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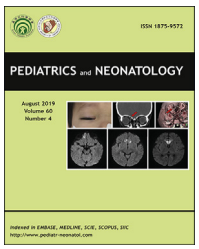

Original Article

Immunotherapy for anti-NMDA receptor encephalitis: Experience from a single center in Taiwan

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Key Words
cyclophosphamide;
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intravenous immunoglobulin;
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Background: Anti-N-methyl-D-aspartate (NMDA) receptor encephalitis is an anti-neuronal antibody-mediated inflammatory brain disease that causes severe psychiatric and neurological deficits in previously healthy patients. The aims of this study were to demonstrate the clinical characteristics of patients diagnosed with anti-NMDA receptor encephalitis and to compare the different treatment strategies among these patients.
Methods: Patients presenting with newly acquired psychiatric and/or neurological deficits were studied retrospectively from 2009 to 2017. Patients with evidence of anti-NMDA receptor antibodies in serum and/or cerebrospinal fluid were enrolled. The modified Rankin scale was

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used to assess the initial status and outcomes of the enrolled patients. Details of the clinical presentations and results of investigations were analyzed.
Results: All (n = 24) of the patients received first-line immunotherapy (steroids, and/or intravenous immunoglobulin, and/or plasma exchange), and 14 patients received second-line immunotherapy (rituximab and/or cyclophosphamide). The mean time between the first- and second-line treatment was 13 days. During the first 6 months, 20 patients (20/24, 83%) achieved a good outcome (modified Rankin Scale score $\leq$2) and 15 patients (15/24, 62.5%) completely recovered. Four patients (17.7%) relapsed, and three patients (12.5%) had associated tumors.
Conclusion: Immunotherapy is an effective treatment for anti-NMDA receptor encephalitis. Rituximab and/or cyclophosphamide are treatment options for those who cannot tolerate or do not respond to first-line immunotherapy. Prospective studies are necessary to investigate the role of rituximab and cyclophosphamide in anti-NMDA receptor encephalitis.

1. Introduction

Anti-N-methyl-D-aspartate (NMDA) receptor encephalitis is an autoimmune disease in which the body produces autoantibodies that act against NMDA receptors in the brain, resulting in both neurological and/or psychiatric symptoms.[1] NMDA receptors are cell surface glutamate receptors that play a pivotal role in memory acquisition and synaptic plasticity. The hallmark of anti-NMDA receptor encephalitis is considered to be depletion of NMDA receptors, a complex mechanism involving crosslinking and internalization.[2]

Anti-NMDA receptor encephalitis has been described as a paraneoplastic syndrome related to ovarian teratoma, specific to young females.[3] Dalmau et al. reported the first 100 patients with anti-NMDA receptor encephalitis (91 female patients) with mainly psychiatric presentations. Of the 98 patients who underwent screening, 59% had tumors, most of which were ovarian teratomas.[4] In 2009, the first study of anti-NMDA receptor encephalitis conducted on children (32 cases) reported a higher percentage of male patients (6/32, 18.8%) than the adults group, and that younger patients were less likely to have tumors.[5]

The clinical presentation of anti-NMDA receptor encephalitis is complex and diverse in each setting.[6] Prior to the development of neuropsychiatric symptoms, which are significant in this disease, most people experience a prodromal period, with symptoms similar to upper respiratory tract infections or a flu-like illness. The neuropsychiatric involvement following the prodromal stage can be lethal and require intensive care unit-level care.[7] Prolonged neuropsychiatric symptoms including seizures, changes in personality, memory deficits, psychosis and sleep disorders are relatively common, although most patients make a nearly complete recovery with early and aggressive treatment.

Anti-NMDA receptor encephalitis can be reversed and treated with an early diagnosis and appropriate therapy. The most well-known treatment for this autoimmune disorder is immunomodulation.[1,8] First-line immunotherapy includes intravenous high-dose steroids (methylprednisolone), intravenous immunoglobulin (IVIG), and/or plasmapheresis. Removing the tumor is indicated in some cases. New regimens or second-line immunotherapy for those who do not respond well to the first-line treatment includes targeted B-cell therapy with rituximab and cyclophosphamide (an alkylating agent which directly inhibits T cell and B cell proliferation). However, there is currently no definite time point at which second-line treatment should be started in non-responders or poor responders. The aims of this study were to demonstrate the demographic data of patients diagnosed with anti-NMDA receptor encephalitis and to compare different treatment strategies among these patients.

2. Methods

This study was a retrospective study involving patients diagnosed with anti-NMDA receptor encephalitis at a tertiary medical center in Taiwan. Patients with evidence of a newly acquired neuropsychiatric deficit compatible with anti-NMDA receptor encephalitis such as psychosis, seizures, movement disorders, and/or autonomic dysfunction, plus additional supportive evidence of inflammation in the blood, cerebrospinal fluid (CSF) and neuroimaging were screened. The patients with confirmed evidence of anti-NMDA receptor antibodies in the serum and/or CSF were included in the study, and those with central nervous system infections prior to the diagnosis of anti-NMDA receptor encephalitis were excluded.

Serial workup including hemogram, biochemical profile, tumor markers, CSF study, electroencephalogram (EEG), brain magnetic resonance imaging (MRI) and abdominal echo were performed in all of the included patients. A cell-based indirect immunofluorescence test was used to detect and quantify IgG antibodies against the NMDA receptor (Euroimmun Diagnosika, Lübeck, Germany). The performance status of each patient was assessed using the modified Rankin Scale (mRS) at the time of diagnosis and after 6 months of follow-up. The patients were defined as having a good outcome if they had an mRS score $\leq$2 at the 6-month follow-up visit. Relapse was defined when the

Table 1 Demographic data of the 24 patients diagnosed with anti-NMDA receptor encephalitis.

	(n = 24)
Gender	
Male (n, %)	8 (33.3%)
Female (n, %)	16 (66.7%)
Age (mean ± SD)	16.62 ± 7.39
Age (years)	
≤ 18 (n, %)	17 (70.8%)
>18 (n, %)	7 (29.2%)
Fever	
Yes (n, %)	10 (41.7%)
No (n, %)	14 (58.3%)
Prodrome	
Yes (n, %)	18 (75.0%)
No (n, %)	6 (25.0%)
Initial presentations	
Neurogenic (n, %)	1 (4.2%)
Psychogenic (n, %)	3 (12.5%)
Both (n, %)	20 (83.3%)
Initial status (mRS)	
2 (n, %)	1 (4.2%)
3 (n, %)	12 (50.0%)
4 (n, %)	9 (37.5%)
5 (n, %)	2 (8.3%)
Anti-NMDA receptor antibody	
Serum (n, %)	8 (33.3%)
Cerebrospinal (n, %)	1 (4.2%)
Both (n, %)	15 (62.5%)
Second-line treatment	14 (58.3%)
1st line to 2nd line (n, mean days)	13 (53.76 ± 103.80)
Seizures	
Yes (n, %)	20 (83.3%)
No (n, %)	4 (16%)
ICU stay	
Yes	17 (70.8%)
No	7 (29.2%)
Length of ICU stay (n, mean day)	17 (37.82 ± 46.47)
Hospitalization days (n, mean day)	24 (60.38 ± 62.20)
Recurrence	
Yes (n, %)	4 (16.7%)
No (n, %)	20 (83.3%)
EEG	
Abnormal	19 (79.2%)
Diffuse slow waves (theta and delta)	10
Delta brush	6
Focal epileptiform discharge	3
Normal	5 (20.8%)
MRI	
Abnormal	8 (33.3%)
T2 hyperintensity of bilateral medial temporal lobe	1
T2 hyperintensity of bilateral occipital lobe	1
T2 hyperintensity of left parietal juxta-cortical region	1
T2 hyperintensity of left frontal temporal region and thalamus	1
T2 hyperintensity of the cerebral cortex and cerebellar folia	1
Diffuse cortical swelling	1
Communicating hydrocephalus	1
Venous malformation over superior cerebellar vermis	1
Normal	16 (66.7%)
CSF	
Abnormal	1 (4.2%)
Pleocytocis (46/uL) with increased protein level (136 mg/dL)	
Normal	23 (95.8%)
Tumor	
Yes (n, %)	3 (12.5%)
No (n, %)	21 (87.5%)
Follow-up at 6 months (mRS score)	
0	15
1	4
2	2
4	3

CSF: cerebrospinal fluid; EEG: electroencephalography; ICU: intensive care unit; MRI: magnetic resonance imaging; mRS: modified Rankin scale; NMDA: N-methyl-D-aspartate.

patients had similar neuropsychiatric symptoms after a period of complete recovery from the disease.

First-line immunotherapy included intravenous high-dose steroids (methylprednisolone), IVIG and/or plasmapheresis. Second-line immunotherapy for those who did not respond well to the first-line treatment included targeted B-cell therapy with rituximab and/or cyclophosphamide.

3. Statistical analyses

Demographic, clinical, and laboratory features (categorical variables) were examined using Pearson's Chi-squared (x2) or Fisher's exact tests. Variables that were not normally distributed were logarithm-transformed and presented as means. Statistical analyses were performed using SPSS version 24.0 and a P-value < 0.05 was considered statistically significant.

4. Results

Twenty-four patients were enrolled in this study, of whom 16 were female. The mean age of the patients was 16.6 ± 7.4 years, and seven were aged ≥18 years. Ten (41.7%) patients had a fever, and 18 (75%) had prodromal symptoms prior to disease onset. During the initial stage of the disease, 20 patients presented with both neurogenic and psychogenic symptoms, one presented with only neurogenic symptoms, and three presented with only psychogenic symptoms. Only one patient had an initial mRS score ≤2.

The results of the tests and investigations are shown in Table 1. Anti-NMDA receptor antibodies were found in the

serum of eight patients, in the CSF of one patient, and in both the CSF and serum in 15 patients. Tumors were detected in three patients. With regard to the treatment modalities, 14 of the 24 patients (58.3%) received second-line treatment after receiving first-line immunotherapy, and the mean time between the two lines of treatment was 53.76 ± 103.8 days. During the study period, 17 patients were admitted to intensive care units for a mean period of 37.82 ± 46.47 days. All of the patients were hospitalized for a mean period of 60.38 ± 62.2 days. Of the 24 patients, four (16.7%) had recurrence or relapse of the disease. With regards to mRS score after 6 months of follow-up, 21 patients had a score of ≤2, and the remaining three patients still had a score of 4.

The most frequent clinical presentations in our patients were psychiatric symptoms, followed by cognitive dysfunction, altered conscious level and seizures (Fig. 1). Twenty-two patients (91.7%) had altered consciousness, 11 (45.8%) had sleep disorders, and eight (33.3%) had dysautonomia.

As shown in Table 2, 10 of the 24 subjects received only first-line treatment, of whom eight were female and older than 12 years of age. In addition, nine of the patients had an initial mRS score >2. Nine patients had seizures, including three with status epilepticus. Of the 14 patients who proceeded to second-line treatment, six were male, and nine were aged between 12 and 17 years. All of these 14 patients had an initial mRS score of >2. Seizure episodes were noted in 11 patients, of whom four had status epilepticus.

In the patients who received first-line treatment only (n = 10), four were diagnosed with respiratory failure, and six were admitted to intensive care units. Most of the cases (7/10) in this group were hospitalized for more than 1 month. None of the patients in this group relapsed, and only one had a tumor.

Of the 14 patients who received second-line treatment, nine were diagnosed with respiratory failure, four relapsed, and two had tumors. Ten (71.4%) of these 14 patients had good outcomes with an mRS score ≤ 2 at 6 months of follow-up (Fig. 2).

5. Discussion

Anti-NMDA receptors encephalitis is an autoimmune disease in which antibodies attack the extracellular portion of NR1 subunits of NMDA receptors, and it can result in a variety of neuropsychiatric symptoms.[4,9] The diagnosis of anti-NMDA receptor encephalitis is confirmed by the detection of specific IgG anti-NR1 antibodies in the CSF and/or blood with associated clinical presentations. Early recognition of the disease with appropriate treatment is crucial for better disease outcomes.

Table 2 Comparison of the two treatment strategies among the 24 patients with anti-NMDA receptor encephalitis.

n = 24		1st		1st + 2nd		*p* value
n		10		14		
Age (years)	≤18	4	23.5%	13	76.5%	0.009*
	>18	6	85.7%	1	14.3%	
Gender	Male	2	25%	6	75%	0.234
	Female	8	50%	8	50%	
Initial mRS score	≤2	1	100%	0	0%	0.417
	>2	9	39.1%	14	60.9%	
Auto-antibodies	Serum	4	50%	4	50%	0.619
	CSF	0	0%	1	100%	
	Both	6	40%	9	60%	
Seizures	Yes	9	45%	11	55%	0.437
	No	1	25%	3	75%	
Status epilepticus	Yes	3	42.9%	4	57.1%	0.643
	No	7	41.2%	10	58.8%	
Respiratory failure	Yes	4	44.4%	5	55.6%	0.582
	No	6	40%	9	60%	
ICU stay	Yes	6	37.5%	10	62.5%	0.439
	No	4	50%	4	50%	
Hospitalization (days)	<30	2	33.3%	4	66.7%	0.381
	30–60	7	53.8%	6	46.2%	
	>60	1	20%	4	80%	
Relapse	Yes	0	0%	4	100%	0.094
	No	10	50%	10	50%	
Tumor	Yes	1	33.3%	2	66.7%	0.629
	No	9	42.9%	12	57.1%	

*$p < 0.05$.
1st: first-line immunotherapy (intravenous high-dose steroids, intravenous immunoglobulin, and/or plasmapheresis).
2nd: second-line immunotherapy (rituximab and/or cyclophosphamide).
CSF: cerebrospinal fluid; ICU: intensive care unit; mRS: modified Rankin Scale; NMDA: N-methyl-D-aspartate.

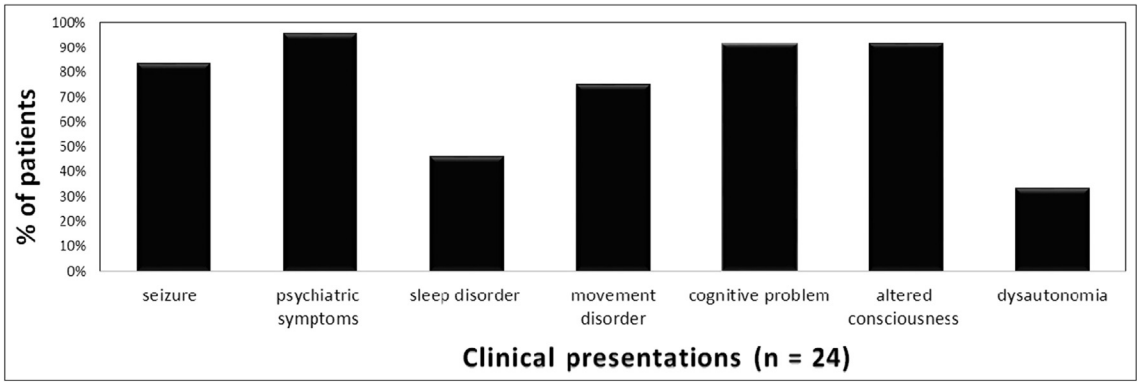

Figure 1 Frequency of clinical presentations among the 24 patients with anti-NMDA receptor encephalitis. The most frequent clinical presentation in our patients was psychiatric symptoms, followed by cognitive dysfunction, altered consciousness level and seizures.

In this study, our patients presented with a flu-like prodrome followed by a continuum of neuropsychiatric features, which is consistent with previous studies.[5,6,10–12] The most frequent clinical presentation in our patients was psychiatric symptoms, followed by cognitive dysfunction, altered consciousness level, and seizures. Although the initial presentation of anti-NMDA receptor encephalitis is generally non-specific, the combination of neurological and psychiatric symptoms should raise suspicion of the disease. In the current study, 17 of the 24 patients required intensive care unit admission due to an altered consciousness level, status epilepticus, and impending respiratory failure. The mean duration of hospitalization in our patients was 60.38 $\pm$ 62.2 days, which is significantly shorter than in a previous study.[13] This may be due to an increased awareness of the physicians in combination with aggressive therapy.

There are currently few established treatment guidelines for anti-NMDA receptor encephalitis focusing on children. In the current study, we used a stepwise approach once the diagnosis of anti-NMDA receptor encephalitis had been confirmed. First-line treatment including methylprednisolone pulse therapy, intravenous immunoglobulin, plasmapheresis, and/or tumor removal (if any) was applied in every patient. Fourteen patients (58.3%) who did not respond to the first-line treatment proceeded to second-line treatment, which included weekly rituximab for 4 weeks and monthly cyclophosphamide for 6 months. The average duration from the first- to second-line treatment in these 14 patients was 13 days, which is consistent with another study.[1] Rapidly proceeding to further treatment options may offer a better outcome for those who do not benefit from first-line treatment.

In the current study, 13 out of the 14 patients who received second-line treatment were aged $<$18 years (Table 2). This may suggest that pediatric patients are more likely to receive second-line treatment, which is consistent with a previous study.[13] Comparing the two treatment groups (Table 2), we found that all of the patients who received both treatments (14/14, 100%) had a higher initial mRS score ($>$2). Among 16 patients who required intensive care unit care, 10 (62.5%) failed to respond satisfactorily to the first-line treatment and subsequently received second-line treatment. Moreover, four of the five patients who were hospitalized for more than 60 days received both first- and second-line treatment. Although it was not clinically significant, this may indicate that those who proceeded to second-line treatment had more severe disease compared to those who received first-line treatment only.

We assessed the outcomes of our patients at 0, 4, 8, and 24 weeks after the diagnosis according to mRS score. Among the 14 patients who received second-line

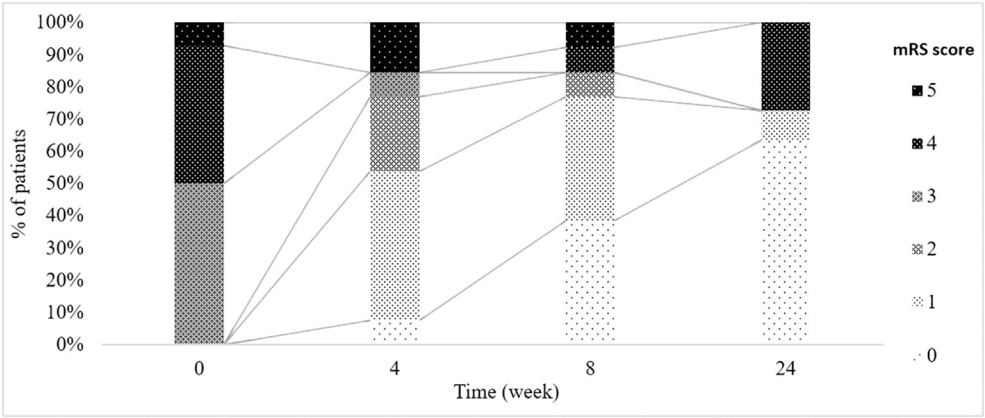

Figure 2 Fourteen patients with anti-NMDA receptor encephalitis received second-line treatment, of whom 10 (71.4%) had good outcomes with an mRS score $\leq$ 2 at 6 months of follow-up.

treatment, 70% had good outcomes with an mRS score $\leq$2 at 6 months of follow-up, which is similar to a previous observational cohort study.[1] This implies that the patients who received additional second-line treatment had worse disease severity, but that they responded well to immunotherapy in second-line treatment with notably good outcomes.

There are several limitations to this study, including the small number of cases, relatively short period of disease follow-up, and lack of titer assessments of auto-antibodies, as this may have indicated the response to the immunotherapy.

In conclusion, this is the first cohort study in Taiwan to focus on the effects of immunotherapy in patients with anti-NMDA receptor encephalitis, especially in the pediatric group. The results showed that rituximab and/or cyclophosphamide played an important role in the treatment of anti-NMDA receptor encephalitis. Further prospective studies are warranted to validate our findings.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pedneo.2018.10.006.