Autoimmune encephalitis is a group of encephalitis syndromes that cause altered mentality, memory decline, or seizures in association with the presence of serum and cerebrospinal fluid (CSF) autoantibodies (auto-Abs). An early diagnosis enables early treatments. The detection of auto-Abs is a confirmatory diagnosis. Tissue-based assay, cell-based immunoassay, and immunoblotting are used to detect various autoantibodies. The CSF test for the presence of antibodies is important because it is more sensitive and reflects disease activity in many autoimmune encephalitis, although antibody tests can be negative even in the presence of autoimmune encephalitis. EEG is often abnormal, but nonspecific. A unilateral or bilateral medial temporal T2 high signal is a common finding in MRI. Fludeoxyglucose-positron emission tomography is sometimes useful for diagnosis in patients with normal MRI. (2016;6:45-50)

Key words: Autoimmune encephalitis, Autoantibody, Laboratory diagnosis, Imaging, EEG
Clues | Advantages | Disadvantages
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Syndrome-based criteria | • Enable early immunotherapy | • Incorrect
| • Useful for inclusion criteria of clinical studies | • Not helpful for differential diagnosis
| | • Can lead to over-immunotherapy | Response to immunotherapy
| • Usef ul in retrospective case analysis | • Many patients with AE do not respond to 1st-line immunotherapy
| | • Not useful for initial decision | Clinicians’ “gut feeling” based on clinical course, MRI, and CSF test
| • Case-specific approaches | • Requires experience
| | • Incorrect | Antibody test
| • Confirmatory | • Time and availability for antibody tests
| • Determines comorbidities, tumors, long-term treatment, and prognosis | • False positive or asymptomatic antibodies

AE, autoimmune encephalitis

*Table 1. The various diagnostic approaches used in autoimmune encephalitis*

**Work-up for systemic tumors**

Many cases of autoimmune encephalitis are associated with systemic tumors (Table 2). Therefore, suitable methods should be applied to detect these tumors (Table 3).

The expression of target antigens by the tumor tissue itself usually contributes to the genesis of auto-Ab, especially in many paraneoplastic autoimmune encephalitis series. The detection of systemic tumors is very important, not only because systemic tumors can be fatal in cases of delayed treatments, but also because the removal of the tumor can facilitate the recovery from autoimmune encephalitis. Sometimes, the size of the cancer or tumor is too small to allow its detection when the initial neurological symptoms and signs appear. In these cases, even though the initial screening for tumors is negative, repeated follow-up assessments are necessary.

**Detection of auto-Ab**

Although about half of all autoimmune encephalitis series are Ab-negative cases, the detection of auto-Ab is a confirmatory diagnostic test. Three basic research techniques are used for this purpose: tissue-based assay, cell-based assay, and immunoprecipitation. The tissue-based assay is a screening method. In this method, mouse-brain tissue sections, such as hippocampal and cerebellar sections, are stained with the patient’s serum or CSF using an indirect immunofluorescence technique. This enables the detection even of unknown Abs, as well as the established Ab (Fig. 2).

**Table 2. Common cancers associated with autoimmune antibodies**

**Table 3. Recommended tests for cancer screening in patients with autoimmune encephalitis**
In the diagnosis of specific autoimmune encephalitis, the cell-based immunoassay is used for the detection of cell-surface or synaptic Abs, and immunoblotting is used for the detection of intracellular paraneoplastic Abs. The cell-based immunoassay is a new technology of autoimmune diagnostics. Four main procedures are involved in this method: the insertion of DNA encoding the target antigens into a plasmid, transfection of this plasmid into vector cells, reaction of vector cells with the patient's serum or CSF, and detection of specific Abs via indirect immunofluorescence (Fig. 3).

For the detection of classical intracellular onconeuronal Abs, the immunoblotting technique is used. Immunoblotting is the method that uses Abs to detect a specific protein from a mixture of several unrelated proteins. The application of this technique in antibody diagnosis involves several steps, including the separation of proteins via electrophoresis, the transfer of the proteins onto a membrane, and the overlay of primary (patients' sample) and secondary Abs onto the membrane, followed by detection using enzymes or radioisotopes.

The diagnostic steps used in our hospital are summarized in Fig. 4. The screening test is performed using the tissue-based assay in all patients with suspected autoimmune encephalitis. For the detection of synaptic and intracellular Abs, the cell-based immunoassay and immunoblotting are used, respectively. In the case of the detection of an unknown Ab in the tissue-based assay (Fig. 5A and B), we perform confirmative tests staining cultured neuronal cells with the patients' sample (Fig. 5C and D). Further immunoprecipitation via liquid chromatography and mass spectrometry enables the identification of a novel specific antigen. The generation of overexpressing cell lines allows the creation of a new system for the diagnosis of the presence of novel Ab.

We created the Korea Autoimmune Synaptic and Paraneoplastic Encephalitis Registry (KASPER). From June 2012 to 2015, more than 2500 samples were collected from 72 hospitals nationwide (Fig. 6A). The positive rate for autoimmune encephalitis was 8.6%. We found more than 10 cases with possible novel antibodies. The most common synaptic autoimmune encephalitis was that associated with the anti-NMDAR Ab (68%), followed by anti-LGI1 Ab encephalitis (22%). Anti-amphiphysin, anti-Yo, and anti-Ma2/Ta encephalitis were common onconeuronal paraneoplastic encephalitis series (Fig. 6B). We observed two age peaks in the occurrence of autoimmune encephalitis (Fig. 7). Anti-NMDAR encephalitis had the highest peak in the third decade, and anti-LGI1 encephalitis had a peak at the seventh decade.

**CSF findings**

CSF findings were abnormal in most cases, albeit nonspecific. Lymphocytic pleocytosis and increased CSF protein was the commonest finding. An oligoclonal band was also found in a quarter of the patients.

Testing for the presence of the anti-NMDAR Ab in CSF is important. The sensitivity of anti-NMDAR Ab testing is higher in CSF compared with serum. Among the patients who were identified as having Ab in CSF, 85.6% had Abs in serum for the anti-NMDAR Ab, whereas 100% of the patients with a positive serum for this Ab also had positive CSF samples. The serum was negative for the anti-NMDAR Ab in 14% of the patients with a positive Ab in the CSF. This was explained by the frequent intrathecal synthesis of Abs in anti-NMDAR encephalitis. The proportion of CD19(+) B cells was reported to be greater than 10%, which is significantly higher than that observed in noninflammatory neurological disorders. The conversion from a high to a low titer and rapidly declining titer in the patients correlated with good outcome and lower relapse rates. A high auto-Ab titer in the CSF and serum was related with a poor outcome.

**Antibody-negative autoimmune encephalitis**

The Ab test can be negative even in the presence of autoimmune encephalitis. Some explanations are available for these negative Ab tests, even with the application of tissue-based analyses. These include antigen denaturation during tissue fixation, false-negative results caused by a small amount of Ab, differences between human and mouse epitopes, and the presence of T-cell-dominant autoimmune encephalitis. The existence of Ab-negative autoimmune encephalitis was supported by the finding that 44% of patients that are rituximab responders have autoimmune encephalitis without detectable Abs.

In contrast, the positive Ab test does not always imply the presence of pathogenic Ab. Some patients with small cell lung cancer and the anti-Hu Ab are asymptomatic. The presence of the anti-TPO Ab in Hashimoto’s thyroiditis is not relevant to the disease process. The Ab against the voltage-gated potassium channel can be present in Miller-Fisher syndrome and Bickerstaff encephalitis. The anti-NMDAR Ab can be present in multiple sclerosis and neuromyelitis optica. Some patients were identified as having demyelinating disorders in addition to anti-NMDAR encephalitis. In some cases, it
is difficult to determine whether these patients have two concurrent neurological conditions or there is a false-positive Ab test.

EEG

EEG was abnormal in 90% of cases of anti-NMDAR encephalitis.19 However, these findings were nonspecific. Nonconvulsive status epilepticus (NCSE) patterns, periodic lateralized epileptiform discharges, or nonspecific slowing were observed. In some cases, continuous EEG is necessary to detect a subclinical ictal rhythm or NCSE pattern. In anti-NMDAR encephalitis, it may be difficult to differentiate NCSE from continuous orofacial dyskinesia without EEG. Generalized rhythmic delta activity with superimposed fast activity (extreme delta brush) is present in some patients with anti-NMDAR encephalitis.31 The presence of an extreme delta brush was suggestive of more prolonged hospitalization. However, other EEG findings failed to differentiate autoimmune encephalitis with a different etiology.32

MRI

Unilateral or bilateral medial temporal T2 high signals with an extrahippocampal cortical or subcortical lesion are common findings in onconeural autoimmune encephalitis (Fig. 8A).33-35 These medial temporal high signals or swelling may be followed by progressive hippocampal atrophy, together with a chronic course.36

In anti-NMDAR encephalitis, MRI abnormalities were found only in 20%-50% of patients.13,37,38 MRI is frequently normal or mild even in patients with anti-NMDAR encephalitis who are in a comatose state. The lesion may involve various areas of the brain.19,39-41 In anti-VGKC encephalitis, MRI did not differentiate anti-LGI1 encephalitis from anti-CASPR2 Ab encephalitis.42-44 Unilateral or bilateral medial temporal T2 high signals were typically found in anti-LGI1 encephalitis in more than 70% of cases.45-47 In some cases, these changes were accompanied by basal ganglia signal changes. Hippocampal atrophy usually progressed along with disease progression in the absence of proper treatment.44,46,48 Unilateral or bilateral medial temporal T2 high signals were also found in the majority of anti-GABAB Ab encephalitis (Fig. 8B).49-51 On rare occasions, brainstem abnormalities were found in anti-GABAB Ab encephalitis with brainstem involvement.52,53

FDG-PET

FDG-PET scan is useful in the detection of abnormalities, even in patients with a normal MRI. It usually shows medial temporal hypometabolic changes in limbic encephalitis.54,55 FDG-PET abnormalities in anti-NMDAR encephalitis were found at various brain sites, such as the frontal, temporal, and occipital lobes, brainstem, and cerebellum.37,39,41,56 The findings ranged from focal hypermetabolism to diffuse hypometabolism.37,39,41,56-58 FDG-PET in anti-LGI1 encephalitis frequently showed basal ganglia and medial temporal hypermetabolism (Fig. 9).47,60

Conclusions

Autoimmune encephalitis is a group of epilepsy and encephalitis syndromes that are associated with serum and CSF auto-Abs. The detection of auto-Abs is a confirmatory diagnosis. A tissue-based assay is used as a screening method, and a cell-based immunoassay is applied for the detection of synaptic Abs, whereas immunoblotting is used for the detection of intracellular paraneoplastic Abs. The Ab tests using both CSF and serum are necessary and CSF sometimes reflects disease activity. The Ab test can be negative even in the presence of autoimmune encephalitis. Efforts aimed at detecting new auto-Abs are needed. EEG is nonspecific. A unilateral or bilateral medial temporal T2 high signal is a common finding in MRI. FDG-PET is sometimes useful as a diagnostic tool in patients with a normal MRI, and exhibit medial temporal hypo- or hypermetabolism and basal ganglia hypermetabolism in anti-LGI1 encephalitis.

References

1. Lancaster E. The diagnosis and treatment of autoimmune encephalitis. J Clin Neurol 2016;12:1-13.
2. Bernal F, Graus F, Pifarré A, Saiz A, Benyahia B, Ribalta T. Immunohistochemical analysis of anti-Hu-associated paraneoplastic encephalomyelitis. Acta Neuropathol 2002;103:509-15.
3. Lancaster E, Dalmau J. Neuronal autoantigens-pathogenesis, associated disorders and antibody testing. Nat Rev Neurol 2012;8:380-90.
4. Bien CG, Vincent A, Barnett MH, et al. Immunopathology of autoantibody-associated encephalitides: clues for pathogenesis. Brain 2012;135(Pt 5):1622-38.
5. Darnell RB, Posner JB. Paraneoplastic Syndromes. New York: Oxford, 2011.
6. Lancaster E, Martinez-Hernandez E, Dalmau J. Encephalitis and antibodies to synaptic and neuronal cell surface proteins. Neurology
1. Dalmau J, Rosenfeld MR. Autoimmune encephalitis update. Neuro Oncol 2014;16:771-8.
2. Wang R, Guan HZ, Ren HT, Wang W, Hong Z, Zhou D. CSF findings in patients with paraneoplastic encephalomyelitis/ sensory neuropathy. Neurology 1991;41:1757-64.
3. Dalmau J, Tüzün E, Wu HY, et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol 2007;61:25-36.
4. Hughes EG, Peng X, Gleichman AJ, et al. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. J Neurosci 2010;30:5866-75.
5. Tüzün E, Kürtüncü M, Lang B, et al. Prevalence of antineuronal antibodies in patients with encephalopathy of unknown etiology: Data from a nationwide registry from Korea. J Neuroimmunol 2016;293:34-8.
6. Hoffberger R. Neuroimmunology: an expanding frontier in autoimmunity. Front Immunol 2015;6:206.
7. Sunwoo JS, Lee ST, Byun JI, et al. Clinical manifestations of patients with CASPR2 antibodies. J Neuroimmunol 2015;281:17-22.
8. Byun JI, Lee ST, Jung KH, et al. Prevalence of antineuronal autoantibodies: current diagnostic challenges. Mult Scler Relat Disord 2014;3:303-20.
9. Magi B, Liberatori S. Immunoblotting techniques. Methods Mol Biol 2005;295:227-54.
10. Sunwoo JS, Lee ST, Byun JI, et al. Clinical manifestations of patients with CASPR2 antibodies. J Neuroimmunol 2015;281:17-22.
11. Dalmau J, Tüzün E, Wu HY, et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol 2007;61:25-36.
12. Hughes EG, Peng X, Gleichman AJ, et al. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. J Neurosci 2010;30:5866-75.
13. Tüzün E, Kürtüncü M, Lang B, et al. Prevalence of antineuronal antibodies in patients with encephalopathy of unknown etiology: Data from a nationwide registry from Korea. J Neuroimmunol 2016;293:34-8.
42. Vincent A, Buckley C, Scott JM, et al. Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. *Brain* 2004;127(Pt 3):701-12.

43. Ances BM, Vitaliani R, Taylor RA, et al. Treatment-responsive limbic encephalitis identified by neuropil antibodies: MRI and PET correlates. *Brain* 2005;128(Pt 8):1764-77.

44. Kotsenas AL, Watson RE, Pittok C, et al. MRI findings in autoimmune voltage-gated potassium channel complex encephalitis with seizures: one potential etiology for mesial temporal sclerosis. *AJNR Am J Neuroradiol* 2014;35:84-9.

45. Lai M, Huijbers MGM, Lancaster E, et al. Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. *Lancet Neurol* 2010;9:776-85.

46. Irani SR, Stagg CJ, Schott JM, et al. Faciobrachial dystonic seizures: the influence of immunotherapy on seizure control and prevention of cognitive impairment in a broadening phenotype. *Brain* 2013;136:3151-62.

47. Shin YW, Lee ST, Shin JW, et al. VGKC-complex/LGI1-antibody encephalitis: clinical manifestations and response to immunotherapy. *J Neuroimmunol* 2013;265:75-81.

48. Andrade DM, Tai P, Dalmau J, Wennberg R. Tonic seizure: A diagnostic clue of anti-LGI1 encephalitis? *Neurology* 2011;76:1355-7.

49. Lancaster E, Lai M, Peng X, et al. Antibodies to GABA(B) receptor in limbic encephalitis with seizures: case series and characterization of the antigen. *Lancet Neurol* 2010;9:67-76.

50. Boronat A, Gelfand JM, Gresa-Arribas N, et al. Encephalitis and antibodies to dipeptidyl-peptidase-like protein-6, a subunit of Kv4.2 potassium channels. *Ann Neurol* 2013;73:120-8.

51. Kim TI, Lee ST, Shin JW, et al. Clinical manifestations and outcomes of the treatment of patients with GABAB encephalitis. *J Neuroimmunol* 2014;270:45-50.

52. Jarius S, Steinmeyer F, Knobel A, et al. GABAB receptor antibodies in paraneoplastic cerebellar ataxia. *J Neuroimmunol* 2013;256:94-6.

53. Mundyranapurath S, Jarius S, Probst C, Stöcker W, Wildemann B, Bösel J. GABA-B-receptor antibodies in paraneoplastic brainstem encephalitis. *J Neuroimmunol* 2013;259:88-91.

54. Scheid R, Linke T, Voltz R, et al. Serial 18-fluoro-2-deoxy-d-glucose positron emission tomography and magnetic resonance imaging of paraneoplastic limbic encephalitis. *Arch Neurol* 2004;61:1785-9.

55. Basu S, Alavi A. Role of FDG-PET in the clinical management of paraneoplastic neurological syndrome: detection of the underlying malignancy and the brain PET-MRI correlates. *Mol Imaging Biol* 2008;10:131-7.

56. Leybold F, Buchert R, Kleiter I, et al. Fluorodeoxyglucose positron emission tomography in anti-N-methyl-d-aspartate receptor encephalitis: distinct pattern of disease. *J Neurol Neurosurg Psychiatry* 2012;83:681-6.

57. Fisher RE, Patel NR, Lai EC, Schulz PE. Two different 18F-FDG brain PET metabolic patterns in autoimmune limbic encephalitis. *Clin Nucl Med* 2012;37:e213-8.

58. Baumgartner A, Rauer S, Mader I, Meyer PT. Cerebral FDG-PET and MRI findings in autoimmune limbic encephalitis: correlation with autoantibody types. *J Neurol* 2013;260:2744-53.

59. Maqbool M, Oleske DA, Huq AH, Salman BA, Khodabakhsh K, Chugani HT. Novel FDG-PET findings in anti-NMDA receptor encephalitis: a case based report. *J Child Neurol* 2011;26:1325-8.

60. Irani SR, Michell AW, Lang B, et al. Faciobrachial dystonic seizures precede LGI1 antibody limbic encephalitis. *Ann Neurol* 2011;69:892-900.