barr virus; CMV: cytomegalovirus.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Conceptualization, Chang Zeng; Data curation, Formal analysis, Chen Zhang; MW; Methodology, WPL; Resources, WPL and BX; Writing (original draft preparation), CZ; Writing (reviewing and editing), CWZ; Supervision, BX. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and analysed during the current study are not publicly available due to the privacy policy of Xiangya hospital but are partially available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Xiangya Hospital's Institutional Review Board approved the study. No consent was required of the patients and proxies because of the retrospective nature of the study.

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Additional Files

Figure e-1. ROC curve of APE^2 and APE^2-CHN scores.

Based on ROC analysis with a cutoff of 4, the sensitivity and specificity of the APE^2 score in predicting the presence of CNS-specific auto-antibodies in cases of epilepsy of unknown etiology (149) were 64.5% and 64.4% respectively, with an area under the curve (AUC) of 0.630. Based on ROC analysis with a cutoff of 5, the sensitivity and specificity of the APE^2-CHN score were 85.5% and 58.9% respectively, with an AUC of 0.688.

Figure e-2. ROC curve of RITE^2 and RITE^2-CHN scores.

RITE^2-CHN score ≥8 had a slight advantage (sensitivity 98.6%; specificity 63.2%; AUC = 0.856) than RITE^2 score ≥6 (sensitivity 95.9%; specificity50.0%; AUC = 0.772) in predicting favorable immunotherapy outcome.

Figures

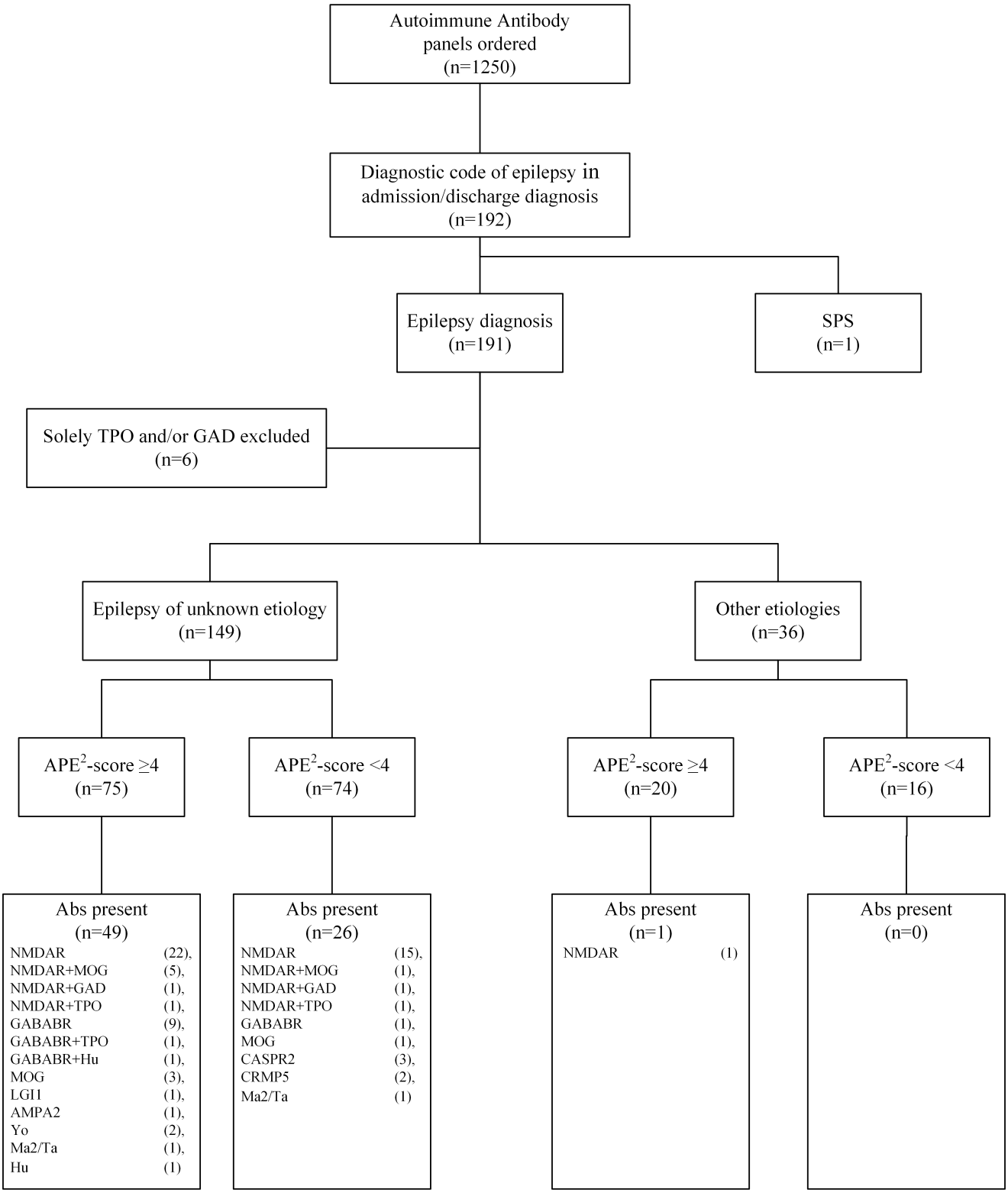

Figure 1

Distribution of neural antibody-positive and antibody-negative patients among reviewed epilepsy cases based on APE2 score. Abs, Antibodies; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; ANNA-1/Hu, antineuronal nuclear type 1; CASPR2, contactin-associated protein-like 2; CRMP-5/CV2, collapsin response mediator protein 5; LGI1, leucine-rich glioma inactivated 1; NMDAR, N-methyl-D-aspartate receptor; GABABR, γ-aminobutyric acid type B; SPS, stiff-person syndrome.

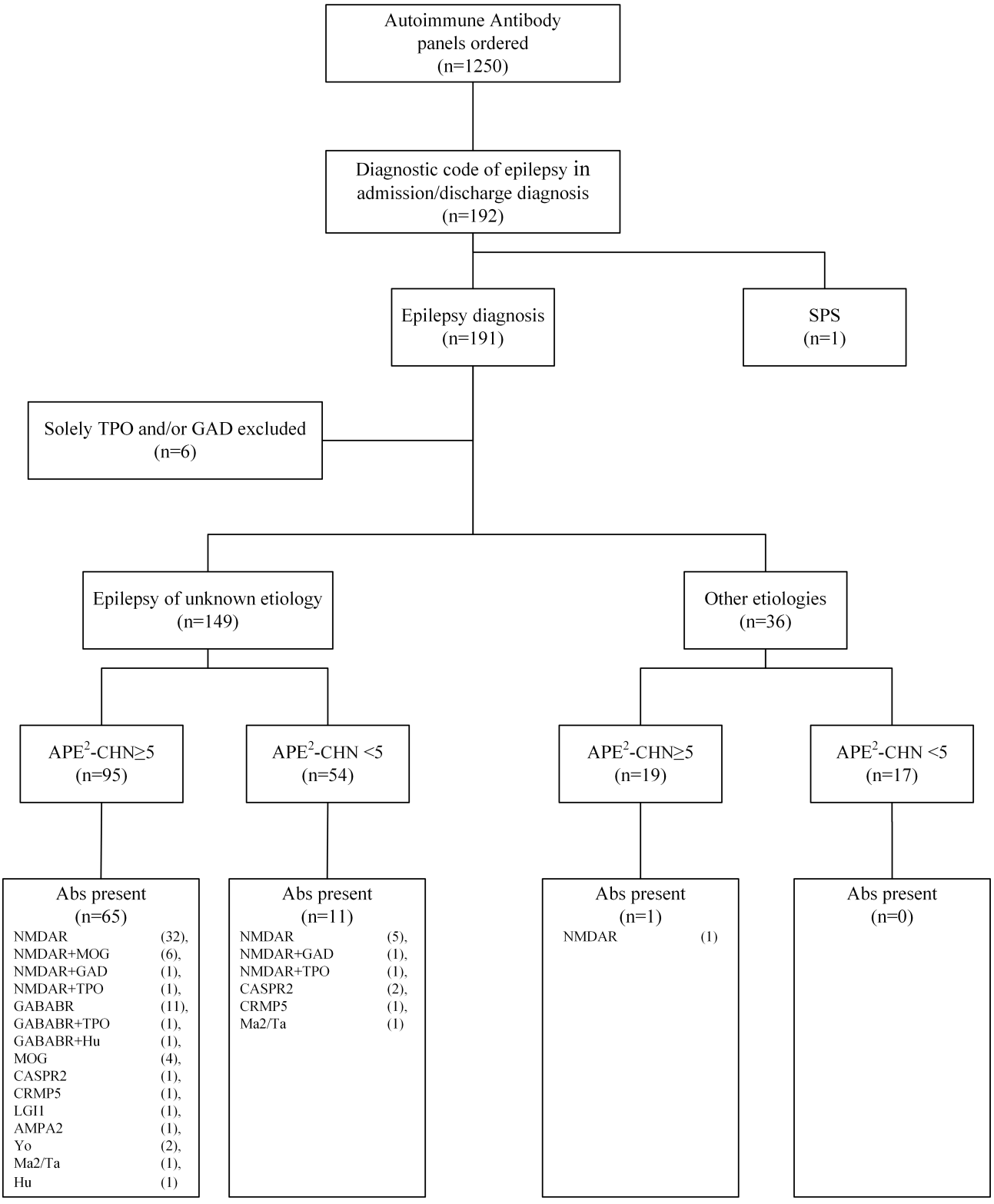

Figure 2

Distribution of neural antibody-positive and antibody-negative patients among reviewed epilepsy cases based on APE2 score. Abs, Antibodies; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; ANNA-1/Hu, antineuronal nuclear type 1; CASPR2, contactin-associated protein-like 2; CRMP-5/CV2, collapsin response mediator protein 5; LGI1, leucine-rich glioma inactivated 1; NMDAR, N-methyl-D-aspartate receptor; GABABR, γ-aminobutyric acid type B; SPS, stiff-person syndrome.

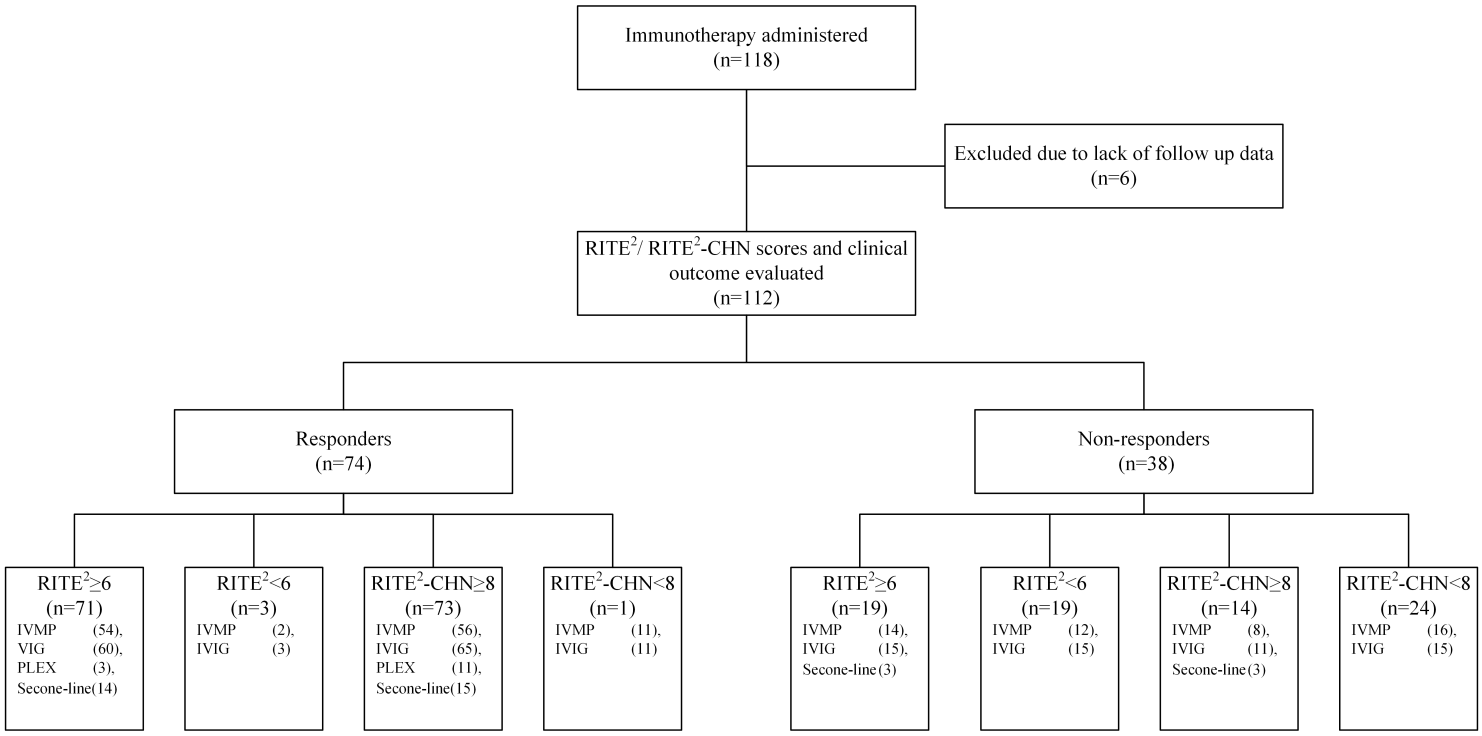

Figure 3

Distribution of patients initiated on immunotherapy regimen based on their seizure outcome. Responders (>50% reduction in seizure frequency) and non-responders are further divided into sub-groups based on their RITE2 and RITE2-CHN scores followed by their choice of initial immunotherapy agent. IVMP, intravenous methylprednisolone, 1 g, i.v., once per day for 5 consecutive days; IVIG, intravenous immunoglobulin, 0.4 g/kg, i.v., once per day for 5 consecutive days; PLEX, plasmapheresis, 5 sessions, every alternate day.

Supplementary Files

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