Journal of the Neurological Sciences 390 (2018) 26–32

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Practical issues in measuring autoantibodies to neuronal cell-surface antigens in autoimmune neurological disorders: 190 cases

Juntaro Kaneko[a], Naomi Kanazawa[a], Naomi Tominaga[a], Atsushi Kaneko[a], Hiroki Suga[a], Ryo Usui[a], Daisuke Ishima[a], Eiji Kitamura[a], Tsugio Akutsu[a], Koji Yoshida[b], Kazutoshi Nishiyama[a], Takahiro Iizuka[a,*]

[a] Department of Neurology, Kitasato University School of Medicine, Sagamihara, Japan
[b] Department of Neurology, Hyogo Brain and Heart Center, Himeji, Japan

ARTICLE INFO

Keywords:
Autoimmune
Encephalitis
Autoantibodies
Epilepsy
Psychosis
Stiffness

ABSTRACT

Objectives: To address practical issues in measuring autoantibodies to neuronal cell-surface antigens (NSAs) in various autoimmune neurological disorders (ANDs).
Methods: We retrospectively reviewed the clinical information of 221 patients with clinically suspected ANDs who underwent antibody testing for NSAs between January 2007 and September 2017. 31 were excluded. In 190 patients, antibody-detection rate (ADR) and antibody-phenotype association were assessed.
Results: Fifty-four patients had NSA-antibodies: NMDA receptor (NMDAR) (n = 39), AMPA receptor (n = 3), leucine-rich glioma inactivated 1 (LGI1) (n = 3), glycine receptor (GlyR) (n = 3), GABA(A) receptor (n = 2), GABA(B) receptor (n = 1), metabotrophic glutamate receptor 5 (n = 1), or unknown (n = 6); 3 had multiple NSA-antibodies. ADR in patients with diagnostic criteria for "possible autoimmune encephalitis (AE)", "probable anti-NMDAR encephalitis", "definite autoimmune limbic encephalitis (ALE)", and "stiff-person spectrum disorder (SPSD)", was 34% (46/134), 85% (34/40), 46% (11/24), and 22% (4/18), respectively, but NSA-antibodies were not identified in 11 patients with systemic autoimmune disorders (SADs). Among 134 patients with "possible AE" criteria, NMDAR-antibodies were more frequently identified in patients with typical anti-NMDAR encephalitis than those without (34/40 [85%] vs. 4/94 [4%], $p < 0.0001$). LGI1-antibodies were identified in patients with ALE but not in the others (3/24 [13%] vs. 0/110 [0%], $p = 0.005$). GlyR-antibodies were identified in those with stiff-person syndrome plus (2/8, 25%) or stiff-limb syndrome (1/6, 17%).
Conclusions: NSA-antibodies were most frequently identified in "probable anti-NMDAR encephalitis", followed by "definite ALE", "possible AE", and "SPSD", but not identified in SADs. NMDAR, LGI1 and GlyR were associated with clinical phenotype. Cell-surface antigens should be determined based on individual phenotype.

1. Introduction

Acute encephalitis is a severe inflammatory disorder of the brain characterized by subacute onset of memory deficits, altered level of consciousness, psychiatric symptoms, and seizures [1]. It is often caused by infection [2] but various immune-mediated causes have been identified as a major cause of non-infectious encephalitis [2,3]. After the discovery of autoantibodies against neuronal cell-surface and synaptic antigens (NSAs) [4–12], the concept of "encephalitis" has changed. A syndrome-based diagnostic approach to autoimmune encephalitis (AE) [1] is proposed to initiate prompt immunotherapy, with diagnostic criteria for "possible AE", "autoantibody-negative but probable AE", "anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis", and "autoimmune limbic encephalitis (ALE)". Among those, the probable anti-NMDAR encephalitis criteria have recently been validated in both pediatric [13] and adult ages [14] with high sensitivity and specificity. However, isolated psychosis or epileptic seizure is known as a "forme fruste" of anti-NMDAR encephalitis [15].

In these criteria [1], "reasonable exclusion of alternative causes" is mandatory even in possible AE criteria; however, it is often difficult to exclude all potential alternative causes at early stage of the disease. Antibody detection rate (ADR) in these diagnostic criteria is unclear. Both rheumatologic and epileptic disorders are listed in the differential diagnosis of AE [1], but the causative relationship of systemic

* Corresponding author at: Department of Neurology, Kitasato University School of Medicine, 1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0374, Japan.
E-mail addresses: kanazawa@med.kitasato-u.ac.jp (N. Kanazawa), naotomi@med.kitasato-u.ac.jp (N. Tominaga), a.kaneko@kitasato-u.ac.jp (A. Kaneko), h.suga@kitasato-u.ac.jp (H. Suga), ryousui@med.kitasato-u.ac.jp (R. Usui), ishima@med.kitasato-u.ac.jp (D. Ishima), ekitamura@med.kitasato-u.ac.jp (E. Kitamura), takutsu@med.kitasato-u.ac.jp (T. Akutsu), kyoshida@hbhc.jp (K. Yoshida), nishiyk@med.kitasato-u.ac.jp (K. Nishiyama), takahiro@med.kitasato-u.ac.jp (T. Iizuka).

https://doi.org/10.1016/j.jns.2018.04.009
Received 1 February 2018; Received in revised form 5 April 2018; Accepted 5 April 2018
Available online 08 April 2018
0022-510X/

autoimmune disorders (SADs) to encephalitis remains unclear. Distinctive epileptic syndromes such as febrile infection-related epilepsy syndrome (FIRES) [16], acute encephalitis with refractory, repetitive partial seizures (AERRPS) [17], and new-onset refractory status epilepticus (NORSE) [18–20] are presumed to be of autoimmune origin [20,21]. It remains controversial whether these epileptic syndromes are included in possible AE. To address these issues in clinical practice, we evaluated NSA-antibodies in our cohort.

2. Methods

2.1. Subjects

We retrospectively reviewed the clinical information of 221 patients with clinically suspected autoimmune neurological disorders (ANDs) who underwent antibody testing for NSAs between January 1, 2007 and September 10, 2017. These patients were admitted to Kitasato University Hospital or other associated hospitals between January 1, 1999 and September 10, 2017. These antibodies were measured using the serum and/or cerebrospinal fluid (CSF) in patients after 2007, and using the archived samples obtained at the acute phase and kept in frozen in patients before 2007.

Thirty patients, who were subsequently diagnosed with non-autoimmune disorders, were excluded from the subjects (Supplementary data 1). The other one female patient with probable anti-NMDAR encephalitis criteria was also excluded because neither serum nor CSF obtained at the active phase was examined for NSA-antibodies (only serum obtained at autopsy 42 months after the onset of symptoms was examined and was negative). Thus, in 190 patients with ANDs (median 37 years [4–91 years]; 119 [63%] female) antibody testing results were evaluated (Fig. 1).

Information on symptoms, neurological signs, CSF, electroencephalogram (EEG), MRI, and treatment, were obtained from the authors or referring physicians. Written or oral informed consent was obtained from patients or their family. The studies were approved by Institutional Review Boards of Kitasato University (B17-144).

2.2. Antibody assays

NSA-antibodies were measured at the laboratory of Josep Dalmau (University of Barcelona); all samples were investigated with two independent assays: rat brain tissue immunohistochemistry and cell-based assays (CBAs) for NMDAR antibodies. The studies were done independently on what the brain tissue immunohistochemistry showed. If the brain tissue immunohistochemistry showed any reactivity, all known NSAs were tested in CBAs. If the brain tissue immunohistochemistry was positive and all CBAs were negative, then the samples were examined with cultured live neurons to determine whether they have antibodies against an unknown antigen. The NSAs tested with CBAs included the NMDAR, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), γ-aminobutyric acid-A receptor (GABAaR), γ-aminobutyric acid-B receptor (GABAbR), metabotrophic glutamate receptor 5 (mGluR5), contactin-associated protein-like 2 (CASPR2), dipeptidyl-peptidase-like protein 6 (DPPX), leucine-rich glioma inactivated-1 (LGI1), and neurexin-3α [4–12]. Both serum and CSF were examined in 187 patients, but only serum in 2 and CSF in 1. Antibodies to NSAs not characterized yet were denoted as “unknown NSA-antibodies”.

In patients with stiff-person spectrum disorder (SPSD) [22,23], autoantibodies to α1-subunit of the glycine receptor (GlyR) were additionally measured with CBAs [23]. In patients with anti-NMDAR encephalitis who had concurrent or past episodes of demyelinating disorder, autoantibodies to myelin oligodendrocyte glycoprotein (MOG) and aquaporin-4 (AQP4) were also measured with CBAs [24]. In patients with paraneoplastic neurological syndrome associated with malignancy, ALE, SPSD, or NORSE, autoantibodies against classical paraneoplastic intracellular antigens (CV2, Ma2/Ta, Ri, Yo, Hu, recoverin, SOX1, titin, Zic4, glutamic acid decarboxylase (GAD65), Tr (DNER), and amphiphysin) were also measured in serum with EUROLINE (Euroimmun AG) at Kitasato University.

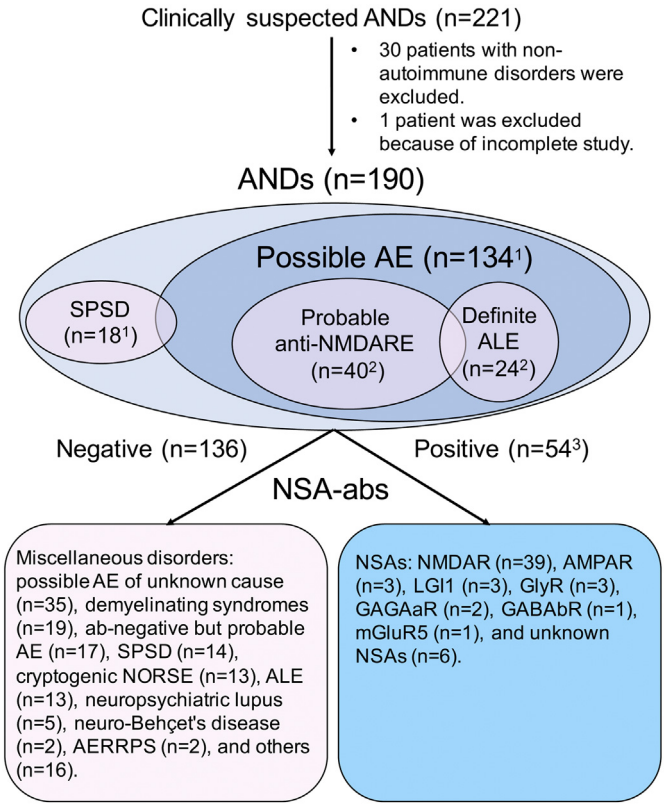

Fig. 1. Summary of 221 patients with clinically suspected ANDs.
This figure shows the summary of 221 patients with clinically suspected ANDs who underwent antibody testing for NSAs. After evaluation, 31 patients were excluded. In 190 patients, antibody detection rate was determined based on 4 major diagnostic criteria for “Possible AE”, “Probable anti-NMDARE”, “Definite ALE”, and “SPSD”. (see text).
[1]One patient with overlapping AE and SPSD is included in both groups of SPSD and possible AE. [2]Six patients are included in both groups of probable anti-NMDARE and definite ALE. [3]Three of 54 patients had multiple NSA-antibodies simultaneously (see Table 1).
ab(s): antibodies; AE: autoimmune encephalitis; AERRPS: acute encephalitis with refractory, repetitive partial seizures; ALE: autoimmune limbic encephalitis; AMPAR: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ANDs: autoimmune neurological disorders; GABAaR: γ-aminobutyric acid-A receptor; GABAbR: γ-aminobutyric acid-B receptor; GlyR: glycine receptor; LGI1: leucine-rich glioma inactivated-1; mGluR5: metabotrophic glutamate receptor 5; NMDAR: NMDA receptor; anti-NMDARE: anti-NMDAR encephalitis; NORSE: new-onset refractory status epilepticus; NSA: neuronal cell-surface antigens and synaptic proteins; SADs: systemic autoimmune disorders; SPSD: stiff-person spectrum disorder.

2.3. Inclusion criteria for patients with clinically suspected ANDs

To evaluate a potential involvement of NSA-antibodies in ANDs, we included all patients who were clinically suspected by the authors or referring physicians to have neurological disorders of autoimmune etiologies. These disorders included anti-NMDAR encephalitis, ALE or other type of AE, acute disseminated encephalomyelitis (ADEM), atypical multiple sclerosis (MS), tumefactive MS, neuromyelitis optica (NMO) or NMO spectrum disorder (NMOSD), myelitis of unknown cause, SPSD, Isaacs' syndrome, post-herpes simplex encephalitis (Post-HSE) [25], possible autoimmune isolated psychosis, Rasmussen encephalitis and other epileptic syndromes including AERRPS and