Ph.D. Dissertation of Medicine

Clinical characteristics of seronegative autoimmune encephalitis and factors associated with outcomes

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August 2023

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Submitting a Ph.D. Dissertation of
Medicine

April 2023

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Seoul National University
Translational medicine Major

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Confirming the Ph.D. Dissertation written by
Woo-Jin Lee
July 2023

Chair _____(Seal)
Vice Chair _____(Seal)
Examiner _____(Seal)
Examiner _____(Seal)
Examiner _____(Seal)

ABSTRACT

Seronegative autoimmune encephalitis (AE) is AE without any identifiable pathogenic antibody. Although it is a major subtype of AE, many unmet clinical needs exist in terms of clinical characteristics, treatments, and prognosis. Here in this institutional cohort study, I analyzed patients diagnosed with seronegative AE with available 2-year outcomes were for the disease course, 2-year outcome prediction system, effect of immunotherapy, necessity of further immunotherapy at 6 or 12 months, and pattern of brain atrophy. Seronegative AE was subcategorized into antibody-negative probable AE (ANPRA), autoimmune limbic encephalitis (LE), and acute disseminated encephalomyelitis (ADEM). Poor 2-year outcome was defined by modified Rankin scale [mRS] scores 3-6, and the 2-year serial data of Clinical Assessment Scales in Autoimmune Encephalitis (CASE) score was used for longitudinal data analyses. A total of 147 patients were included. The frequency of achieving a good 2-year outcome (mRS 0-2) was 56.5%. The ANPRA subtype exhibited the poorest outcomes, although the baseline severity was similar among the subtypes. The RAPID score, consisting of five early utilizable clinical factors, refractory status epilepticus, age of onset ≥ 60 years, probable AE (ANPRA subtype), infratentorial involvement, and delay of immunotherapy ≥ 1 month,

was associated with poorer 2-year outcomes. Any immunotherapy was associated with clinical improvement in the patients with low risk for poor 2-year outcomes (RAPID scores 0-1), and the combination immunotherapy of steroid, immunoglobulin, rituximab, and tocilizumab was associated with better outcomes in the patients with high risk for poor 2-year outcomes (RAPID scores 2-5). In patients with persistent disease at 6 months, continuing immunotherapy was associated with more improvement, while the effect of continuing immunotherapy for more than 12 months was unclear. In the longitudinal analysis of MRI, the development of cerebellar atrophy indicated poor outcomes, while the absence of diffuse cerebral atrophy or medial temporal atrophy indicated the possibility of a good outcome. From this study, I newly demonstrated the clinical characteristics and courses, the effect of immunotherapy and its duration, and prognostic factors in seronegative AE.

Keyword: seronegative autoimmune encephalitis; immunotherapy; outcome prediction; prognosis;

Student Number: 2019-36969

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1. INTRODUCTION

1.1. Overview of autoimmune encephalitis

Encephalitis is defined as inflammation that involves the brain parenchyma which results in a significant deterioration in the brain function.[1, 2] The incidence of encephalitis is 10–15/100,000 per year, which is lower than other central nervous system (CNS) diseases, such as stroke, dementia, or epilepsy. However, due to the high severity of the disease the potential risk of mortality, it is a major cause of disease that requires treatment in the intensive care unit.[3]

Encephalitis is classified into infectious encephalitis and non-infectious encephalitis, and most of the non-infectious encephalitis corresponds to autoimmune encephalitis (AE). Over the last decade, AE has become the major etiology of encephalitis. According to a recent community-based study, the incidence of AE between 2006 and 2015 tripled the incidence between 1995 and 2005 and its prevalence became comparable to that of the infectious encephalitis.[3] Considering the concurrent decrement of encephalitis of unknown origin, the rapid expansion of AE might be explained by the establishment of its diagnostic criteria, ongoing discovery of the novel autoantibodies for AE, and expanding clinicians' awareness on the disease.[4]

AE is classified into seropositive and seronegative AE. Seropositive AE is further classified according to the locations of the

target antigen of the specific autoantibodies, into intracellular and extracellular antigens. Autoantibodies against intracellular antigens are often caused by paraneoplastic syndrome.[1, 4] Although its detection is fundamental for the diagnosis of disease, intracellular autoantibodies do not have direct role in AE pathogenesis. Meanwhile, autoantibodies against cell surface antigens bind to the receptors in on the synaptic surface of neuron and interfere with signal transduction of the neuron causing the functional derangement of the CNS. In addition to the specific autoantibodies of AE, it is recently identified that anti-myelin oligodendrocyte glycoprotein (MOG) antibody which causes myelitis or optic neuritis, anti-ganglioside antibodies which causes peripheral neuropathy, can also cause AE.[1, 5]

1.2. Recent advances in the treatment of autoimmune encephalitis

Immunotherapy is fundamental for the treatment of AE. First-line immunotherapy regimen incudes intravenous immunoglobulin (IVIg) and high-dose corticosteroids.[6, 7] Previously, clinical responsiveness to the first-line immunotherapy was determined after 2-4 weeks from the treatment, and second-line immunotherapy such as rituximab or cyclophosphamide were performed for the cases with insufficient clinical improvement after the first-line immunotherapy.[6, 8] The frequency of improvement of encephalitis in response to first-

line immunotherapy is 50%. Additional improvement and good long-term outcome can be achieved in 50-65% of patients who do not respond to first-line immunotherapy by using second-line immunotherapy.[8, 9] If clinical response to second-line immunotherapy is insufficient, tocilizumab can be used as a third-line immunotherapy. In this case, clinical improvement and good long-term prognosis can be achieved in 50-65% of the patients.[10]

At 2-year, good neurologic outcome is achieved in 75-80% of AE caused by cell surface autoantibodies and in 60-65% of AEs caused by intracellular autoantibodies or seronegative AE.[4, 8, 10-12] Given that active immunotherapy in the early stages of autoimmune encephalitis is very important to improve the prognosis, it is recently reported that the early combination of immunotherapy consisting of steroid, IVIG, rituximab, and tocilizumab (SIRT) provides better clinical outcomes in anti-N-methyl-D-Aspartate receptor (anti-NMDAR), evaluated using a comprehensive clinical severity scale for AE, the Clinical Assessment Scale in Autoimmune Encephalitis (CASE).[11] Therefore, the early administration of SIRT regimen has become a standard treatment for AE of moderate to high severity.[4, 6, 11] However, about 10-15% of AE dose not respond to the combination immunotherapy regimen and have devastating clinical outcome. In those refractory cases, 26S proteasome inhibitor bortezomib, regulatory T cell agonist interleukin-2, and Interleukin-1 receptor antagonist anakinra, can be considered as rescue treatment.[7]

1.3. Seronegative autoimmune encephalitis

Seronegative autoimmune encephalitis (AE) is AE without any identifiable pathogenic antibody and can be defined by the recently established operational criteria for probable autoimmune encephalitis.[13] Based on the criteria, seronegative AE is categorized into three subtypes: autoimmune limbic encephalitis (LE), acute disseminated encephalomyelitis (ADEM), and antibody-negative probable AE (ANPRA).[13]

While the clinical spectrum of AE has rapidly expanded,[13-16] seronegative AE has become the major subtype of AE. The incidence and prevalence of seronegative AE might be similar to those of seropositive AE.[16] Although novel autoantibodies that have not yet been identified might account for some portion of seronegative AE, seronegative AE is a major portion of AE with distinct clinical features and pathomechanism.[15, 17]

Nevertheless, many critical unmet needs exist for seronegative AE. In seropositive AE, the disease mechanism, clinical features and courses, prognosis, and effect of combination immunotherapy are established for each autoantibody-mediated disease.[18-23] Furthermore, antibody titre changes and seroconversion serve as biomarkers that help to determine the duration of immunotherapy.[15, 19, 24, 25] However, in seronegative AE, a highly heterogeneous pathomechanism within the disease entity and lack of

autoantibodies have been the major obstacles preventing adequate outcome prognostication, decisions on immunotherapy regimens and durations, and optimisation of the effect of each immunotherapy regimen, which are the key elements for treatment.[15, 17, 26, 27]

1.4. Purpose of research

In this study, I aimed to address those unmet needs to enable more standardized care for seronegative AE. Thus, I collected 2-year data on clinical severity changes assessed using a recently developed comprehensive clinical severity scale for AE, the Clinical Assessment Scale in Autoimmune Encephalitis (CASE).[28] Based on these data, I evaluated the clinical characteristics according to the subtypes, effect of each immunotherapy regimen, and prognosis in seronegative AE. Furthermore, I investigated the factors available at an early clinical stage that predict 2-year outcomes, markers to monitor the disease course, and the effect of immunotherapy in chronic phases in patients with persistent disease.

2. MATERIALS AND METHODS

2.1. Study population

Based on the prospective cohort of patients with clinical suspicion of AE at Seoul National University Hospital (SNUH) from January 1, 2014, to March 1, 2020, I included all consecutive patients who met the following criteria: (1) admitted in SNUH, a national referral centre for encephalitis, presenting with acute or subacute onset of working memory deficits, altered mental status (decreased or altered level of consciousness, lethargy, or personality change), or psychiatric symptoms,[1] (2) satisfied the diagnostic criteria for possible AE after reasonably excluding infectious etiologies or etiologies other than encephalitis, (3) satisfied the diagnostic criteria for probable AE along with negative results of tests for AE-associated autoantibodies, and (4) and available 2-year clinical outcomes.

2.2. Testing for autoimmune encephalitis associated autoantibody

Along with brain MRI and CSF evaluations, the diagnostic work-up for AE included tests for AE-associated autoantibodies and investigations of other etiologies of encephalitis or encephalopathy. For the detection of autoantibodies, patient's serum and CSF sample were screened for the presence of autoantibodies using 3,3'-diaminobenzidine immunohistochemical staining on rat brain

sections.[29, 30] For the diagnosis of specific AE-associated autoantibodies, cell-based analysis was used for the detection of cell-based analyses for synaptic/cell-surface antibodies against NMDAR, leucine-rich-glioma-inactivated 1 (LGI1), contactin-associated protein 2 (CASPR2), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic-acid-receptor-1 and 2 (AMPA 1 and 2), dipeptidyl-peptidase-like protein 6 (DPP6), anti-amphiphysin, and γ-aminobutyric-acid-receptor-B ($GABA_B$) (Euroimmune Ag, Germany) and immunoblotting was used for detecting intracellular antibodies including collapsin response mediator protein 5 (CRMP5), Ma2, Ri, Yo, Hu, Recoverin, Sox-1, Titin, Zic4, and glutamic-acid decarboxylase-65 (GAD65) (Euroimmune Ag).[26, 31-36]

For cell-based analysis, four main procedures were performed as follows: (1) insertion of DNA encoding the target antigens into a plasmid, (2) transfection of this plasmid into vector cells, (3) reaction of vector cells with the patient's serum or CSF, and (4) detection of specific Abs via indirect immunofluorescence.

For immunoblotting, four main procedures were performed as follows: (1) separation of proteins via electrophoresis, (2) transfer of the proteins onto a membrane, (3) overlay of primary (patients' sample) and secondary antibodies onto the membrane, and (4) detection of using enzymes or radioisotopes.[26, 31-36]

2.3. Testing for other etiologies of encephalitis

An investigation panel for the other etiologies of encephalitis/encephalopathy included culture, polymerase chain reaction (PCR), or antibody assays for bacteria and major viruses, including Herpesviridae, Enterovirus, respiratory virus, John Cunningham (JC) virus, measles, Japanese B virus, Mycobacterium, fungus, mycoplasma, Toxoplasma, Cryptococcus, and other infectious etiologies, if suspected, using patient serum and CSF samples. Based on the patients' clinical and laboratory profiles, toxic/metabolic etiologies, systemic or primary central nervous system (CNS) vasculitis, demyelinating diseases associated with anti-aquaporin 4 or myelin oligodendrocyte glycoprotein antibodies, prion disease, and CNS neoplasm were carefully excluded, if indicated, using relevant diagnostic modalities.[13, 34] Hashimoto's encephalopathy and Bickerstaff encephalitis were not included in this study population because those two diseases represent clearly distinct clinical features. This study was approved by the institutional review board of SNUH, and written informed consent was obtained from all enrolled patients or their next of kin.

2.4. Analysis of clinical profiles

Time course data of seronegative AE over 2 years were collected using the modified Rankin scale (mRS) score (range 0–6) and CASE scores (ranges 0–27) obtained at each of the following time points:

at the time that the essential symptoms of encephalitis developed (baseline), every week (time window of ±2 days) for 12 weeks, every month (time window of ±5 days) for the next nine months, and then every three months (time window of ±2 weeks) for the remaining 12 months.[23] Two experts in AE (W.-J.L. and S.-T.L. or K.C.) evaluated the CASE scores based on the medical records because the data was missing in the prospective cohort. Consensuses were reached after a discussion for the discrepant cases. Data at a time point without available records were left blank.

Along with the disease subtypes, the presence of symptoms of encephalitis constituting the nine CASE score domains was evaluated.[28] Refractory status epilepticus (RSE) was defined as persistent status epilepticus despite using an appropriate dose of ≥2 intravenous antiepileptic drugs, including benzodiazepine.[23, 26]

2.5. Analysis of brain imaging and cerebrospinal fluid parameters

Two neurologists (W.-J.L. and S.-T.L.) reviewed patients' baseline MRI. Based on T2-weighted fluid-attenuated inversion recovery images, lesions were present in the cortex, medial temporal cortex, subcortex/white matter, striatum/capsule, thalamus, and infratentorium, including the brainstem and cerebellum, and spine. Significant diffusion restriction or gadolinium enhancement in the parenchymal lesion was also assessed. CSF parameters included the

protein level, leukocyte level, and presence of any abnormalities in CSF (leukocyte count ≥5 cells/μL or protein level of ≥40 mg/dL).[13, 35-38]

2.6. Analysis of treatment profiles

For the treatment of seronegative AE, corticosteroids and IVIG (SI) as first-line immunotherapy,[35-37] rituximab as second-line immunotherapy, and tocilizumab or cyclophosphamide as next-line immunotherapy were administered sequentially or in combination.[35-37, 39] The timing, number of courses, and dosages of each immunotherapy regimen were determined by treating physicians based on the disease severity, responsiveness to prior immunotherapy, and safety. Steroids were administered at 1 g daily for 3–5 consecutive days, IVIG at 1–2 g/kg over 1–5 days, rituximab at 375 mg/m^2 weekly for 4 courses with or without additional monthly maintenance,[27, 35-37, 39] and tocilizumab at 8 mg/kg monthly (initial dose divided into two 4 mg/kg injections). Reduced or split doses of immunotherapy were applied in patients susceptible to haematologic or infectious complications based on the physicians' decision.[23, 27, 36] The time from disease onset to the administration of each immunotherapy regimen was obtained, along with the number of treatment courses.[23, 35, 36]

2.7. Analysis of the predictors of 2-year outcomes

Univariate analyses and subsequent logistic regression analyses were performed to identify the factors associated with poor 2-year clinical outcomes (mRS scores 3-6).[35-39] Using variables that remained significant in the logistic regression model, a prediction score for 2-year outcomes was constructed. Although an unweighted scoring system (assigning each factor 1 point) or a weighted scoring system (assigning points according to the odds ratio [OR, exponential B] of the factor in the regression model) were both evaluated, an unweighted scoring system was chosen for simplicity if both scoring systems showed a significant association with 2-year outcomes. Patients with scores of 3-5 were pooled to overcome the small number of patients with each score. The Cuzick-Wilcoxon test for trends was used to evaluate the association between the prediction score and outcome in the total study population or in each disease subtype. The validation of the scoring system was not performed due to the rarity of the disease and the small number of patients.

2.8. Analysis of the effect of immunotherapy

Based on the time-series data of the CASE score, the effects of each treatment regimen on the CASE score change over time was evaluated using a linear mixed model (LMM), in the total study population or subgroups with a low or high risk for poor outcomes divided by the 2-year outcome prediction score. Repeated measure

analysis of covariance (RM-ANCOVA) was performed to compare the CASE scores at given time points before and after the immunotherapy regimens, which are at 4 weeks before the initiation, at the time of initiation, at 4 weeks after the initiation, and at 8 weeks after the initiation of rituximab or tocilizumab, adjusting for the outcome associated factors. Any adverse event was reviewed along with the regimens used at the time of its development, and the severity was classified using the Common Terminology Criteria for Adverse Events (CTCAE v5.0).[40]

2.9. Effect of further immunotherapy on persistent disease

Persistent disease at 6 months or at 12 months was defined as mRS score of ≥ 3 at each time point. To evaluate the effect of administering further immunotherapy at those time points in patients with persistent disease, logistic regression analyses for achieving a further improvement (≥1 score) in the outcome scores at 2 years were used, adjusting for the outcome-predicting factors. RM-ANCOVA for the score changes adjusting for the outcome factors was also performed. Analyses were separately performed for mRS and CASE score improvements. For patients with CSF data within 6±1 months from the onset, CSF protein and leukocyte levels were compared between the groups with or without further immunotherapy, and between the groups with or without further mRS improvement

after further immunotherapy.

2.10. Serial brain MRI analysis

Baseline and follow-up MRI were performed using 1.5-T or 3.0-T units with protocols that included T1-weighted, T2-weighted, T2 fluid-attenuated inversion recovery (FLAIR), and diffusion weighted image along with apparent diffusion coefficient (DWI/ADC). T1-weighted images were obtained with spine-echo sequences using the following parameters: number of slices = 25–30, slice thickness/gap size = 4.0–5.0/1.0–1.2 mm, repetition time/echo time = 466–2822/7.8–26 milliseconds, field of view = 185–229 × 220–229 mm, and matrix = 320–352 × 192–256.

MRI data were reviewed by two neurologists (W.-J.L. and S.-T.L.), blinded to outcomes and other clinical data, to evaluate the development of brain atrophy in the cortex, cerebellum, and medial temporal area. Diffuse cortical atrophy (DCA) was assessed using the scale of Pasquier *et al.*[41] based on axial T1-weighted images (range 0–3),[41] cerebellar hemisphere atrophy using the scale of Naka *et al.*[42] based on sagittal T1-weighted images (range 0–3),[42] and medial temporal atrophy (mTA) using the De Leon *et al.*[43] scale based on axial T1-weighted images (range 0–3).[43] In every assessment, MRI images were compared to the template images displayed in the reference articles and moderate to severe atrophy (grade 2–3) was designated significant atrophy.[42, 44-46] The numbers of patients who were identified to have developed atrophy until 3, 6, 12, and 24

months were assessed along with their association with poor 2-year outcomes.

2.11. Statistical analysis

Data are presented as the mean ± standard deviation, median [interquartile range, IQR], or number (percentage). R software version 4.0.3 (2021; R team, Vienna, Austria) and SPSS 25.0 (IBM Corp., Armonk, NY) were used for statistical analyses, and a *P*-value <0.05 was considered significant. T-tests, Mann-Whitney U tests, or analysis of variance (ANOVA) for continuous variables or χ^2 or Fisher's exact tests for categorical variables were used for the intergroup comparisons. Variables with *P* values <0.10 in the univariate analyses for poor 2-year clinical outcomes were entered in multivariate logistic regression analysis using the backward elimination method. Age, sex, time from onset, and baseline CASE scores were included in LMM analyses to evaluate the effect of each treatment regimen. The Bayesian information criterion (BIC) value was used to examine the fitness of the LMM. To validate the models derived from the logistic regression, LMM, or RM-ANCOVA analyses, bootstrapping with 1,000 iterations of random resampling was used. The final model was refit for each iteration, and the mean values of the statistical parameters were calculated.

3. RESULTS

3.1. Patient characteristics

Of the 454 patients admitted to SNUH presenting with acute or subacute onset of working memory deficits, altered mental status, or psychiatric symptoms and initially examined, 82 with an identified infectious etiology, 19 diagnosed with etiologies other than encephalitis, and 57 who did not meet the diagnostic criteria for possible AE were excluded. Among the remaining 296 patients with possible AE, 119 with antibody-positive definitive autoimmune encephalitis, 16 who did not meet the diagnostic criteria for probable AEs (ANPRA, LE, or ADEM), and 14 with a follow-up duration of less than 2 years were sequentially excluded. Finally, 147 patients with seronegative AE (69 [46.9%] female, 78 [53.1%] male, median age 40.0 [24.0–58.0] years) were included in the study analysis (**Fig. 1**).

Every patient was followed up for more than 24 months, except for the five patients who died during the first 24-month of follow-up. Among the 147 patients, a total of 3,753 (98.2%) of 3,822 (147 patients x 26 time points) clinical data points were obtained. At baseline, the median CASE score was 13 [9–20], and the median mRS score was 5 [4–5]. Three patients had underlying malignancy including small cell lung cancer, anaplastic thyroid cancer, and pancreatic neuroendocrine tumour each.

117 (79.6%) patients were classified as ANPRA, 23 (15.6%)

as LE, and seven (4.8%) as ADEM subtype. Among the 117 patients with the ANPRA subtype, 117 (100.0%) had a brain MRI abnormality, 84 (71.8%) had CSF pleocytosis (≥5 cells/μL), 37 (31.6%) had elevated CSF immunoglobulin G index (≥0.7), 11 (9.4%) had CSF restricted oligoclonal bands, and 3 (2.6%) were confirmed by brain biopsy demonstrating brain inflammatory infiltration and exclusion of other disorders.[1] There was no significant difference in the demographic profiles and baseline severity among the subtypes (Table 1).

Immunotherapy was initiated at 8.0 [3.5–17.5] days from the onset. A total of 142 (96.6%) patients received IVIG treatment, and 117 (80.1%) received steroids. Rituximab was administered to 113 (78.5%) patients for 5 [4–8] courses. Fifty-nine (40.4%) patients received tocilizumab treatment for 4 [2–6] courses, and 15 (10.2%) patients received cyclophosphamide treatment for 4 [2.5–5.5] courses.

For the total seronegative AE patients (n=147), the median CASE score at 2 years was 3 [1–9.5], and the median mRS score was 2 [0–4]. The frequency of favourable 2-year outcomes (mRS scores 0–2) was 83 (56.5%) (Fig. 2A and B). There was no significant difference in the treatment profiles among the subtypes. When CASE score changes in each subtype were plotted over time, there was a trend of decreasing CASE score over time, although the improvement was no more evident after 12-month from the onset. Additionally, the ANPRA subtype was associated with higher mRS scores and a

lower frequency of favourable outcomes (**Fig. 2C**). Comparisons of clinical, laboratory, brain MRI, treatment, and outcome profiles among AE subtypes are summarised in **Table 1**.

3.2. Factors associated with 2-year outcomes and the construction of RAPID scores

In univariate analysis, the patients with poor 2-year outcomes were associated with a higher age; higher frequency of ANPRA subtype; brainstem dysfunction and weakness; any CSF profile abnormality; higher CSF protein levels; MRI abnormalities in the subcortex/white matter, striatum/capsule, thalamus, and infratentorium; diffusion-restriction lesions; delay of immunotherapy ≥ 1 month; cyclophosphamide treatment, compared to the patients with favourable 2-year outcomes, although the baseline severity was comparable (**Table 2**). In subsequent logistic regression analyses, RSE (odds ratio [OR] 4.171, 95% confidence interval [CI] 1.656-10.503, P=0.002), age of onset ≥ 60 years (OR 4.110, 95% CI 1.594-10.598, P=0.003), ANPRA subtype (OR 4.789, 95% CI 1.411-16.254, P=0.012), infratentorial involvement in brain MRI (OR 10.225, 95% CI 3.110-33.616, P<0.001), and delay of immunotherapy for ≥ 1 month (OR 7.379, 95% CI 2.383-22.843, P=0.001) were significantly associated with poor 2-year outcomes (**Table 3**). The bootstrap validation performed with 1,000 iterations reproduced the same results (**Table 4**).

Five factors, RSE, Age of onset ≥ 60 years, Probable AE (ANPRA) subtype, Infratentorial involvement, and Delay of immunotherapy for ≥ 1 month, were used to construct a 2-year outcome scoring system, the RAPID score. As associations with outcomes were similar between the unweighted and weighted scoring systems (both, $P<0.001$, Cuzick-Wilcoxon test for trends), an unweighted system assigning each factor one point was chosen (score range 0-5). Among the 147 patients, 15 (10.2%) patients had a RAPID score of 0, 52 (35.4%) had a score of 1, 59 (40.1%) had a score of 2, 19 (12.9%) had a score of 3, and 2 (1.4%) had a score of 4. While a RAPID score of 0 was associated with an 86.7% frequency of good 2-year outcomes, higher scores were progressively associated with poor clinical courses and lower frequencies of good 2-year outcomes (**Fig. 3**). Receiver operating characteristic analysis returned that the RAPID score cut-off of 2 (0-1 vs. 2-5) best discriminates the group with poor 2-year outcomes (sensitivity 81.3%, specificity 66.3%).

When we analyzed the validity of RAPID score in each disease subtype (ANPRA, LE, and ADEM), RAPID score correlated well with the 2-year outcomes in each ANPRA and LE subtype, but not in the ADEM subtype (Cuzick-Wilcoxon test $P<0.001$, $P=0.004$, and $P=0.102$ for ANPRA, LE, ADEM subtypes, respectively), possibly due to the low number of patients in the ADEM subtype (**Fig. 4**). Nevertheless, the RAPID score was sensitive for predicting poor outcomes in the ANPRA subtype (sensitivity 86.0%), and highly

specific for predicting poor outcomes in the LE and ADEM subtypes (both specificity 100.0%)

3.3. Analysis of the effect of immunotherapy

In the LMM model, time was inversely (P<0.001) correlated with CASE score changes, indicating that the immunotherapy improved the patients in overall (Table 5). Age (P=0.008) and baseline CASE score (P<0.001) were positively correlated with longitudinal CASE scores. In the analysis of each immunotherapy regimen administered up to each time point, the SI regimen had no outperforming effect in lowering the longitudinal CASE scores (fixed effect [FE] 0.204, 95% confidence interval [CI] −0.353–0.762, P=0.472) after adjusting time and other confounders. This LMM analysis does not mean that the SI regimen has no effect, but means that the effect of SI regimen is fully incorporated into the time−dependent recovery. Meanwhile, adding rituximab (FE −1.454, 95% CI −1.967–−0.941, P<0.001) or adding tocilizumab (FE −1.372, 95% CI −1.950–−0.794, P<0.001) was associated with lower longitudinal CASE scores even after adjusting time and other confounders, indicating that adding these regimens might have accelerated the recovery. The bootstrap validation performed with 1,000 iterations reproduced the same results (Table 6).

When the LMM analyses were repeated for the patients with low risk for poor 2−year outcomes (RAPID scores 0–1), the SI regimen (P=0.001), adding rituximab (P<0.001), and adding

tocilizumab (P<0.001) all significantly were associated with lower longitudinal CASE scores. However, in the patients with a high risk for poor 2-year outcomes (RAPID scores of 2-5), the combined immunotherapy using all of the steroids, immunoglobulin, rituximab, and tocilizumab was associated with lower longitudinal CASE scores (P=0.013) (Table 7). The bootstrap validation performed with 1,000 iterations reproduced the same results (Table 8).

To directly compare the pre- and post-CASE score change after the use of rituximab or tocilizumab, we performed RM-ANCOVA analyses adjusted for RAPID score factors. In patients treated with rituximab, 97/113 (85.8%) had available CASE score data at 4 weeks before the initiation, at the time of initiation, and at 4 and 8 weeks after the initiation of rituximab. The remaining 16/113 patients initiated rituximab in 4 weeks from the onset and were excluded from the analysis. While there was no significant change in CASE score for 4 weeks before the rituximab administration (mean change 0.6, 95% CI -0.5-1.7, P=0.257), CASE scores decreased at 4 and 8 weeks after the initiation of rituximab (mean change 2.2, 95% CI 1.5-3.0, P<0.001 and mean change 1.4, 95% CI 1.0-1.8, P<0.001, respectively) (Fig. 5A and Table 9).

For tocilizumab, 55/59 (93.2%) patients had available CASE score data at each time points (at -4, 0, 4, and 8 weeks). While there was no significant change in CASE score for 4 weeks before tocilizumab (mean change 0.7, 95% CI -0.4-1.7, P=0.222), CASE scores decreased at 4 and 8 weeks after the initiation of tocilizumab

(mean change 2.1, 95% CI 1.1–3.1, P<0.001 and mean change 0.9, 95% CI 0.5–1.3, P<0.001, respectively) (Fig. 5B and Table 9).

A total of 147 adverse events developed during the follow-up. Pneumonia was the most common, followed by leukopenia, acute liver injury, urinary tract infection, acute kidney injury, and thrombocytopenia. Sixty-four (43.5%) events occurred during SI, 48 (32.7%) occurred after adding rituximab, and 35 (23.8%) occurred after adding tocilizumab. Serious adverse events (CTCAE Grade 4) developed in four (2.7%) patients (Table 10).

3.4. Effect of further immunotherapy on persistent disease

Eighty-four (57.1%) patients exhibited persistent disease at 6 months. Among them, 45 (53.6%) patients received further immunotherapies, which were IVIG in 10 (22.2%) patients, rituximab in 29 (64.4%), and tocilizumab in 29 (64.4%). For 39 (46.4%) patients who did not receive further immunotherapy, the major reasons for withholding immunotherapy included infectious complications in 14 (35.9%), respiratory failure/intensive care unit admission in 11 (28.2%), and leukopenia in 8 (20.5%). The baseline characteristics were similar between the patients with and those without further immunotherapy (Table 11). Available CSF profiles at 6±1 months were also similar between the groups although the sample sizes were small and the indication of lumbar puncture was

not controlled (n=13 for those with further immunotherapy and 5 for those without further immunotherapy). In RM-ANCOVA adjusting for RAPID scores, administrating further immunotherapy beyond 6 months was associated with more improvement in the mRS (F=9.29, P<0.001) and CASE (F=13.45, P=0.001) scores until 2 years (**Fig. 6A** and **6B**). Bootstrap validation performed with 1,000 iterations reproduced the results (improvement in mRS: F=7.15, P=0.009 and in CASE: F=5.43, P=0.022). In the logistic regression analyses adjusting for the factors included in the RAPID score, administrating further immunotherapy beyond 6 months were associated with achieving a further improvement in the mRS (OR 3.381, 95% CI 1.306-8.750, P=0.012) and CASE (OR 5.320, 95% CI 1.977-14.315, P=0.001) scores (**Table 12**). The bootstrap validation performed with 1,000 iterations reproduced the same results (**Table 13**). Available CSF protein and leukocyte levels at 6±1 months were similar between the patients with and those without mRS improvement after further immunotherapy (n=7 and 6, respectively, **Table 14**).

For the 72 (49.0%) patients who exhibited persistent disease at 12 months, administrating further immunotherapy beyond 12 months was not significantly associated with improvement in outcome scores at 2 years in RM-ANOVA (P>0.05, **Fig. 6C** and **6D**, see **Table 15** for the profiles of further immunotherapy, reasons for withholding immunotherapy, and comparison of the baseline clinical characteristics).

3.5. Serial brain MRI analysis

The number of follow-up MRI evaluations was 3 [2-5]. During follow-up, DCA developed in 63 (42.9%) patients after a median of 2 [1-3.5] months, cerebellar atrophy in 33 (22.4%) at 2 [1.5-4] months, and mTA in 82 (55.8%) at 2 [1-3] months. The development of either DCA or mTA until 6, 12, and 24 months was associated with poor 2-year outcomes with a sensitivity of 78.1%, 89.1%, and 90.6%, respectively, and a negative predictive value of 80.8%, 89.4%, and 90.1%, respectively. The development of cerebellar atrophy until 6, 12, and 24 months was associated with poor 2-year outcomes with a specificity of 97.6%, 97.6%, and 96.4%, respectively, and a positive predictive value of 92.9%, 93.1%, and 90.6%, respectively (**Fig. 7A** and **7B**, see **Fig. 8** for representative cases).

4. DISCUSSION

This study comprehensively describes the features, courses, and prognosis of seronegative AE based on a large cohort defined by established diagnostic criteria. The major findings of the current study provide some important information on the current issues with the diagnosis and treatment of seronegative AE. First, the frequency of good 2-year outcomes was 56.5%, and the ANPRA subtype exhibited the poorest outcomes. Second, RAPID scores consisting of five early utilizable clinical factors (RSE, Age of onset ≥ 60 years, Ab-negative Probable AE (ANPRA) subtype, Infra-tentorial involvement in brain MRI, and Delay of immunotherapy for ≥ 1 month) were associated with poorer 2-year clinical outcomes. Third, the immunotherapy using steroids, IVIG, rituximab, or tocilizumab was effective in the disease, and the combined immunotherapy was feasible, especially in patients with a high risk for poor outcomes at baseline (RAPID scores of 2–5). Fourth, further immunotherapy might be effective for improving outcomes in cases of persistent disease at 6 months, while the effect of further immunotherapy after 12 months was unclear. Fifth, the development of cerebellar atrophy indicated poor outcomes, while the absence of DCA or mTA indicated a possibility of recovery.

The frequency of good 2-year outcomes was low in the ANPRA subtype. Compared to the data from NMDAR-antibody encephalitis (NMDAR encephalitis) cohort, good 2-year outcomes

were less frequent in the current seronegative AE cohort (56.5% vs. 74.4%), although the baseline CASE score was less severe in the seronegative AE cohort (13 [9-20] vs. 18.5 [15-23]).[23] A possible explanation might be that seronegative AE might have a more irreversible and cytotoxic pathomechanism, whereas the antibody-mediated functional disruption of NMDAR encephalitis is largely reversible. Poor outcomes of the ANPRA subtype might be because of more heterogeneous and mixed pathomechanisms that are refractory to conventional immunotherapy. Therefore, the early diagnosis and prognostication might be crucial to improving the treatment and outcomes of seronegative AE.

The RAPID score can be a useful tool to predict 2-year clinical outcomes. Among the factors included in the RAPID scoring system, RSE and delay of immunotherapy for ≥ 1 month have been suggested as prognostic factors in NMDAR encephalitis,[23, 38] while the prognostic association of other factors is newly recognized. The onset age of ≥ 60 years might reflect lower brain functional reserves, intractable disease subtypes, higher risk of medical complications or lower tolerance to immunotherapy. Additionally, age-related alterations in the CNS immune system, which include amplified activation and impaired regulation of microglia,[47, 48] might augment the susceptibility for irreversible and cytotoxic mechanisms of seronegative AE. The infratentorial area is where the critical brain function is highly concentrated, and the infratentorial involvement in brain MRI represents the involvement of this susceptible area and the

risk of progression. The use of the RAPID score can aid in the clinical stratification of patients for treatment selection and provide outcome information.

Although the combination immunotherapy that adds rituximab and tocilizumab to steroids and IVIG led to better outcomes, especially in patients with a high risk for poor outcomes at baseline (RAPID scores of 2–5), this uncontrolled study does not mean that steroid and IVIG are insufficient for the treatment. In the LMM analysis, the CASE score improved continuously over time and this time-dependent improvement was likely to be initiated by the first lint treatment of steroids and IVIG. This study only suggests that the synergistic interaction of each immunotherapy regimen with different mechanisms might effectively maximize the chance of addressing the diverse pathomechanisms of seronegative AE. Steroids exert broad-spectrum immunomodulation by inhibiting both lymphocyte and myelocyte cells, and inflammatory cytokines.[27, 49] IVIG promotes pathogenic IgG clearance by saturating neonatal Fc receptors,[50, 51] neutralizes autoantibodies, downregulates the inflammatory cytokine network, and suppresses both T cells and B cells.[50, 51] Rituximab targets CD20 and thereby inhibits activated and memory B cells. Tocilizumab suppresses B cells, plasma cells, cytotoxic T cells, T helper 17 cells, and microglia while facilitating regulatory T cell function and inhibiting interleukin (IL)-6-mediated proinflammatory cytokine production.[26, 27, 36, 52-55] In further studies, other treatment options, such as cyclophosphamide,[37] an IL-1 receptor antagonist

(anakinra),[56] a Janus kinase inhibitor (tofacitinib),[57] proteasome inhibitor (bortezomib),[58] anti-CD19 agents (such as inebilizumab),[59] or a novel IL-6 blocker (satralizumab),[60, 61] could be considered based on the disease status and presumed pathomechanism. Adverse events were frequent during the disease course, but combination immunotherapy was well tolerated in most cases, and severe adverse events were uncommon. However, leukopenia and thrombocytopenia were associated with a high degree of combination immunotherapies and warrant regular surveillance and management.[62, 63]

Our study also provides some evidence about the duration of immunotherapy in seronegative AE. Further immunotherapy might be effective in improving outcomes in cases of persistent disease at 6 months, while it was unclear after 12 months. Currently, there is no consensus on the protocol of immunotherapy in autoimmune encephalitis and it is a major issue to decide whether to continue immunotherapy in patients with the persistent disease with suboptimal treatment responses in seronegative AE. Our finding might provide a time criterion for deciding when to cease immunotherapy in cases that are refractory despite continuing immunotherapy. Nevertheless, the duration of immunotherapy must be decided on a patient-by-patient basis by carefully considering the patient's clinical situation. Although we observed that CSF inflammatory markers are not associated with the further use of immunotherapy or the response to further immunotherapy, it is possible that this negative result is due to the small sample size or

the indication bias of CSF re-evaluation at 6 months. Those patients with negative treatment responses or marginal CSF abnormality in prior lumbar punctures might have undergone repeated CSF evaluations, decreasing the biomarker value of CSF profiles. Future studies should still aim to identify biomarkers that aid the decision of immunotherapy duration and optimal regimens.

The development of cerebellar atrophy indicates poor outcomes, and the absence of DCA or mTA indicates a possibility of recovery. This finding suggests that serial follow-up MRI might provide information for long-term prognostication and treatment decisions. When significant cerebellar atrophy develops, the probability of achieving a good outcome might be low despite the administration of further immunotherapy. In contrast, the absence of DCA or mTA indicates that further treatments might improve the outcomes. Despite the underlying mechanism of different clinical implications between cerebellar and cortical atrophy, the irreversibility and negative prognostic value of cerebellar atrophy were also demonstrated in NMDAR encephalitis.[64, 65] However, potential selection bias should be taken into account, as patients with poorer clinical courses might have a higher chance of undergoing repetitive MRI evaluations.

There is an institutional effect to be addressed in this study. In this study, the number of seronegative AE patients was higher than that of seropositive AE. This might be explained by the fact that this cohort is from a national referral centre for autoimmune encephalitis

in South Korea, where atypical, severe, or refractory cases choose to be referred in. However, this trend provided a good environment for investigating seronegative AE. The frequency of diffusion restrictions at the initial brain MRI was high not only in ADEM but also in LE and ANPRA subtypes. The main involved areas of diffusion restriction were the cortex for the ANPRA subtype and the medial temporal cortex for the LE subtype, with subtle low apparent diffusion coefficient values. This might reflect the high frequency of prolonged intractable seizures, or status epilepticus in the referred patients, which provokes cytotoxic edema by neuronal energy failure (**Fig. 9**).[66]

The current study has several limitations. First, the different timing of administration among first-line immunotherapy, rituximab, and tocilizumab might contribute to the relative underestimation of the effect of first-line immunotherapy. As IVIG and steroids are typically given first at the beginning of the illness, the decrement of CASE scores by IVIG or steroid might at the initial phases have been interpreted as the effect of time in the LMM analyses. Additionally, the delayed effect of IVIG and steroids might have been estimated as the effect of further immunotherapy regimens in patients who failed to improve in the initial phases. Although LMM is a method to partially overcome these issues by adjusting the effect of time and other clinical variables, the uncontrolled study is not sufficient to evaluate the effect of each immunotherapy. Therefore, the results should not be interpreted as that SI regimen is not effective whereas rituximab

and tocilizumab are. Second, the effect of immunotherapy should be interpreted considering the possibility of selection bias, given that the treating physician decided the use and timing of each immunotherapy regimen based on the severity, clinical course, responsiveness to prior immunotherapy, and adverse events. Propensity score matching for the clinical factors might be useful to partially adjust for the selection bias, especially in the RM-ANOVA analyses in evaluating the effect of each immunotherapy regimen or of further immunotherapy in persisting disease. However, much larger number of patients might be needed for those matching process. Third, external validation cohorts to confirm the effect of immunotherapy or the predictive value of RAPID scores on the outcomes are necessary. Given the practical challenges of performing a prospective randomized controlled study for combination immunotherapy in this rare disease, reproducing the findings of the current study with other large cohorts might be the best way to validate our findings.[21] Additionally, more advanced immunotherapy regimens or specifically designed treatment strategies based on biomarkers, such as CSF biomarkers or quantitative MRI analysis in different brain segments, might be necessary to improve the outcomes of seronegative AE in the future.[26, 27]

Chapter 5. CONCLUSION

The current study provides some important information on the current issues with the diagnosis and treatment of seronegative AE. First, the frequency of good 2-year outcomes was 56.5%, and the ANPRA subtype exhibited the poorest outcomes. Second, RAPID scores consisting of five early utilizable clinical factors were associated with poorer 2-year clinical outcomes. Third, the immunotherapy using steroids, IVIG, rituximab, or tocilizumab was effective in the disease, and the combined immunotherapy was feasible, especially in patients with a high risk for poor outcomes at baseline (RAPID scores of 2–5). Fourth, further immunotherapy might be effective for improving outcomes in cases of persistent disease at 6 months, while the effect of further immunotherapy after 12 months was unclear. Fifth, the development of cerebellar atrophy indicated poor outcomes, while the absence of DCA or mTA indicated a possibility of recovery.

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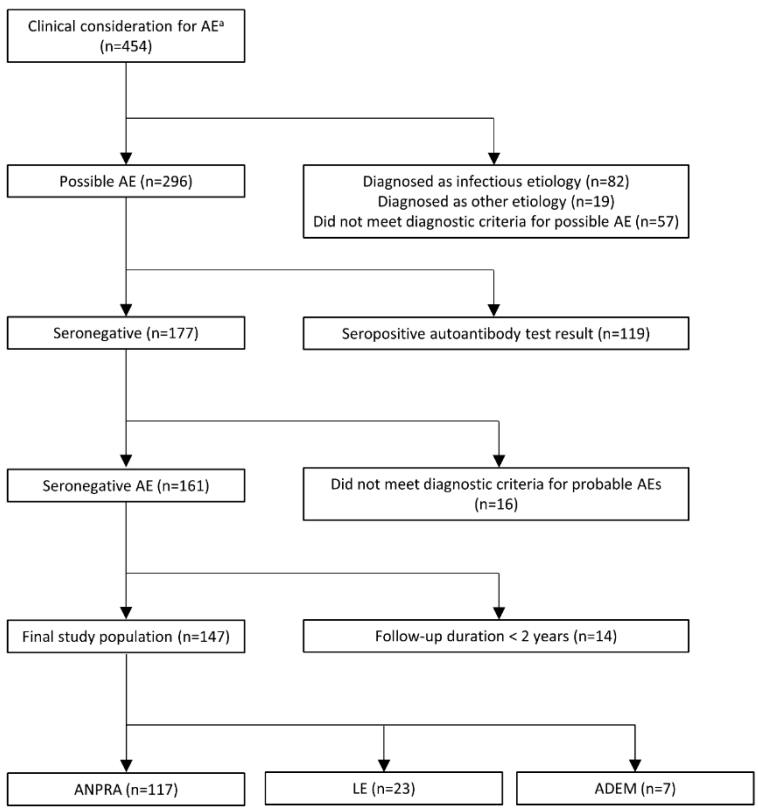
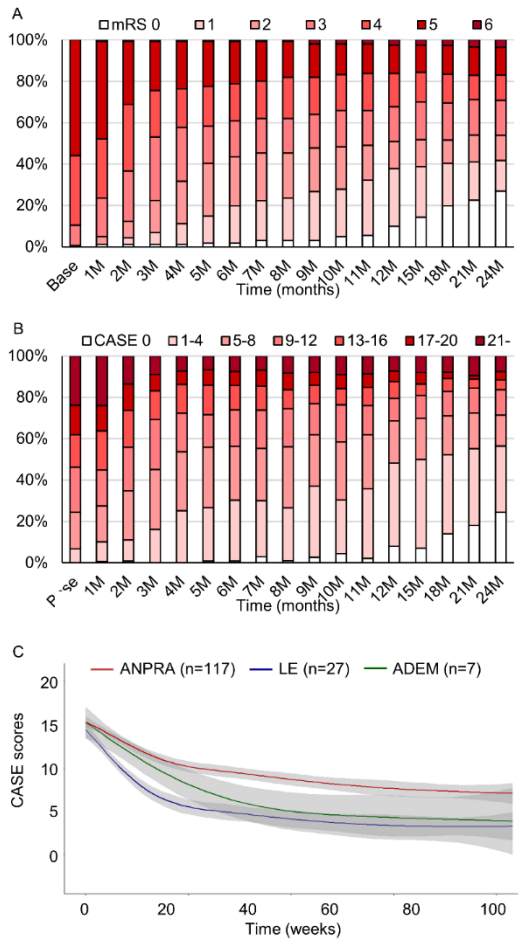
7. FIGURES AND FIGURE LEGENDS

Fig. 1. A flow chart illustrating the process for defining the study population



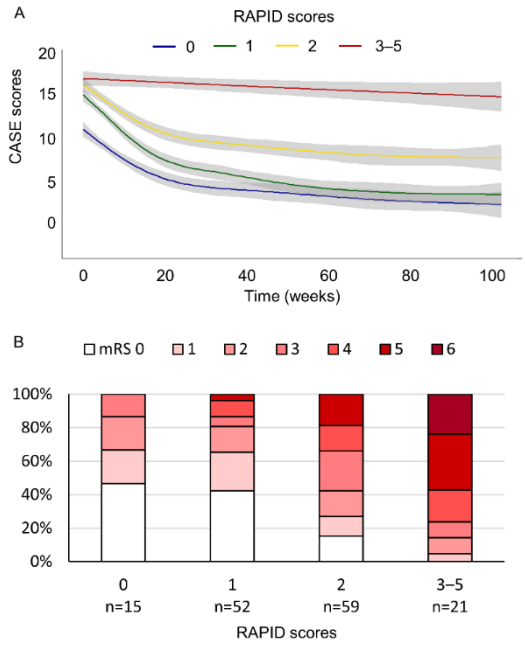
AE: autoimmune encephalitis, ANPRA: antibody−negative probable AE, LE: limbic encephalitis, and ADEM: acute disseminated encephalomyelitis. [a]Admitted to Seoul National University Hospital, a national referral centre for encephalitis, presenting with acute or subacute onset of working memory deficits, altered mental status (decreased or altered level of consciousness, lethargy, or personality change), or psychiatric symptoms

Fig. 2. Clinical courses and outcomes of the study population.



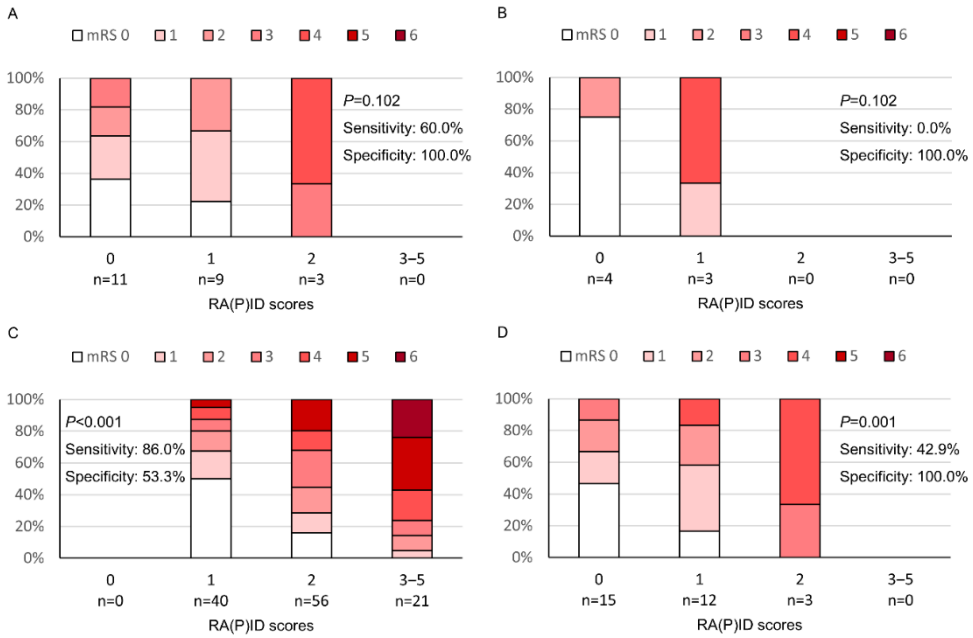
The mRS score profiles (**Panel A**), CASE score profiles (**Panel B**), and CASE score changes in each subtype (**Panel C**) during the follow-up period. mRS: modified Rankin scale, CASE: Clinical Assessment Scale in Autoimmune Encephalitis, LE: autoimmune limbic encephalitis, ADEM: acute disseminated encephalomyelitis, and ANPRA: antibody-negative probable autoimmune encephalitis. The grey-filled areas indicate the 95% confidence interval of the trend lines.

Fig. 3. The association of the RAPID scores with 2-year outcomes.



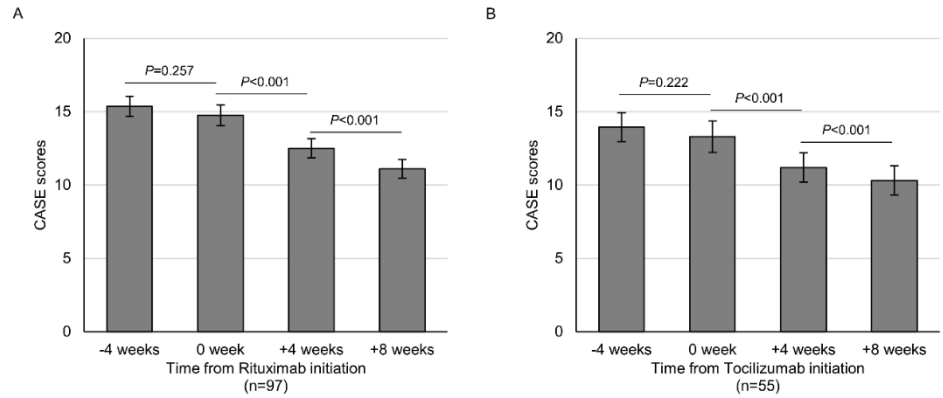
The CASE score changes in each RAPID score subgroup during the 2-year follow-up period (**Panel A**) and distribution of 2-year mRS scores according to the RAPID score subgroups (**Panel B**). CASE: Clinical Assessment Scale in Autoimmune Encephalitis, mRS: modified Rankin scale, RAPID: **R**SE, **A**ge of onset ≥ 60 years, Ab-negative **P**robable AE (ANPRA) subtype, **I**nfratentorial involvement, and **D**elay of immunotherapy for ≥ 1 month. The grey-filled areas indicate the 95% confidence interval of the trend lines.

Fig. 4. Association of the RAPID scores with 2-year outcomes in each disease subtype.



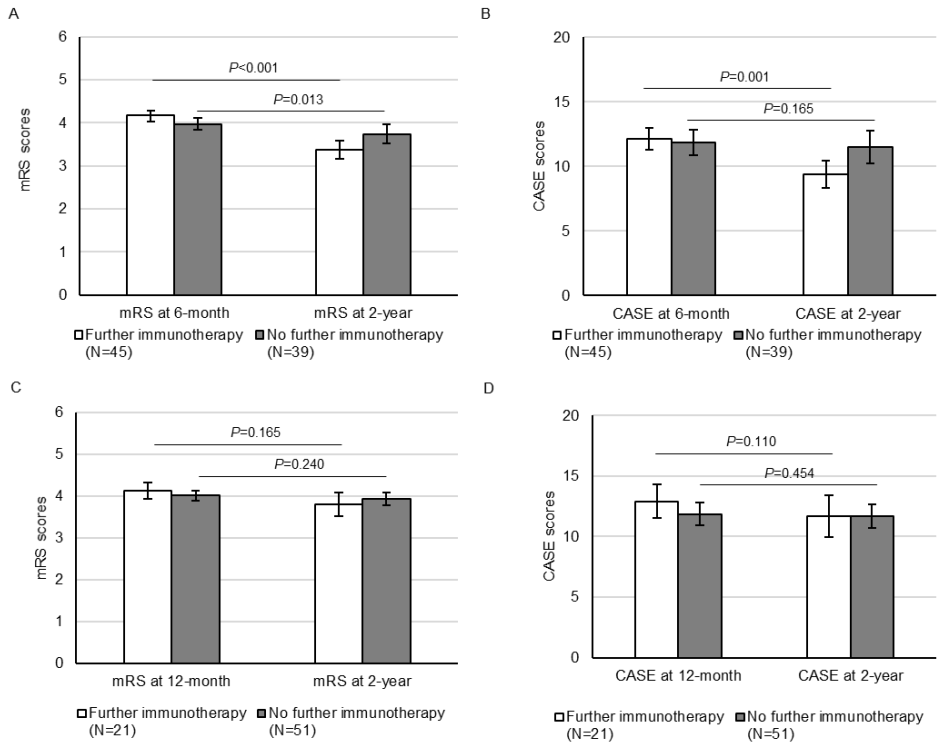
Distribution of 2-year mRS scores according to the RAPID scores in the LE (Panel A), ADEM (Panel B), ANPRA (Panel C), and LE+ADEM (Panel D) subgroups. mRS: modified Rankin scale, LE: limbic encephalitis, ADEM: acute disseminated encephalomyelitis, RAPID: RSE, Age of onset ≥ 60 years, Ab-negative Probable AE (ANPRA) subtype, Infratentorial involvement, and Delay of immunotherapy for ≥ 1 month. *P* values are from the Cuzick-Wilcoxon test for trends.

Fig. 5. The change in CASE scores before and after the initiation of rituximab or tocilizumab regimens.



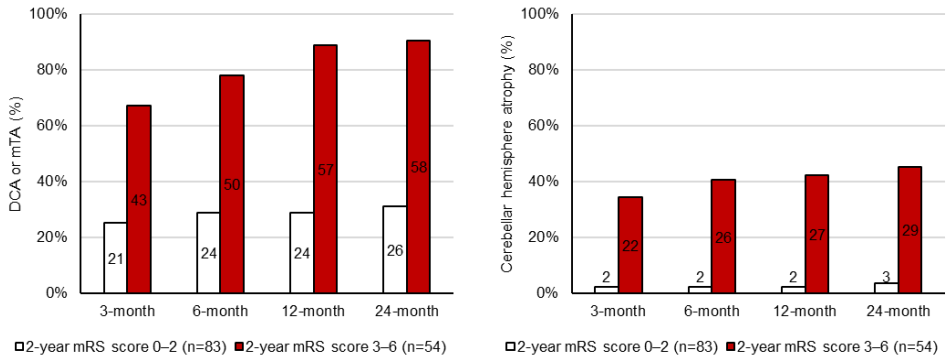
CASE scores at 4 weeks before the initiation, at the time of initiation, at 4 weeks after the initiation, and at 8 weeks after the initiation of rituximab (**panel A**) or after the initiation of tocilizumab (**panel B**). CASE: Clinical Assessment Scale in Autoimmune Encephalitis. Error bars indicate the standard error of the mean. *P* values were derived from Repeated measure analysis of covariance analyses (RM-ANCOVA) analyses.

Fig. 6. The effect of further immunotherapy on persistent disease at 6 months and at 12 months.



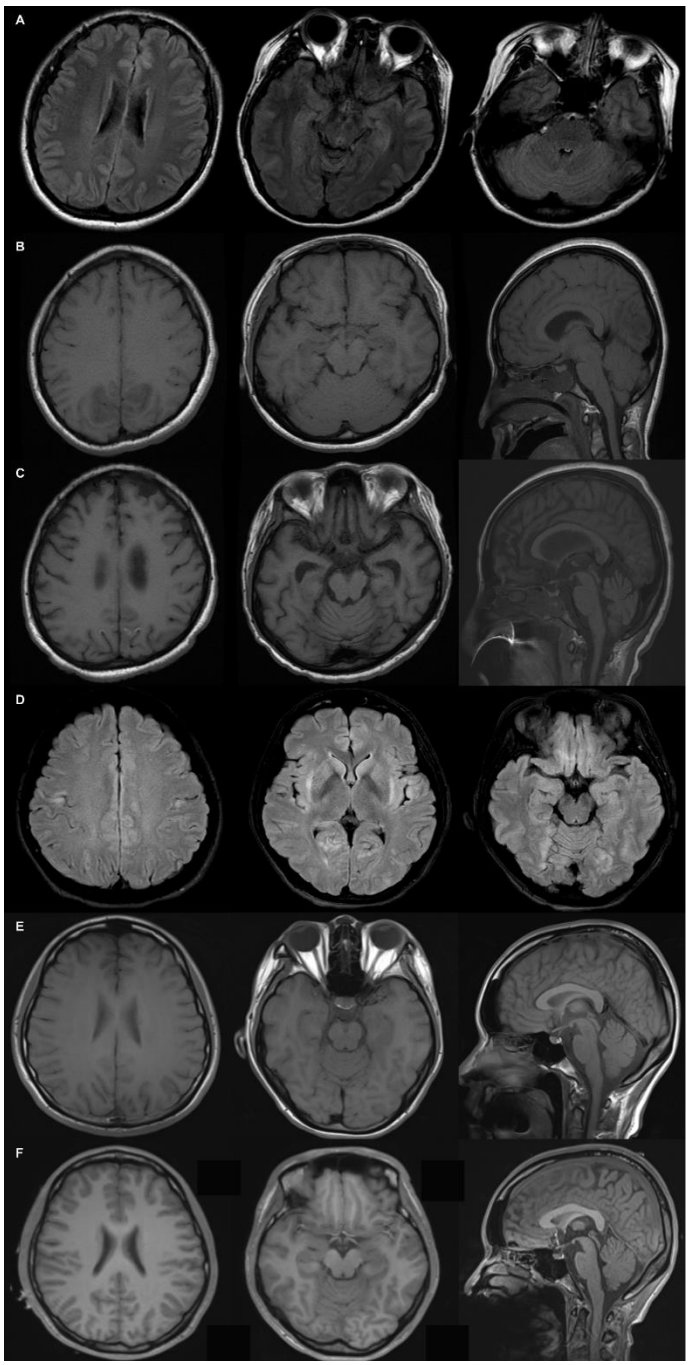
Comparison of the changes in mRS scores (Panel A) and CASE scores (Panel B) from 6 months to 2 years between groups with or without further immunotherapy. Comparison of the changes in mRS scores (panel C) and CASE scores (panel D) from 12 month to 2 years between groups with or without further immunotherapy. mRS: modified Rankin scale and CASE: Clinical Assessment Scale in Autoimmune Encephalitis. Error bars indicate the standard error of the mean. *P* values were derived from Repeated measure analysis of covariance analyses (RM-ANCOVA) analyses.

Fig. 7. The frequency of the development of brain atrophy in serial brain MRI follow-up and its association with the clinical outcomes.



Frequency of patients who developed DCA or mTA (**Panel E**) and cerebellar hemisphere atrophy (**Panel F**) at 3, 6, 12, and 24 months, in subgroups with favourable (RAPID scores 0–2) or poor (RAPID scores 3–6) 2-year mRS scores. mRS: modified Rankin scale, CASE: Clinical Assessment Scale in Autoimmune Encephalitis, DCA: diffuse cortical atrophy, and mTA: medial temporal atrophy. Error bars indicate the standard error of the mean. *P* values were derived from Repeated measure analysis of covariance analyses (RM-ANCOVA) analyses.

Fig. 8. Brain MRI findings of representative patients.

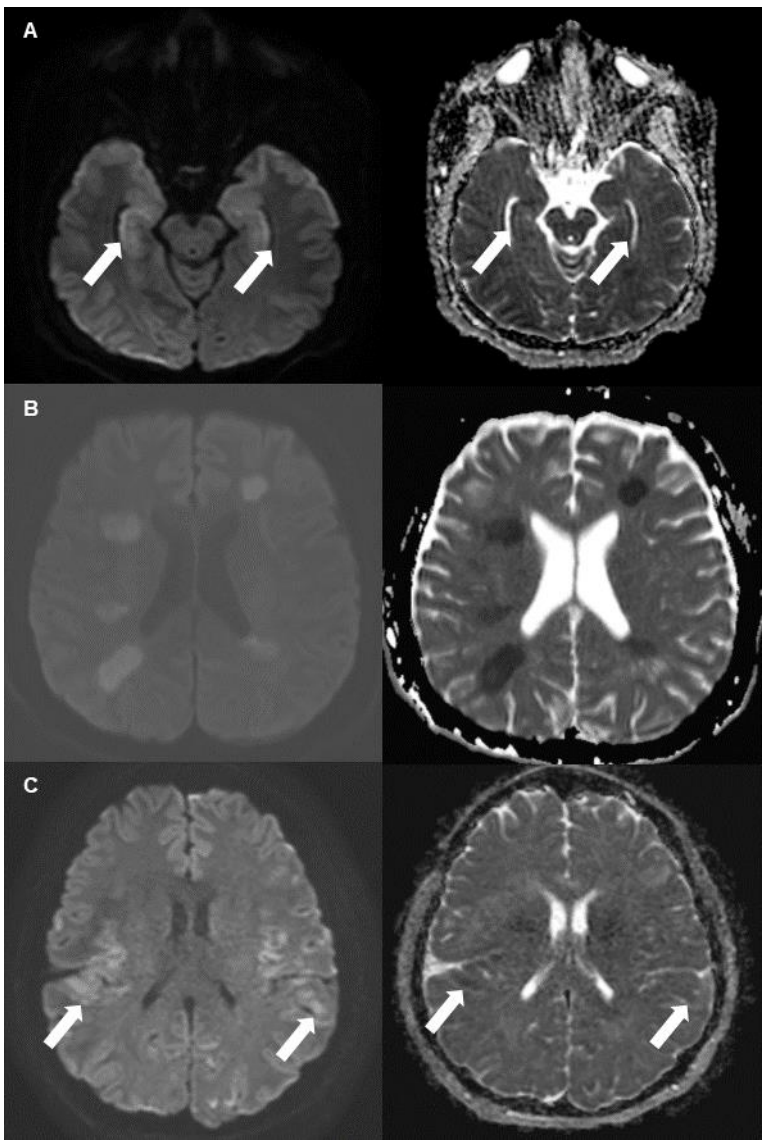


A twenty-three-year-old woman presented with a decreased level of consciousness and refractory status epilepticus (RSE) and was

diagnosed with antibody-negative probable autoimmune encephalitis (ANPRA). The baseline Clinical Assessment Scale in Autoimmune Encephalitis (CASE) score was 27. In the initial brain MRI, T2-fluid attenuated inversion recovery (FLAIR, **panel A**) showed diffuse high signal intensity in the cortex including the bilateral medial temporal area, whereas no atrophy was found in axial and sagittal T1 images (**panel B**). The patient was refractory to combination immunotherapy including steroid, IVIG, rituximab, and tocilizumab. Follow-up MRI at 3 months (**panel C**) exhibited development of mild DCA with Pasquier scale score 1, moderate mTA with De Leon scale score 2, and moderate cerebellar atrophy with Naka scale score 2. The patient did not improve despite continuing immunotherapy and the 2-year CASE score was 24.

A twenty-six-year-old man presented with a decreased level of consciousness and refractory status epilepticus and was diagnosed as ANPRA. The baseline CASE score was 27. In the initial brain MRI, FLAIR showed diffuse high signal intensity along the cortex including the bilateral medial temporal area (**panel D**) without atrophy (**panel E**). The patient partially improved during the administration of combination immunotherapy but symptoms persisted for 6 months (CASE score 16). In follow-up MRI at 7-month (**panel F**), there was no significant atrophy in the cortex, medial temporal area, or cerebellum. The patient slowly improved along with continuing immunotherapy and the 2-year CASE score was 2.

Fig. 9. Illustrative diffusion restriction MRI finding in each disease subtype.



A twenty-year-old woman presented with memory decline and psychiatric symptoms, followed by decreased level of consciousness and refractory status epilepticus (RSE), and was diagnosed with autoimmune limbic encephalitis. In initial brain MRI,

high signal intensity in bilateral medial temporal on the diffusion-weighted image (DWI) along with subtle low apparent diffusion coefficient (ADC) value was found (white arrows, **panel A**).

A twenty-six-year-old man was diagnosed with acute disseminated encephalomyelitis. Initial brain MRI shows scattered DWI high signal intensity lesions with low ADC value (**panel B**).

A fifty-two-year-old man presented with a decreased level of consciousness and refractory status epilepticus and was diagnosed as antibody-negative probable AE. In the initial brain MRI, DWI showed high signal intensity in bilateral frontal and parietal cortices with heterogeneous but subtle low ADC value (white arrows, **panel C**).

8. TABLES

Table 1. Comparison of clinical, laboratory, treatment, and outcome profiles among the disease subgroups.

	Total (N=147)	ANPRA (A, N=117)	LE (B, N=23)	ADEM (C, N=7)	*P*	
Age of onset (years)	43.0 [24.5–58.0]	44.0 [24.0–60.0]	44.0 [30.5–57.5]	37.0 [30.0–43.5]	0.710	
Male Sex (%)	78 (53.1)	65 (55.6)	10 (43.5)	3 (42.9)	0.488	
Clinical profiles						
Initial CASE scores	13 [9–20]	13 [9–20]	15 [9–18.5]	15 [9.5–18.5]	0.982	
Initial mRS scores	5 [4–5]	5 [4–5]	4 [4–5]	5 [4–5]	0.674	
Refractory status epilepticus (%)	44 (29.9)	35 (29.9)	8 (34.8)	1 (14.3)	0.584	
Symptom profiles (Frequency [%], median)						
Seizure (%)	119 (81.0)	96 (82.1)	18 (78.3)	5 (71.4)	0.737	
Memory dysfunction (%)	137 (93.2)	110 (94.0)	22 (95.7)	5 (71.4)	0.062	
Psychiatric symptoms (%)	112 (76.2)	90 (76.9)	19 (82.6)	3 (42.9)	0.089	
Impaired consciousness (%)	118 (80.3)	94 (80.3)	17 (73.9)	7 (100.0)	0.315	
Language problem (%)	105 (71.4)	86 (73.5)	16 (69.6)	3 (42.9)	0.214	
Dyskinesia/Dystonia (%)	37 (25.2)	33 (28.2)	3 (13.0)	1 (14.3)	0.246	
Gait instability and ataxia (%)	117 (79.6)	96 (82.1)	14 (60.9)	7 (100.0)	0.027*	A, C > B
Brainstem dysfunction (%)	78 (53.1)	66 (56.4)	7 (30.4)	5 (71.4)	0.045*	C > B
Weakness (%)	86 (58.5)	73 (62.4)	9 (39.1)	4 (57.1)	0.117	

CSF/MRI profiles						
CSF protein level (mg/dL)	57.0 [43.0–84.0]	60.0 [45.0–90.0]	53.0 [36.5–64.5]	73.0 [58.0–82.0]	0.241	
CSF leukocyte level (cells/μL)	10.0 [2.0–29.0]	11.0 [4.0–34.0]	9.0 [3.0–15.5]	2.0 [0.0–16.5]	0.102	
Any Abnormality in MRI (%)	134 (91.2)	107 (91.5)	20 (87.0)	7 (100.0)	0.550	
Cortex (%)	118 (80.3)	88 (75.2)	23 (100.0)	7 (100.0)	0.168	
Medial temporal cortex (%)	102 (69.4)	75 (64.1)	23 (100.0)	4 (57.1)	0.009**	B > A, C
Subcortex / White−matter (%)	63 (42.9)	55 (47.0)	2 (8.7)	6 (85.7)	<0.001**	C > A > B
Striatum / Capsule (%)	33 (22.4)	28 (23.9)	2 (8.7)	3 (42.9)	0.115	C > B
Thalamus (%)	23 (15.6)	21 (17.9)	0 (0.0)	2 (28.6)	0.060	C > B
Infra−tentorium (%)	19 (12.9)	17 (14.5)	0 (0.0)	2 (28.6)	0.074	C > B
Spine (%)	7 (4.8)	5 (4.3)	0 (0.0)	2 (28.6)	0.007**	C > B, A
Parenchymal enhancement (%)	11 (7.5)	11 (9.4)	0 (0.0)	0 (0.0)	0.218	
Diffusion restriction (%)	47 (32.0)	36 (30.8)	6 (26.1)	5 (71.4)	0.065	C > B, A
Treatment profiles						
Onset to immunotherapy (days)	8.0 [3.5–17.5]	7.0 [3.0–17.0]	12.0 [7.5–22.5]	7.0 [3.0–9.0]	0.197	
Delay of immunotherapy ≥ 1 month (%)	21 (14.3)	18 (15.4)	3 (13.0)	0 (0.0)	0.519	
IVIG treatment (%)	142 (96.6)	112 (95.7)	23 (100.0)	7 (100.0)	0.515	
IVIG courses	1 [1–2]	1 [1–2]	1 [1–3.5]	1 [1–1.5]	0.502	
Steroid treatment (%)	117 (80.1)	92 (79.3)	19 (82.6)	6 (85.7)	0.872	
Steroid courses	1 [1–1]	1 [1–1]	1 [1–1]	1 [1–1]	0.323	
Rituximab treatment (%)	113 (78.5)	88 (77.2)	21 (91.3)	4 (57.1)	0.120	
Onset to Rituximab	31.0 [16.0–	30.0 [15.0–	33.0 [23.0–	34.5 [13.5–	0.682	

(days)	74.0]	77.0]	73.0]	55.5]		
Rituximab courses	5.0 [4.0–8.0]	5 [4–8]	7 [4–8]	6 [3–8]	0.481	
Tocilizumab treatment (%)	59 (40.4)	50 (43.1)	8 (34.8)	1 (14.3)	0.268	
Onset to Tocilizumab (days)	73.0 [25.5–169.5]	79.0 [25.0–201.0]	75.5 [42.0–133.0]	14.0 [14.0–14.0]	0.467	
Tocilizumab courses	4 [2–6]	4 [2–6]	4.5 [4–5.5]	1 [1–1]	0.272	
Outcomes profiles						
Follow–up duration (months)	29.0 [25.0–48.0]	26.0 [25.0–48.0]	32.0 [27.0–46.0]	41.0 [26.5–48.5]	0.791	
2–year CASE scores	3 [1–9.5]	4 [1–11]	2 [0.5–5]	2 [0–7]	0.136	
2–year mRS scores	2 [0–4]	2 [1–4]	1 [0.5–2]	1 [0–3]	0.069	A > B, C
Favorable 2–year mRS score (%)	83 (56.5)	60 (51.3)	18 (78.3)	5 (71.4)	0.042*	B, C > A

Data are presented as mean±standard deviation or as median [interquartile range, IQR]. ANPRA: antibody–negative probable autoimmune encephalitis, LE: autoimmune limbic encephalitis, ADEM: acute disseminated encephalomyelitis, AE: autoimmune encephalitis, CASE: Clinical Assessment Scale in Autoimmune Encephalitis, mRS: modified Rankin scale, CSF: cerebrospinal fluid, and IVIG: intravenous immunoglobulin. $^{*}P<0.05$ and $^{**}P<0.01$.

Table 2. Comparison between groups with or without poor 2-year outcomes.

	Favorable 2-year outcome (mRS scores 0-2, N=83)	Poor 2-year outcome (mRS scores 3-6, N=64)	*P*
Age of onset (years)	37.1±16.7	51.6±18.4	<0.001**
Male Sex (%)	38 (45.8%)	40 (62.5%)	0.065
Clinical profiles			
Initial CASE scores	13.0 [10.0-19.0]	14.5 [8.0-21.0]	0.382
Initial mRS scores	5 [4-5]	5 [5-5]	0.356
Refractory status epilepticus (%)	19 (22.9%)	25 (39.1%)	0.085
Subtypes			0.042*
ANPRA (%)	60 (72.3%)	57 (89.1%)	
LE (%)	18 (21.7%)	5 (7.8%)	
ADEM (%)	5 (6.0%)	2 (3.1%)	
Symptom profiles (Frequency [%], median)			
Seizure	69 (83.1%)	50 (78.1%)	0.579
Memory dysfunction	76 (91.6%)	61 (95.3%)	0.573
Psychiatric symptoms	61 (73.5%)	51 (79.7%)	0.497
Impaired consciousness	64 (77.1%)	54 (84.4%)	0.374
Language problem	56 (67.5%)	49 (76.6%)	0.305
Dyskinesia/Dystonia	18 (21.7%)	19 (29.7%)	0.359
Gait instability and ataxia	61 (73.5%)	56 (87.5%)	0.060
Brainstem dysfunction	36 (43.4%)	42 (65.6%)	0.012*
Weakness	38 (45.8%)	48 (75.0%)	0.001*
CSF/MRI profiles			
CSF protein level[a] (mg/dL)	51.0 [36.0-75.0]	73.5 [53.0-102.0]	0.018*
CSF leukocyte level[a] (cells/μL)	10.0 [2.0-23.0]	12.5 [2.0-72.5]	0.168
Any CSF profile abnormality[a] (%)	70 (84.3%)	63 (98.4%)	0.009**
Any Abnormality in MRI[b] (%)	80 (86.0)	42 (97.7)	0.008**
Cortex (%)	67 (80.7%)	48 (75.0%)	0.527
Medial temporal cortex (%)	59 (71.1%)	42 (65.6%)	0.597
Subcortex / White-matter (%)	20 (24.1%)	43 (67.2%)	<0.001**
Striatum / Capsule (%)	8 (9.6%)	25 (39.1%)	<0.001**
Thalamus (%)	6 (7.2%)	17 (26.6%)	0.003**

Infra-tentorium (%)	5 (6.0%)	14 (21.9%)	0.010*
Spine (%)	3 (3.6%)	4 (6.2%)	0.724
Parenchymal enhancement (%)	5 (6.0%)	6 (9.4%)	0.653
Diffusion restriction (%)	17 (20.5%)	30 (46.9%)	0.001**
Treatment profiles			
Onset to immunotherapy (days)	7.0 [4.0-15.0]	9.0 [2.0-37.0]	0.366
Delay of immunotherapy ≥ 1 month (%)	5 (6.0%)	16 (25.0%)	0.003**
IVIG treatment (%)	81 (97.6%)	61 (95.3%)	0.767
Steroid treatment (%)	66 (79.5%)	51 (81.0%)	0.995
Rituximab treatment (%)	61 (75.3%)	52 (82.5%)	0.399
Onset to rituximab (days)	27 [17-57]	43 [15-100]	0.087
Tocilizumab treatment (%)	31 (37.3%)	28 (44.4%)	0.487
Onset to tocilizumab (days)	73 [28-156]	71 [21.3-145.3]	0.792
Cyclophosphamide treatment (%)	2 (2.4%)	13 (20.3%)	0.001**
Outcomes profiles			
2-year CASE scores	1.0 [0.0- 2.0]	11.0 [7.0-17.0]	<0.001**
2-year mRS scores	1.0 [0.0- 2.0]	4.0 [3.0- 5.0]	<0.001**
Diffuse cortical atrophy (%)	16 (19.3%)	47 (73.4%)	<0.001**
Cerebellar atrophy (%)	3 (3.6%)	30 (46.9%)	<0.001**
Medial temporal atrophy (%)	27 (32.5%)	55 (85.9%)	<0.001**

Data are presented as mean±standard deviation or as median [interquartile range, IQR]. CASE: Clinical Assessment Scale in Autoimmune Encephalitis, mRS: modified Rankin scale, LE: autoimmune limbic encephalitis, ADEM: acute disseminated encephalomyelitis, ANPRA: antibody-negative probable autoimmune encephalitis, CSF: cerebrospinal fluid, and IVIG: intravenous immunoglobulin. *P<0.05 and **P<0.01.

Table 3. Logistic regression analysis for the factors associated with the poor clinical course.

	Odd Ratio (95% CI)	*P*
Refractory status epilepticus (n=44, 29.9%)	4.171 (1.656–10.503)	0.002**
Age of onset ≥ 60 years (n=34, 23.1%)	4.110 (1.594–10.598)	0.003**
Probable AE (ANPRA) subtype (n=117, 79.6%)	4.789 (1.411–16.254)	0.012**
Infra-tentorium involvement in brain MRI (n=19, 12.9%)	10.225 (3.110–33.616)	<0.001**
Delay of immunotherapy ≥ 1 month (n=21, 14.3%)	7.379 (2.383–22.843)	0.001**

R^2=0.422 and *P*<0.001 for the logistic regression equation. AE: autoimmune encephalitis and ANPRA: antibody-negative probable autoimmune encephalitis. **P<0.01.

Table 4. Bootstrap validation for the logistic regression analysis of the factors associated with the poor clinical course.

	B (95% CI)	*P*
Refractory status epilepticus	1.189 (0.391–2.244)	0.006**
Age of onset ≥ 60 years	1.518 (0.664–2.507)	0.001**
Probable AE (ANPRA) subtype	2.979 (1.237–7.171)	0.015*
Infra-tentorium involvement in brain MRI	2.257 (1.113–4.274)	0.001**
Delay of immunotherapy ≥ 1 month	2.005 (0.873–3.874)	0.002**

The bootstrap result is based on 1,000 bootstrap samples. AE: autoimmune encephalitis, ANPRA: antibody-negative probable autoimmune encephalitis. *P<0.05 and **P<0.01.

Table 5. Linear mixed models for the longitudinal CASE score changes.

	Coefficient for the fixed effect (mean±SE)	95% Confidence interval	*P*
Intercept	3.017±1.536	−0.016–6.05	0.051
Age (years)	0.058±0.022	0.015–0.100	0.008**
Male sex	0.559±0.792	−1.004–2.123	0.481
Baseline CASE scores	0.523±0.064	0.397–0.649	<0.001**
Time (weeks)	−0.074±0.003	−0.08–−0.069	<0.001**
Regimens			
SI regimen	0.204±0.284	−0.353–0.762	0.472
Adding Rituximab	−1.454±0.262	−1.967–−0.941	<0.001**
Adding Tocilizumab	−1.372±0.295	−1.950–−0.794	<0.001**

Bayesian information criterion (BIC) values: 19312.2. CASE: Clinical Assessment Scale in Autoimmune Encephalitis, SI: steroid and IVIG, and CSF: cerebrospinal fluid. *P<0.05 and **P<0.01.

Table 6. Bootstrap validation for the linear mixed models for the longitudinal CASE score changes.

	Coefficient for the fixed effect (mean±SE)	95% Confidence interval	*P*
Intercept	3.017±0.381	2.251–3.75	0.001**
Age (years)	0.058±0.004	0.05–0.065	0.001**
Male sex	0.559±0.159	0.251–0.864	0.004**
Baseline CASE scores	0.523±0.012	0.5–0.546	0.001**
Time (weeks)	−0.074±0.003	−0.081–−0.068	0.001**
Regimens			
SI regimen	0.204±0.357	−0.434–0.98	0.578
Adding Rituximab	−1.454±0.284	−2.032–−0.919	0.001**
Adding Tocilizumab	−1.372±0.314	−2.001–−0.772	0.001**

The bootstrap result is based on 1,000 bootstrap samples. CASE: Clinical Assessment Scale in Autoimmune Encephalitis, and SI: steroid and IVIG. *P<0.05 and **P<0.01.

Table 7. Linear mixed models for the longitudinal CASE score changes in subgroups divided by RAPID scores.

model 1: RAPID scores 0–1	Coefficient for the fixed effect (mean±SE)	95% Confidence interval	*P*
Intercept	3.468±1.746	−0.012–6.949	0.051
Age (years)	0.072±0.031	0.009–0.134	0.025*
Male sex	2.407±0.883	0.645–4.169	0.018**
Baseline CASE scores	0.457±0.072	0.312–0.601	<0.001**
Time (weeks)	−0.087±0.004	−0.094–−0.079	<0.001**
Regimens			
SI regimen	−1.354±0.394	−2.127–−0.580	0.001**
Adding Rituximab	−2.742±0.332	−3.393–−2.090	<0.001**
Adding Tocilizumab	−1.756±0.375	−2.491–−1.020	<0.001**
model 2: RAPID scores 2–5	**Coefficient for the fixed effect (mean±SE)**	**95% Confidence interval**	***P***
Intercept	7.617±2.515	2.616–12.619	0.003**
Age (years)	0.013±0.029	−0.045–0.071	0.651
Male sex	−2.470±1.164	−4.786–−0.155	0.037*
Baseline CASE scores	0.480±0.094	0.294–0.667	<0.001**
Time (weeks)	−0.065±0.004	−0.073–−0.056	<0.001**
Regimens			
SI regimen	0.873±0.384	0.119–1.626	0.023*
Adding Rituximab	−0.372±0.377	−1.111–0.366	0.323
Adding Tocilizumab	−1.057±0.423	−1.887–−0.228	0.013*

Bayesian information criterion (BIC) values: model 1= 8022.9 and model 2= 11063.2 CASE: Clinical Assessment Scale in Autoimmune Encephalitis, and SI: steroid and IVIG. *P<0.05 and **P<0.01.

Table 8. Bootstrap validation for the linear mixed models for the longitudinal CASE score changes in the subgroups divided by RAPID scores

model 1: RAPID scores 0–1	Coefficient for the fixed effect (mean±SE)	95% Confidence interval	*P*
Intercept	3.468±0.564	2.340–4.541	0.001**
Age (years)	0.072±0.007	0.059–0.085	0.001**
Male sex	2.407±0.204	1.990–2.817	0.001**
Baseline CASE scores	0.457±0.018	0.422–0.495	0.001**
Time (weeks)	−0.087±0.004	−0.094–−0.080	0.001**
Regimens			
SI regimen	−1.354±0.494	−2.265–−0.315	0.013*
Adding Rituximab	−2.742±0.389	−3.501–−2.027	0.001**
Adding Tocilizumab	−1.756±0.401	−2.554–−0.982	0.001**
model 2: RAPID scores 2–5	**Coefficient for the fixed effect (mean±SE)**	**95% Confidence interval**	***P***
Intercept	7.617±0.515	6.550–8.615	0.001**
Age (years)	0.013±0.005	0.003–0.023	0.010*
Male sex	−2.470±0.210	−2.875–−2.073	0.001**
Baseline CASE scores	0.480±0.017	0.447–0.513	0.001**
Time (weeks)	−0.065±0.005	−0.074–−0.056	0.001**
Regimens			
SI regimen	0.873±0.464	−0.058–1.817	0.065
Adding Rituximab	−0.372±0.378	−1.092–0.408	0.335
Adding Tocilizumab	−1.057±0.457	−2.046–−0.222	0.020*

The bootstrap result is based on 1,000 bootstrap samples. CASE: Clinical Assessment Scale in Autoimmune Encephalitis, and SI: steroid and IVIG. *P<0.05 and **P<0.01.

Table 9. Repeated measure analysis of covariance for the comparison of the CASE scores before and after the use of rituximab or tocilizumab.

Rituximab (RTX, n=97)	CASE scores (mean±SD)	Score change from the prior time point (mean [95% CI])	*P*
At 4 weeks before the RTX initiation	15.4±6.7	–	–
At the RTX initiation	14.8±7.0	0.6 [−0.5–1.7]	0.257
At 4 weeks after the RTX initiation	12.5±6.4	2.2 [1.5–3.0]	<0.001**
At 8 weeks after the RTX initiation	11.1±6.3	1.4 [1.0–1.8]	<0.001**
Tocilizumab (TOC, n=55)	**CASE scores (mean±SD)**	**Score change from prior time point (mean [95% CI])**	***P***
At 4 weeks before the TOC initiation	13.9±7.4	–	–
At the TOC initiation	13.3±8.0	0.7 [−0.4–1.7]	0.222
At 4 weeks after TOC initiation	11.2±7.4	2.1 [1.1–3.1]	<0.001**
At 8 weeks after TOC initiation	10.3±7.4	0.9 [0.5–1.3]	<0.001**

Repeated measures of analysis of covariance (RM−ANCOVA) were performed after adjusting RAPID scores. CASE: Clinical Assessment Scale in Autoimmune Encephalitis, SD: standard deviation, and CI: Confidence interval. **P<0.01.

Table 10. Profiles of the adverse events.

	Total	CTCAE grade	Onset (Days)	During SI	During SIR	During SIRT
Pneumonia	53 (36.1%)	3 [3–3]	16 [10–40.5]	35 (66.0%)	12 (22.6%)	6 (11.3%)
Leukopenia	30 (20.4%)	3 [2–3]	77.5 [34–200.5]	4 (13.3%)	12 (40.0%)	14 (46.7%)
Acute liver injury	27 (18.4%)	2 [1–2]	23 [14–52]	11 (40.7%)	12 (44.4%)	4 (14.8%)
Urinary tract infection	20 (13.6%)	3 [3–3]	39 [13–150]	6 (30.0%)	8 (40.0%)	6 (30.0%)
Acute kidney injury	11 (7.5%)	2 [2–3]	72 [13–259]	7 (63.6%)	2 (18.2%)	2 (18.2%)
Thrombocytopenia	6 (4.1%)	3 [2–3.3]	221.5 [54.5–390]	1 (16.7%)	2 (33.3%)	3 (50.0%)
Any serious adverse event†	4 (2.7%)	–	208 [85–255]	1 (25.0%)	1 (25.0%)	2 (50.0%)

Data are presented as median [interquartile range, IQR]. CTCAE: Common Terminology Criteria for Adverse–Events, SI: steroid and IVIG, SIR: steroid, IVIG, and rituximab, SIRT: steroid, IVIG, rituximab, and Tocilizumab. † 1 pneumonia, 2 leukopenia, 1 thrombocytopenia, and 1 acute kidney injury.

Table 11. Comparison of clinical, laboratory, treatment, and outcome profiles according to the groups with or without further immunotherapy, within the patients with mRS scores ≥3 at 6-month.

	Further immunotherapy after 6-month (N=45)	No further immunotherapy after 6-month (N=39)	*P*
Age of onset (years)	47.0 [26.0-61.0]	52.0 [33.0-63.5]	1.000
Male Sex (%)	25 (55.6%)	26 (66.7%)	0.415
Clinical profiles			
Subypes			0.166
LE (%)	5 (11.1%)	4 (10.3%)	
ADEM (%)	0 (0.0%)	3 (7.7%)	
ANPRA (%)	40 (88.9%)	32 (82.1%)	
Initial CASE scores	15 [7-21]	16 [9.5-21]	0.641
Initial mRS scores	5 [4-5]	4 [4-5]	0.327
6-month CASE scores	11 [7-16]	11 [7.5-16]	0.946
6-months mRS scores	4 [4-5]	4 [3-5]	0.335
Refractory status epilepticus (%)	16 (35.6%)	16 (41.0%)	0.772
Profiles of further immunotherapy †			
IVIG treatment (%)	10 (22.2)		
Rituximab treatment (%)	29 (64.4)		
Tocilizumab treatment (%)	29 (64.4)		
Reasons for withholding immunotherapy §			
Infectious complication (%)		14 (35.9)	
Leukopenia (%)		8 (20.5)	
Respiratory failure / ICU admission (%)		11 (28.2)	
Patient's decision (%)		2 (5.1)	
Death (%)		1 (2.6)	
Infusion adverse events (%)		1 (5.1)	
Other medical problems (%)		2 (5.1)	
Symptom profiles			
Seizure (%)	33 (73.3%)	32 (82.1%)	0.490
Memory dysfunction (%)	39 (86.7%)	38 (97.4%)	0.166
Psychiatric symptoms (%)	34 (75.6%)	32 (82.1%)	0.648
Impaired consciousness (%)	34 (75.6%)	35 (89.7%)	0.159
Language problem (%)	31 (68.9%)	30 (76.9%)	0.563
Dyskinesia/Dystonia (%)	14 (31.1%)	10 (25.6%)	0.756

Gait instability and ataxia (%)	37 (82.2%)	35 (89.7%)	0.503
Brainstem dysfunction (%)	26 (57.8%)	26 (66.7%)	0.541
Weakness (%)	29 (64.4%)	32 (82.1%)	0.119
CSF/MRI profiles			
CSF protein level at baseline (mg/dL)	58.5 [46.0–98.0]	67.0 [51.0–96.0]	0.389
CSF leukocyte level at baseline (cells/μL)	8.0 [2.0–30.0]	14.0 [3.5–73.0]	0.406
Any CSF profile abnormality at baseline (%)	42 (93.3%)	39 (100.0%)	0.293
CSF protein level at 6−month (mg/dL) [†a]	66.7±27.7 (N=13)	66.8±24.3 (N=5)	0.992
CSF leukocyte level at 6−month (cells/μL) [†a]	21.1±43.8 (N=13)	2.0±2.8 (N=5)	0.354
Any Abnormality in MRI (%)	42 (93.3%)	38 (97.4%)	0.714
Cortex (%)	32 (71.1%)	33 (84.6%)	0.225
Medial Temporal Cortex (%)	30 (66.7%)	26 (66.7%)	1.000
Subcortex / White−matter (%)	22 (48.9%)	24 (61.5%)	0.346
Striatum / Capsule (%)	15 (33.3%)	13 (33.3%)	1.000
Thalamus (%)	9 (20.0%)	10 (25.6%)	0.723
Infra−tentorium (%)	9 (20.0%)	7 (17.9%)	1.000
Spine (%)	4 (8.9%)	3 (7.7%)	1.000
Outcomes profiles			
2−year CASE scores	8 [4–12]	11 [5–16]	0.205
2−year mRS scores	3 [2– 4]	4 [3– 5]	0.224
CASE scores changes	2 [1– 5]	1 [0– 3]	0.022*
mRS scores changes	1 [0– 1]	0 [0– 1]	0.031*

Data are presented as median [interquartile range, IQR] or mean±standard deviation. LE: autoimmune limbic encephalitis, ADEM: acute disseminated encephalomyelitis, ANPRA: antibody−negative probable autoimmune encephalitis, CASE: Clinical Assessment Scale in Autoimmune Encephalitis, mRS: modified Rankin scale, and ICU: intensive care unit. *P<0.05. †CSF data available within ±1 month from the 6−month time point, Datasets available: [a]18.

Table 12. Logistic regression analysis for the factors associated with the improvement in mRS or CASE scores after 6-months until 2-year, in the patients with mRS scores ≥3 at 6-month.

†mRS score improvement	Odd Ratio (95% CI)	*P*
Further immunotherapy after 6-month	3.381 (1.306-8.750)	0.012*
Refractory status epilepticus	0.393 (0.140-1.102)	0.076
Age of onset ≥ 60 years	0.416 (0.139-1.240)	0.116
Probable AE (ANPRA) subtype	0.986 (0.385-2.523)	0.976
Infra-tentorium involvement in brain MRI	0.390 (0.109-1.398)	0.148
Delay of immunotherapy ≥ 1 month (%)	0.331 (0.089-1.225)	0.098
§CASE score improvement	Odd Ratio (95% CI)	*P*
Further immunotherapy after 6-month	5.320 (1.977-14.315)	0.001**
Refractory status epilepticus	0.689 (0.248-1.912)	0.475
Age of onset ≥ 60 years	0.462 (0.160-1.334)	0.154
Probable AE (ANPRA) subtype	1.387 (0.546-3.528)	0.492
Infra-tentorium involvement in brain MRI	0.366 (0.099-1.348)	0.131
Delay of immunotherapy ≥ 1 month (%)	0.794 (0.219-2.882)	0.725

†R^2=0.229 and P<0.001 for the logistic regression equation.

§R^2=0.280 and P<0.001 for the logistic regression equation.

mRS: modified Rankin scale, CASE: Clinical Assessment Scale in Autoimmune Encephalitis, AE: autoimmune encephalitis, and ANPRA: antibody-negative probable autoimmune encephalitis.

*P<0.05 and **P<0.01.

Table 16. Bootstrap validation for the logistic regression analyses for the factors associated with the improvement in mRS or CASE scores from 6−months to 2−year, within the patients with mRS scores ≥3 at 6−month.

mRS score improvement	B (95% CI)	*P*
Further immunotherapy after 6−month	1.319 (0.25–2.922)	0.019*
Refractory status epilepticus	−1.261 (−3.019−−0.199)	0.025*
Age of onset ≥ 60 years	−1.20000 (−3.014–0.008)	0.041*
Probable AE (ANPRA) subtype	−0.719 (−2.716–0.787)	0.319
Infra−tentorium involvement in brain MRI	−1.263 (−3.342−−0.036)	0.058
Delay of immunotherapy ≥ 1 month (%)	−1.035 (−2.946–0.519)	0.130
CASE score improvement	**B (95% CI)**	***P***
Further immunotherapy after 6−month	1.889 (0.800–3.771)	0.001**
Refractory status epilepticus	−1.096 (−2.984–0.254)	0.089
Age of onset ≥ 60 years	−0.955 (−2.472–0.207)	0.085
Probable AE (ANPRA) subtype	−1.444 (−21.678–0.316)	0.081
Infra−tentorium involvement in brain MRI	−1.699 (−4.208−−0.203)	0.028*
Delay of immunotherapy ≥ 1 month (%)	−0.717 (−2.888–1.513)	0.351

The bootstrap result is based on 1,000 bootstrap samples. mRS: modified Rankin scale, CASE: Clinical Assessment Scale in Autoimmune Encephalitis, ANPRA: antibody−negative probable AE, and AE: autoimmune encephalitis. *P<0.05 and **P<0.01.

Table 14. Comparison of cerebrospinal fluid profiles at 6-month between the groups with or without additional mRS improvement after further immunotherapy, within the patients with mRS scores ≥3 at 6-month.

At 6-month	mRS improvement (N=7)	No mRS improvement (N=6)	*P*
CSF protein level (mg/dL) †	66.4±36.4	66.9±15.9	0.976
CSF leukocyte level (cells/μL) †	40.2±61.2	4.7±6.2	0.216

Data are presented as mean±standard deviation. mRS: modified Rankin scale and CSF: cerebrospinal fluid. *P<0.05. †CSF data available within ±1 months from the 6-month time point, Datasets available: 13.

Table 15. Comparison of clinical, laboratory, treatment, and outcome profiles according to the groups with or without further immunotherapy, within the patients with mRS scores ≥3 at 12-month.

	Further immunotherapy after 12-month (N=21)	No further immunotherapy after 12-month (N=51)	*P*
Age of onset (years)	46.0 [23.0-61.0]	55.0 [35.5-65.0]	0.535
Male Sex (%)	13 (61.9%)	32 (62.7%)	1
Clinical profiles			
Subtypes			0.494
LE (%)	1 (4.8%)	5 (9.8%)	
ADEM (%)	0 (0.0%)	2 (3.9%)	
ANPRA (%)	20 (95.2%)	44 (86.3%)	
Initial CASE scores	15 [7-21]	13 [8.5-21]	0.620
Initial mRS scores	5 [4-5]	4 [5-5]	0.554
12-month CASE scores	11 [8-16]	10 [7-15.5]	0.378
12-months mRS scores	4 [3-5]	4 [3-5]	0.765
Refractory status epilepticus (%)	7 (33.3%)	20 (39.2%)	0.841
Profiles of further immunotherapy †			
IVIG treatment (%)	2 (9.5)		
Rituximab treatment (%)	7 (33.3)		
Tocilizumab treatment (%)	11 (52.4)		
Reasons for withholding immunotherapy §			
Infectious complication (%)		25 (49.0)	
Leukopenia (%)		6 (11.8)	
Respiratory failure / ICU admission (%)		10 (19.6)	
Patient's decision (%)		6 (11.8)	
Death (%)		1 (2.0)	
Infusion adverse events (%)		4 (7.8)	
Other medical problems (%)		1 (2.0)	
Symptom profiles			
Seizure (%)	15 (71.4%)	40 (78.4%)	0.741
Memory dysfunction (%)	17 (81.0%)	50 (98.0%)	0.037*
Psychiatric symptoms (%)	12 (57.1%)	44 (86.3%)	0.017*

Impaired consciousness (%)	14 (66.7%)	45 (88.2%)	0.068
Language problem (%)	14 (66.7%)	40 (78.4%)	0.454
Dyskinesia/Dystonia (%)	9 (42.9%)	13 (25.5%)	0.241
Gait instability and ataxia (%)	17 (81.0%)	46 (90.2%)	0.493
Brainstem dysfunction (%)	11 (52.4%)	35 (68.6%)	0.301
Weakness (%)	11 (52.4%)	42 (82.4%)	0.020*
CSF/MRI profiles			
CSF protein level at baseline (mg/dL)	63.5 [46.0–84.0]	73.0 [51.5–109.5]	0.271
CSF leukocyte level at baseline (cells/μL)	8.0 [1.0–38.0]	18.0 [5.5–79.0]	0.184
Any CSF profile abnormality at baseline (%)	19 (90.5%)	51 (100.0%)	0.148
Any Abnormality in MRI (%)	19 (90.5%)	49 (96.1%)	0.706
Cortex (%)	16 (76.2%)	39 (76.5%)	1.000
Medial Temporal Cortex (%)	15 (71.4%)	32 (62.7%)	0.666
Subcortex / White-matter (%)	13 (61.9%)	30 (58.8%)	1.000
Striatum / Capsule (%)	7 (33.3%)	20 (39.2%)	0.841
Thalamus (%)	4 (19.0%)	13 (25.5%)	0.780
Infra-tentorium (%)	4 (19.0%)	12 (23.5%)	0.917
Spine (%)	0 (0.0%)	6 (11.8%)	0.241
Outcomes profiles			
2-year CASE scores	9 [7–16]	10 [6–15.5]	0.941
2-year mRS scores	4 [3–5]	4 [3–5]	0.503
CASE scores changes	1 [0– 2]	0 [0– 0]	0.095
mRS scores changes	0 [−1– 0]	0 [0– 0]	0.022*

Data are presented as median [interquartile range, IQR] or mean±standard deviation. LE: autoimmune limbic encephalitis, ADEM: acute disseminated encephalomyelitis, ANPRA: antibody-negative probable autoimmune encephalitis, CASE: Clinical Assessment Scale in Autoimmune Encephalitis, mRS: modified Rankin scale, and CSF: cerebrospinal fluid. *P<0.05.

9. 국문초록

항체음성자가면역뇌염(Seronegative autoimmune encephalitis)은 뇌염의 원인 항체가 검출되지 않는 자가면역뇌염임. 최근 자가면역뇌염의 임상적 범위가 확대되면서, 항체음성자가면역뇌염이 자가면역뇌염의 주요 아형으로 대두되고 있지만, 항체음성자가면역뇌염의 임상 특성, 치료법 및 예후가 아직 규명되지 않았음. 저자는 뇌염 환자에 대한 기관 코호트를 기반으로 항체음성자가면역뇌염 환자 2년 간의 장기 임상 경과를 분석하였으며, 2년 후 임상적 예후 예측 시스템을 개발하였음. 또한 주요 면역치료 요법의 효과, 6개월 또는 12개월 시점에서 불충분한 회복이 있는 환자에서 추가 면역 요법의 효과, 뇌 MRI 상 관찰되는 뇌의 위축과 장기적 예후와의 연관성 등을 규명하고자 하였음.
항체음성자가면역뇌염은 최근 제시된 분류 체계에 의해 항체음성유력자가면역뇌염(antibody-negative probable autoimmune encephalitis, ANPRA), 자가면역변연뇌염(autoimmune limbic encephalitis, LE) 및 급성파종뇌척수염(acute disseminated encephalomyelitis, ADEM)으로 분류하였음. 발병 후 2년 시점에서 불량한 임상결과는 발병 후 2년 시점의 수정 Rankin 척도[modified Rankin scale, mRS] 점수 3~6으로 정의되었으며, 임상 양상의 시계열 분석을 위해 자가면역뇌염 임상 평가 척도 (clinical assessment scales in autoimmune encephalitis, CASE) 점수체계를 활용하여 환자 별 2년 임상 경과 데이터베이스를 구축하였음. 총 147명의 환자를 최종 분석하였으며, 이 중 2년 시점에서 양호한 임상결과(mRS 0-2)를

달성한 빈도는 56.5%였음. 초기 질병 심각도는 세 가지 질병 유형 간 유사하였으나, ANPRA 유형은 가장 불량한 임상결과를 나타내음. 질병 초기에 평가 가능한 5가지 임상 요인인, 불응성뇌전증지속상태(refractory status epilepsitucs), 발병 연령 60세 이상, ANPRA 하위 유형, 뇌 MRI 상 천막하 부위 병변 침범, 발병 후 1개월 이상 면역치료 지연으로 구성된 RAPID 점수는 불량한 2년 임상결과를 예측할 수 있었음. 불량한 2년 임상결과의 위험이 낮은 환자(RAPID 점수 0-1점)에서는 고용량 스테로이드, 면역글로불린, 리툭시맙(rituximab), 토실리주맙(tocilizumab)등 면역 치료제제 각각이 모두 임상 경과 개선과 관련이 있었던 반면, 불량한 2년 임상결과의 위험이 높은 환자(RAPID 점수 2-5점)에서는 스테로이드, 면역 글로불린, 리툭시맙 및 토실리주맙을 모두 조합한 치료만이 임상 경과 개선에 효과가 있었음. 뇌염 병증이 지속되어 발병 후 6개월 시점에 mRS점수가 3점 이상인 환자에서 면역 요법을 지속하면 2년 시점 임상결과가 추가적으로 개선되나, 12개월 시점에 mRS점수가 3점 이상인 환자에서의 면역 요법을 지속은 효과가 불분명했음. 뇌 MRI 추적 검사에서 중등도 이상의 소뇌 위축의 발생은 나쁜 예후를 높은 확률로 예측하였으나, 미만성대뇌위축이나 내측두엽위축이 발생하지 않은 경우 임상적 회복 및 좋은 2년 임상결과 획득의 가능성을 시사하였음. 본 연구를 통해 항체음성자가면역뇌염의 임상적 특징과 경과, 면역요법의 효과와 기간, 예후인자 등을 종합적으로 새롭게 규명함.

주요어: 항체음성자가면역뇌염; 면역치료; 임상결과예측; 예후

Student Number: 2019-36969