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Effectiveness of treatment for 31 patients with seropositive autoimmune autonomic ganglionopathy in Japan

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Abstract

Background: Autoimmune autonomic ganglionopathy (AAG) is characterized by serum autoantibodies against the ganglionic acetylcholine receptor (gAChR). Immunomodulatory treatments may alleviate AAG symptoms, but the most appropriate treatment strategy is unclear.

Objective: This study aimed to confirm the effectiveness of treatments, particularly immunotherapy, in patients with seropositive AAG in Japan, as well as to determine the most effective treatment and the best assessment method for clinical response to treatment.

Methods: We collected data from a previous cohort study of patients with seropositive AAG. The clinical autonomic and extra-autonomic symptoms were objectively counted and subjectively assessed using the modified Composite Autonomic Symptom Score. Post-treatment changes in the gAChR antibody level were evaluated.

Results: Thirty-one patients received immunotherapy. Among them, 19 patients received intravenous methylprednisolone; 27, intravenous immunoglobulin; 3, plasma exchange; 18, oral steroids; 2, tacrolimus; 1, cyclosporine; and 1, mycophenolate mofetil. Patients who received immunotherapy showed improvements in the total number of symptoms (from 6.2 ± 2.0 to 5.1 ± 2.0) and modified Composite Autonomic Symptom Score (from 37.4 ± 15.3 to 26.6 ± 12.8). Orthostatic intolerance, sicca, and gastrointestinal symptoms were ameliorated by immunotherapy. Immunotherapy decreased the antibody levels (gAChRα3 antibodies, from 2.2 ± 0.4 to 1.9 ± 0.4, $p = 0.08$; gAChRβ4 antibodies, from 1.6 ± 0.1 to 1.0 ± 0.2, $p = 0.002$), but antibody levels increased in 10 patients despite immunotherapy. The rate of improvement in the total number of symptoms was higher in patients with combined therapy than in patients with non-combined therapy (70.7% vs 28.6%).

Conclusions: The scores in many items on the rating scale decreased after immunotherapy in patients with seropositive AAG, particularly in the combined immunotherapy group. However, more accurate assessment scales for clinical symptoms and multicenter randomized, placebo-controlled prospective studies are warranted to establish future treatment strategies.

Keywords: autoantibody, autoimmune autonomic ganglionopathy, ganglionic acetylcholine receptor, immunotherapy, treatment

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Introduction

Autoimmune autonomic ganglionopathy (AAG) is a rare acquired immune-mediated neurological disease that results in various autonomic symptoms. AAG is induced by autoantibodies for the α3 subunits and β4 subunits of the ganglionic acetylcholine receptor (gAChR).[1] These autoantibodies can interfere with synaptic transmission in all peripheral autonomic ganglia in the autonomic nervous system[2] and are present in approximately half of patients with idiopathic autonomic neuropathy.[3,4]

AAG is a potentially treatable disease, and the efficacy of several types of immunotherapy,

including intravenous immunoglobulin (IVIg), intravenous methylprednisolone (IVMP), plasma exchange (PLEX), rituximab, oral prednisolone (PSL), and other immunosuppressants, has been reported.[2,5,6] In terms of therapeutic strategies, the efficacy of combined immunomodulatory therapy for AAG has been reported, including by our research group.[7,8] However, the sample sizes in these studies were small. Several studies found that symptoms may worsen in response to treatment after an initial improvement and suggested that longer treatment could prevent relapse and maintain improvement.[9–12] In view of the pathophysiological similarities with myasthenia gravis, the efficacy of acetylcholinesterase inhibitors, such as pyridostigmine, has also been investigated.[2] However, no evidence-based treatment recommendations for AAG are available, probably owing to its rarity, the often complicated autonomic function tests required, the difficulty of differentiating AAG from similar disorders, and the lack of widespread use of gAChR antibody testing. In recent years, the research environment for AAG has improved, with reports of quantitative multimodal autonomic biomarkers being used to guide therapeutic decision-making and to determine the response to treatment and establish novel techniques for detection of gAChR antibodies.[13] Therefore, an evidence-based treatment for AAG, not only in Japan but also internationally, is needed.

In this context, we focused on clinical symptoms as a parameter for evaluation of the effectiveness of immunotherapy in patients with AAG. The aim of this study was to assess the effectiveness of immunotherapy in patients with seropositive AAG according to changes in severity of clinical symptoms and gAChR antibody levels after treatment.

Methods

LIPS assay for anti-gAChR antibodies

Serum gAChRα3 and gAChRβ4 antibodies were detected using the luciferase immunoprecipitation system (LIPS) assay, which was developed by a National Institutes of Health group as an efficient quantitative approach to analyze antibodies against human autoantigens in serum samples from patients. We have previously established and reported on the use of the LIPS assay for AAG diagnosis on the basis of IgGs to both the α3 and β4 gAChR subunits in human serum samples.[4] We measured the gAChR antibody levels at the Nagasaki Kawatana Medical Center and Kumamoto University Hospital, as described elsewhere.[4,14] Autoantibody levels were expressed as an antibody index which was calculated as the ratio of the relative luminescence units of the sample to the relative luminescence units of the cut-off value. The normal antibody index value was established to be < 1.0 based on data from healthy individuals.

Study participants

Data of patients with AAG included in a Japanese multicenter cohort registry from January 2012 to August 2019 were retrospectively analyzed. Clinical diagnoses were made by neurologists at the respective hospitals. Questionnaires and consent forms were sent to referring neurologists, and the data were sorted and analyzed in Tokyo, Japan. The questionnaire included six categories: (1) age, sex, clinical diagnosis, age at onset of disease, antecedent infection, and mode of symptom onset; (2) autonomic manifestations; (3) extra-autonomic manifestations; (4) comorbid diseases (endocrine disorders, tumors, and autoimmune diseases); (5) autonomic testing; and (6) other laboratory findings.[4,14,15] With regard to the mode of symptom onset, acute onset and subacute onset were defined as autonomic symptoms reaching a peak within 3 months. Chronic onset was defined as autonomic symptoms reaching a peak after 3 months.[15]

One hundred and thirty-one of 204 patients with autonomic dysfunction who tested positive for gAChR autoantibodies but had only a single autoantibody evaluation were excluded. Of the 73 patients with seropositive AAG who were evaluated more than once, those with sufficient data for clinical profile, modified Composite Autonomic Symptom Score (mCOMPASS), and measured gAChR autoantibody levels both before and after treatment were extracted. Thirty-one patients were finally enrolled in the analysis (Figure 1).

Clinical assessment of autonomic and extra-autonomic manifestations

We determined autonomic dysfunction, which is an objective evaluation method based on bedside examinations, review of patient records, and interviews with patients' families. The

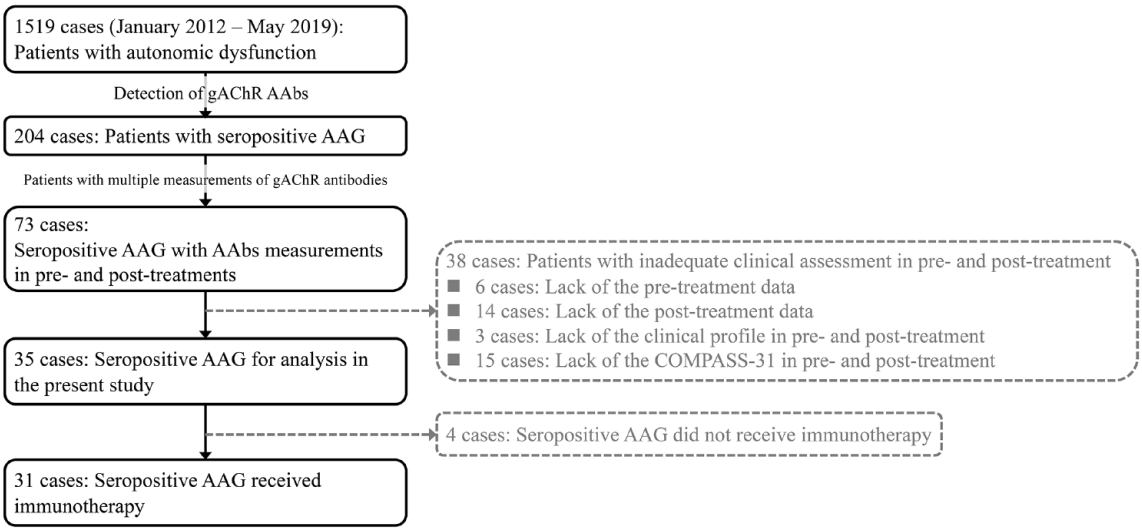

Figure 1. Study flow chart. Two hundred and four cases with seropositive autoimmune autonomic ganglionopathy were extracted from 1519 cases with autonomic dysfunction, and 31 patients with full data at two points were included in the analysis.

clinical profile includes the following autonomic manifestations: syncope or orthostatic hypotension (OH) and orthostatic intolerance (OI); pupillary dysfunction; sicca complex; coughing episodes; skin dryness or hypohidrosis/anhidrosis indicating heat intolerance; upper gastrointestinal (GI) problems; lower GI problems, including diarrhea or constipation; bladder dysfunction requiring catheterization for dysuria or urinary retention; and sexual dysfunction.[15] OH was defined as systolic blood pressure decline $\geqslant$ 30 mmHg within 10 min of rising from a supine position; this was assessed using the head-up tilt test provided that the patient's general health status allowed this assessment. Constipation was considered to be present if there were no stools for $>$ 3 days. Urinary symptoms were estimated by nocturnal or diurnal urinary frequency, urgency, urinary incontinence, voiding difficulty, and retention.

We also investigated the following extra-autonomic manifestations,[15,16] namely, four symptoms in the sensory disturbance category: paresthesia, dysesthesia, nerve conduction study abnormalities, and no symptoms. Sensory examinations included pinprick, temperature, light touch, vibration sense, and joint position sense. After excluding neurodegenerative diseases, patients with cerebral, cerebellar, brainstem, and/or spinal cord symptoms were considered to have central nervous system (CNS) involvement. We distinguished CNS involvement from other neurological disorders, such as multiple system atrophy, based on the results of clinical assessments, laboratory tests, and imaging studies.

In this study, with input from the attending physician, we objectively evaluated the presence or absence of 10 autonomic symptoms (OH, OI, pupillary dysfunction, sicca complex, coughing episodes, dryness, upper GI problems, lower GI dysfunction, bladder dysfunction, and sexual dysfunction) and five extra-autonomic manifestations (endocrine dysfunction, sensory disturbance, CNS involvement, tumors, and autoimmune disease) as the clinical profile, for a total possible score of 15, before and after immunotherapy.

Modified COMPASS

The Composite Autonomic Symptom Score (COMPASS) 31 is a subjective evaluation method that is derived from the responses to a self-administered questionnaire.[17] COMPASS 31 is a shortened version of the Composite Autonomic Symptom Score, which was designed to quantitatively assess autonomic symptoms. It has six subscale-weighted scores in the following domains: OI (four items; range, 0–40), vasomotor (three items; range, 0–5), secretomotor

(four items; range, 0–15), gastrointestinal (12 items; range, 0–25), bladder (three items; range, 0–10), and pupillomotor (five items; range, 0–5). We excluded questions related to the vasomotor and pupillomotor domains because judging skin color changes in Japanese patients is occasionally difficult on an individual basis, and wearing sunglasses or tinted glasses in Japan is not customary for middle-aged and older people (mCOMPASS; supplementary data 1).[18] Total pre-immunotherapy and post-immunotherapy scores were calculated by summation of the individual item scores, with a possible maximum score of 90.[14,15,19]

Immunotherapy

The majority of patients received helpful symptom-targeted treatments. Almost all of them received IVIg (gamma globulin 400 mg/kg/day for 5 days), IVMP (500–1000 mg/day for 3–5 days), and/or PLEX as first-line therapy because of their known effectiveness for autoimmune neurological diseases. If a patient did not respond to first-line treatment or showed only a transient improvement in autonomic symptoms, a second treatment was started and followed by a third if necessary. Courses of IVIg, IVMP, and/or PLEX were followed by PSL when they were not sufficient to achieve stable clinical improvement. Other immunosuppressant agents were subsequently administered if patients did not benefit from PSL alone. Oral administration of PSL and/or immunosuppressants was positioned as second-line therapy. Outcomes for the 31 seropositive AAG cases were obtained from each neurologist via questionnaires and follow-up e-mail if necessary. The 31 patients were divided into two groups according to whether they received combined first- and second-line immunotherapy (combined immunotherapy) or either first- or second-line immunotherapy (non-combined immunotherapy). The clinical profile and mCOMPASS results were obtained before and after immunotherapy. We also assessed the gAChR antibody levels in follow-up serum samples and compared these values with baseline levels.

We deemed immunotherapy to have been effective when a decrease of at least one point was observed in the clinical profile score after treatment and to have been ineffective when no change or an increase of at least one point was noted. The same procedure was performed for the mCOMPASS assessment before and after immunotherapy.

Statistical analysis

Differences between groups were assessed using the chi-square or Wilcoxon rank-sum test as appropriate. Wilcoxon signed-rank test was used to evaluate the relationship between symptoms and laboratory tests before and after treatment. All statistical analyses were performed using JMP 13 software (SAS Institute Inc., Cary, NC, USA). Statistical significance was set at $p < 0.05$.

Results

Clinical features of patients with seropositive AAG

We identified 31 patients with seropositive AAG (gAChRα3 antibody-positive, $n = 20$; gAChRβ4 antibody-positive, $n = 1$; and double antibody-positive cases, $n = 10$; Table 1).

The median age at onset was 52.6 years. Seventeen (54.8%) of the 31 patients were men. Seven patients (22.6%) reported an antecedent illness shortly before the onset of autonomic symptoms, 25.8% presented with other autoimmune diseases, and none had cancer. IVIg was used most often (27/31, 87.1%), followed by IVMP (19/31, 61.3%), whereas PLEX was used in only 9.6% of cases (3/31) as an acute treatment. Oral PSL was administered in 58.1% (18/31), and other immunosuppressive drugs were given in 12.9% (4/31), including tacrolimus in 2, cyclosporine in 1, and mycophenolate mofetil in 1. Combined immunotherapy was performed in approximately half of the cases (17/31, 54.8%).

Clinical profile before and after immunotherapy

With input from the attending physician, we objectively evaluated the differences in autonomic and extra-autonomic manifestations before and after immunotherapy based on the clinical profile. Following immunotherapy, the autonomic clinical profile score decreased from 5.1 ± 0.3 to 4.2 ± 0.3. Similarly, the total clinical profile score before immunotherapy decreased from 6.2 ± 0.4 to 5.1 ± 0.4 (Figure 2). The frequencies of improvement in autonomic symptoms pre- and post-immunotherapy were as follows: OH (77.4% and 64.5%, respectively), OI (87.1% and 64.5%, respectively), sicca (54.8% and 43.5%, respectively), and lower gastrointestinal dysfunctions (83.9% and 67.7%, respectively; Supplementary data 2-1). In terms

Table 1. Clinical features.

	Total AAG ($n=31$)	Seropositive for gAChRα3 antibodies ($n=20$)	Seropositive for gAChRβ4 antibodies ($n=1$)	Seropositive for gAChRα3 and gAChRβ4 antibodies ($n=10$)
Age at onset, years, average ± SE	52.6 ± 4.1	45.6 ± 5.2	31	68.8 ± 4.3
Male gender, *n* (%)	17 (54.8)	13 (65.0)	0 (0)	4 (40.0)
Acute or subacute onset, *n* (%)	10 (32.3)	6 (30.0)	0 (0)	4 (40.0)
Antecedent events, *n* (%)	7 (22.6)	5 (25.0)	0 (0)	2 (20.0)
Comorbid diseases, *n* (%)				
Tumors	0 (0)	0 (0)	0 (0)	0 (0)
Autoimmune diseases	8 (25.8)	3 (15.0)	1 (100)	4 (40.0)
Positive antibodies other than gAChR antibodies, *n* (%)	9 (29.0)	4 (20.0)	0 (0)	5 (50.0)
Time from onset to first measurement, average month ± SE	47.3 ± 11.7	59.2 ± 16.4	84	17.0 ± 8.2
Time from measurement to treatment, average month ± SE	3.4 ± 0.8	2.5 ± 0.9	8	3.8 ± 1.5
Time from first treatment to remeasurement, average month ± SE	3.4 ± 0.8	3.4 ± 1.1	0	4.0 ± 0.9
Treatment				
IVMP, *n* (%)	19 (61.3)	13 (65.0)	0 (0)	6 (60.0)
IVIg, *n* (%)	27 (87.1)	17 (85.0)	1 (100)	9 (90.0)
PLEX, *n* (%)	3 (9.7)	1 (5.0)	0 (0)	2 (20.0)
Oral steroid, *n* (%)	18 (58.1)	11 (55.0)	4.0 (0)	7 (70.0)
Other immunosuppressants, *n* (%)	4 (12.9)	2 (10.0)	0 (0)	2 (20.0)
Symptomatic treatment, *n* (%)	27 (87.1)	18 (90.0)	1 (100)	8 (80.0)
Combined immunotherapy, *n* (%)	17 (54.8)	11 (55.0)	0 (0)	6 (60.0)

AAG, autoimmune autonomic ganglionopathy; gAChR, ganglionic acetylcholine receptor; SE, standard error; IVMP, intravenous methylprednisolone; IVIg, intravenous immunoglobulin; PLEX, plasma exchange.

of extra-autonomic manifestations, endocrine symptoms improved (12.9% and 3.2%, respectively; Supplementary data 2-1).

mCOMPASS before and after immunotherapy

The mCOMPASS is a comprehensive questionnaire-based scale that is used to assess autonomic symptoms across multiple domains. We compared pre- and post-immunotherapy scores for each domain and the total mCOMPASS in all 31 patients, the scores for OI (from 20.3 ± 2.5 to 15.2 ± 1.8), secretomotor (from 4.8 ± 0.6 to 3.1 ± 0.5), gastrointestinal (from 7.7 ± 0.1 to 6.2 ± 0.6), and total scores (from 37.4 ± 2.8 to 26.6 ± 2.3) decreased following immunotherapy. Almost no change was noted in bladder function after immunotherapy (Figure 3).

The following mCOMPASS items showed improvement from pre- and post-treatment: OI (mCOMPASS 3, 6.7 ± 0.8, 5.7 ± 0.8, respectively;

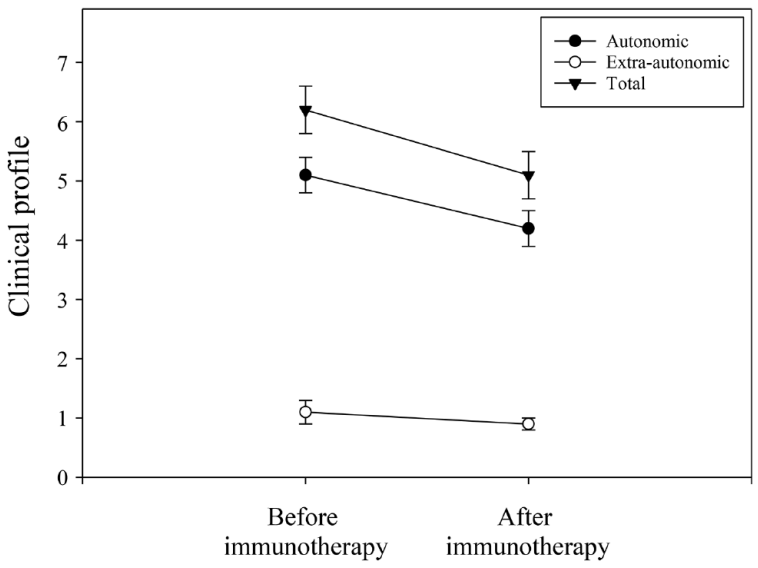

Figure 2. Clinical profile pre-immunotherapy and post- immunotherapy. The number of autonomic symptoms and symptoms in all patients decreased with immunotherapy. Extra-autonomic manifestations did not change after immunotherapy.

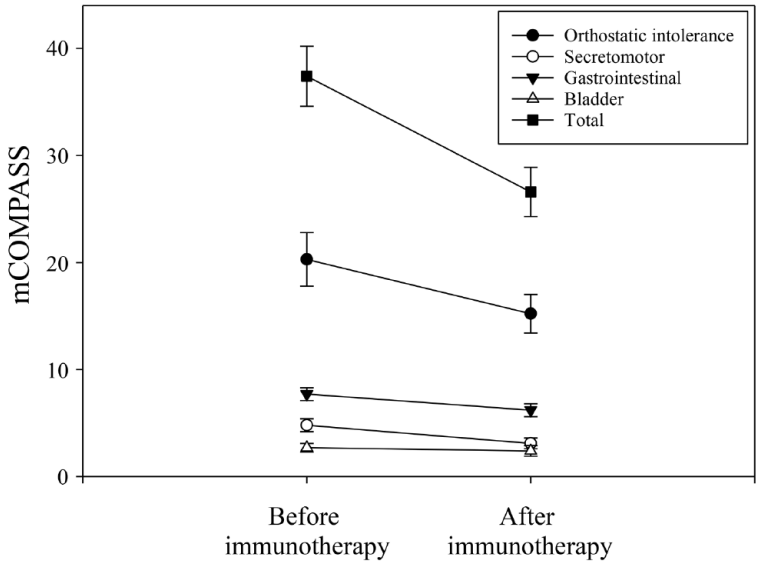

Figure 3. mCOMPASS obtained pre-immunotherapy and post-immunotherapy. The sections for orthostatic intolerance, secretomotor, gastrointestinal, and total scores were decreased by immunotherapy. Only bladder symptoms showed almost no change after immunotherapy. mCOMPASS, modified Composite Autonomic System Score.

mCOMPASS 4, 5.5 ± 0.8, 0.9 ± 0.4, respectively), dry mouth (mCOMPASS 7, 1.2 ± 0.2, 0.7 ± 0.2; mCOMPASS 8, 1.9 ± 0.3, 1.2 ± 0.2, respectively), vomiting (mCOMPASS 13, 0.4 ± 0.1, 0.2 ± 0.1, respectively), diarrhea (mCOMPASS 15, 0.6 ± 0.2, 0.3 ± 0.1, respectively; mCOMPASS 16, 0.5 ± 0.1, 0.1 ± 0.1, respectively), constipation (mCOMPASS 20, 0.9 ± 0.2, 0.5 ± 0.1, respectively), and urinary disturbance (mCOMPASS 22, 1.0 ± 0.2, 0.8 ± 0.2, respectively) (Supplementary Data 2-2).

gAChR antibody levels before and after immunotherapy

Figure 4 shows the changes in gAChR antibody levels after immunotherapy. The median levels of gAChRα3 antibodies showed a downward trend by immunotherapy (from 2.2 ± 0.4 to 1.9 ± 0.4, $p = 0.08$), and those of gAChRβ4 antibodies were significantly decreased (from 1.6 ± 0.1 to 1.0 ± 0.2, $p = 0.0002$). Increased levels were found in 10 (32.2%) of 31 patients with gAChRα3 antibodies and in one (3.2%) with gAChRβ4 antibodies.

Effectiveness of immunotherapy

Clinical features were observed in the immunotherapy effective group and immunotherapy ineffective group based on the respective clinical profile (Supplementary data 3-1) and mCOMPASS results (Supplementary data 3-2).

The immunotherapy effective group had more patients with combined therapy than patients with non-combined immunotherapy based on the clinical profile (70.6% vs 28.6%); however, between-group difference was not found based on the mCOMPASS results (76.5% vs 71.4%; Tables 2 and 3, respectively). Of the 20 patients with reduced antibody levels, 13 (65.0%) had received combined immunotherapy and seven (35.0%) had received non-combined immunotherapy. By contrast, four of 10 patients with increased antibody levels received combined immunotherapy and six received non-combined immunotherapy. The IVMP + IVIg + PSL combination was used most frequently (9/31, 29.0%), and seven patients (7/9, 77.8%) showed a therapeutic response to combined immunotherapy. The nine patients treated with the IVMP + IVIg + PSL combination showed no deterioration in the total clinical profile score, although no change was observed in two patients (cases 2 and 9; Table 4). In these two cases, the autonomic clinical profile remained unchanged or worsened. However, looking at the mCOMPASS, two cases of deterioration (patients 7 and

9; Table 4) were noted. According to the clinical profile assessment, patient 7 improved, whereas patient 9 remained unchanged. In the mCOMPASS study of each domain, bladder function was unchanged (patients 1, 3, 5, and 9; Table 4) or worsened (patients 2, 4, 6, and 7; Table 4) in eight of nine patients.

Discussion

Previous studies reported that immunotherapies for AAG, such as IVIg, IVMP, and PLEX, are effective and used as first-line treatment.[2,5,6] However, these treatments need to be repeated often to maintain remission in several patients with AAG.[9–11] As second-line treatment, administration of oral PSL or other immunosuppressants is required alone or in combination with first-line treatment because the patient needs continuous immunosuppression therapy to prevent relapse.[6–9] The present study identified two important issues in clinical practice. First, immunotherapy improved autonomic symptoms in patients with seropositive AAG, and combined immunotherapy may be more useful than monotherapy. Second, subjective and objective evaluation methods varied in their ability to judge immunotherapy effectiveness. Thus, establishing a standard for immunotherapy is necessary for patients with AAG.

Although AAG treatment has been discussed in small case studies or case reports, no relevant controlled treatment trials have been performed. In this study, we analyzed 31 patients with seropositive AAG who received immunotherapy in general and teaching hospitals throughout Japan. The antibody levels of these patients were measured before and after treatment. Analysis of the clinical profile and mCOMPASS results showed that immunotherapy improved symptoms in patients with seropositive AAG. The autonomic symptoms common to both clinical profile and mCOMPASS that improved after immunotherapy were OH/OI, sicca, and lower GI dysfunction. This finding was consistent with that in previous reports,[9,13,18,20] and these autonomic symptoms are likely to respond to immunotherapy. Furthermore, no significant change was found in bladder dysfunction after treatment. Koay *et al.*[13] reported that the bladder dysfunction item in mCOMPASS does not improve with immunotherapy. Extra-autonomic manifestations were assessed using clinical profile, but a therapeutic effect was shown for endocrine disorders. We have encountered cases in which

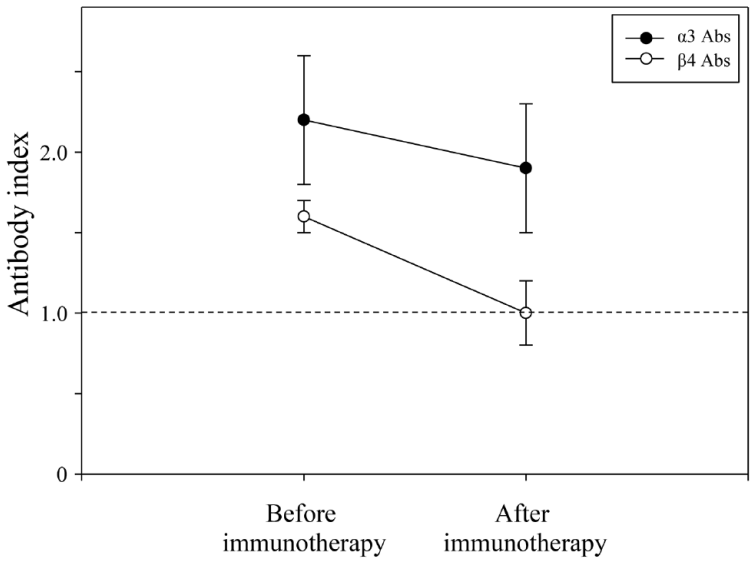

Figure 4. Pre-immunotherapy and post-immunotherapy ganglionic acetylcholine receptor antibody levels. gAChRα3 antibody levels had a downward trend and gAChRβ4 antibody levels significantly decreased with immunotherapy. gAChR, ganglionic acetylcholine receptor.

immunotherapy can improve endocrine disorders and autonomic symptoms in patients with seropositive AAG. This is consistent with the course of endocrine disorders associated with neuroimmune diseases.[21–23] Analysis of further extra-autonomic manifestations, particularly those without comorbid CNS involvement and sensory disturbance, is warranted to accumulate more seropositive AAG cases.

In this study, we found that immunotherapy, particularly combined therapy, may be effective in AAG treatment, which is consistent with previous reports indicating the effectiveness of combined immunotherapy.[7,8] Although the improvement in autonomic symptoms was accompanied by decreased gAChR antibody levels after immunotherapy in two-thirds of the cases, approximately one-third of the patients had unexpectedly elevated antibody levels in the LIPS assay. The autonomic symptoms improved in most of these patients; thus, the antibody levels would have possibly decreased if the LIPS assay was performed a little later. However, the increased gAChR antibody levels after immunotherapy are important to consider in AAG pathogenesis because this raises the possibility of true pathogenicity of gAChR autoantibodies and the presence of other additional pathogenic agents. A

Table 2. Comparison of treatment between effective and ineffective group using clinical profile.

Variable	Effective group (*n* = 16)	Ineffective group (*n* = 15)
Combined therapy, *n* (%)	**12 (70.6)**	**5 (29.4)**
IVMP + PSL	1 (5.9)	1 (5.9)
IVIg + PSL	3 (17.6)	0 (0)
IVIg + PSL + immunosuppressants	0 (0)	1 (5.9)
IVMP + IVIg + PSL	7 (41.2)	2 (11.8)
IVMP + IVIg + PSL + immunosuppressants	1 (5.9)	0 (0)
IVMP + IVIg + PLEX + PSL + immunosuppressants	0 (0)	1 (5.9)
Non-combined therapy, *n* (%)	**4 (28.6)**	**10 (71.4)**
IVMP	1 (7.1)	0 (0)
IVIg	2 (14.3)	5 (35.7)
IVMP + IVIg	0 (0)	3 (21.4)
IVMP + IVIg + PLEX	1 (7.1)	1 (7.1)
PSL + immunosuppressants	0 (0)	1 (7.1)

IVMP, intravenous methylprednisolone; PSL, prednisolone; IVIg, intravenous immunoglobulin; PLEX, plasma exchange.

Table 3. Comparison of treatment between effective and ineffective group using mCOMPASS.

Variable	Effective group (*n* = 23)	Ineffective group (*n* = 8)
Combined therapy, *n* (%)	**13 (76.5)**	**4 (23.5)**
IVMP + PSL	2 (11.8)	0 (0)
IVIg + PSL	2 (11.8)	1 (5.9)
IVIg + PSL + immunosuppressants	1 (5.9)	0 (0)
IVMP + IVIg + PSL	7 (41.2)	2 (11.8)
IVMP + IVIg + PSL + immunosuppressants	0 (0)	1 (5.9)
IVMP + IVIg + PLEX + PSL + immunosuppressants	1 (5.9)	0 (0)
Non-combined therapy, *n* (%)	**10 (71.4)**	**4 (28.6)**
IVMP	1 (7.1)	0 (0)
IVIg	7 (50.0)	0 (0)
IVMP + IVIg	1 (7.1)	2 (14.3)
IVMP + IVIg + PLEX	1 (7.1)	1 (7.1)
PSL + immunosuppressants	0 (0)	1 (7.1)

mCOMPASS, modified Composite Autonomic System Score; IVMP, intravenous methylprednisolone; PSL, prednisolone; IVIg, intravenous immunoglobulin; PLEX, plasma exchange.

Table 4. Changes in parameters before and after treatment in the combination of 'IVMP + IVIg + PSL' group.

Case	Age/sex	Clinical profile						mCOMPASS										Levels of gAChR antibodies			
		Autonomic		Extra-autonomic		Total		Orthostatic intolerance		Secretomotor		Gastrointestinal		Bladder		Total		α3		β4	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	75/F	4	2	1	1	5	3	36	28	6	2	5	6	2	2	50	39	1.57	1.33	1.65	0.78
2	28/M	7	7	0	0	7	7	36	16	6	0	8	7	3	6	54	29	1.30	1.27	0.44	0.34
3	73/M	5	2	1	0	6	2	28	20	6	0	6	7	4	4	45	32	2.87	4.92	1.80	1.79
4	54/M	5	3	2	2	7	5	36	28	4	2	7	5	7	8	54	43	1.06	0.99	0.42	0.25
5	56/M	8	2	3	3	11	5	28	24	6	4	12	4	3	3	49	35	1.34	0.81	0.25	0.28
6	75/F	6	3	0	0	6	3	0	28	11	0	7	8	1	3	47	39	13.4	3.37	2.00	1.01
7	83/F	5	1	2	1	7	2	0	0	4	6	10	7	4	10	19	24	1.74	0.44	1.05	0.30
8	13/M	5	1	1	0	6	1	24	0	0	0	8	2	2	0	34	2	1.59	0.56	1.59	0.16
9	9/M	5	6	1	0	6	6	0	24	2	0	0	6	0	0	2	30	3.02	0.55	3.02	0.64

The total score is calculated using weighting factors based on the points of each score,[17] so that the total scores are not always equal in integer display. IVMP, intravenous methylprednisolone; IVIg, intravenous immunoglobulin; PSL, prednisolone; mCOMPASS, modified Composite Autonomic System Score; gAChR, ganglionic acetylcholine receptor; F, female; M, male.

trend toward a higher proportion of patients with a decreased gAChR antibody level in the combined immunotherapy group and a higher proportion of patients with an increased gAChR antibody level in the non-combined immunotherapy group was observed. Nine of the patients treated with combined immunotherapy received a combination of IVMP + IVIg + PSL; analysis of the clinical profile data showed that none of these patients had worsening AAG, although some remained unchanged. By contrast, two cases of deterioration were observed in the analysis of the mCOMPASS data. The IVMP + IVIg + PSL combination had an effectiveness rate of approximately 80%, demonstrating its usefulness. The only autonomic symptom that did not respond to this combination was bladder dysfunction in the mCOMPASS. As previously mentioned, bladder dysfunction may be less responsive to immunotherapy, but the reason for this is unknown. Other similar reports are available, and the reasons for this need to be considered in the future.

Comparison of the clinical profile and mCOMPASS assessment methods according to immunotherapy effectiveness showed inconsistent scores with the actual items. This phenomenon was also observed when comparing the effectiveness of combined immunotherapy and non-combined immunotherapy. Analysis of the clinical profile data showed that patients with AAG who received combined immunotherapy had a higher rate of improvement than their counterparts who received non-combined immunotherapy. However, analysis of the mCOMPASS data did not show such a trend. This study aimed to analyze the effects of immunotherapy by focusing on clinical symptoms, and a discrepancy emerged between clinical profile scores and mCOMPASS results. The clinical profile is a mere count of the autonomic and extra-autonomic manifestations of each symptom, without any appropriate weighing factor. However, the mCOMPASS is a subjective scoring system whereby each autonomic nervous symptom domain has an appropriate weighing factor. Therefore, the mCOMPASS is probably more appropriate for assessing severity. Although several studies have used mCOMPASS to evaluate symptoms of seropositive AAG,[9,13,15,18,24] few have used it to assess treatment effects.[9,13,18] However, the mCOMPASS does not allow for assessment of extra-autonomic manifestations in AAG and is not suitable for use in infants and young children or in patients with cognitive impairment. The mCOMPASS assessment was poor in two (patients 7 and 9; Table 3) of the nine patients treated with the IVMP + IVIg + PSL combination. Patient 7 was an 83-year-old woman with CNS involvement that manifested as cognitive decline, and patient 9 was a 9-year-old boy who had OI episodes but scored zero in the OI domain in both their initial response to the mCOMPASS. These patients may not have been able to appropriately self-assess their symptoms, which is a limitation of this study. However, Koay *et al.*[13] succeeded in capturing the initial deficits and treatment response using quantitative multimodal biomarkers. In the future, autonomic function tests should be combined with objective and quantitative assessments in these patients.

This study has several limitations. First, it is retrospective in nature, using data from a cohort study that excluded cases with insufficient data. Since this was a retrospective study, the duration of immunotherapy and the timing of evaluation varied for each case, which may have affected the treatment efficacy. Similarly, the treatment effectiveness was not predefined for the retrospective design. While improvement in symptoms was assumed to vary greatly depending on the course, type, and severity of symptoms, as well as on the evaluator, the present study defined clear improvement as a decrease of one or more points in CP or mCOMPASS. Future prospective studies need to discuss the treatment protocol and its efficacy. Second, the sample size was small. Statistical analyses were not performed for many comparisons between groups due to the small number of cases included in the study, which may lead to a weak statistical power. Finally, important differences were found in the antibody detection methods used. Although radioimmunoprecipitation assays in the United States,[1–3] LIPS assays in Japan,[4,14] and flow cytometric assays in Australia[25,26] are commonly used to detect gAChR antibodies, the sensitivity and specificity of each method vary, and validation studies should be performed. In the future, a multicenter prospective randomized, placebo-controlled study within an international framework is warranted to overcome these limitations to standardize treatment of patients with AAG.

In conclusion, the findings in this study demonstrate the possibility of the effectiveness of immunotherapy in patients with seropositive AAG. Adequate combined immunotherapy may lead to improvement in the clinical symptoms of AAG.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Nippon Medical School Ethics Review Committee (approval number: B-2021-434). Written informed consent was obtained from all patients whose data were included in the study.

Consent for publication

Not applicable.

Author contribution(s)

Toshiyuki Hayashi: Conceptualization; Formal analysis; Methodology; Writing – original draft.

Shunya Nakane: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Visualization; Writing – original draft; Writing – review & editing.

Akihiro Mukaino: Data curation; Investigation; Methodology; Writing – review & editing.

Osamu Higuchi: Data curation; Investigation; Methodology; Project administration; Writing – review & editing.

Makoto Yamakawa: Data curation; Investigation; Methodology; Writing – review & editing.

Hidenori Matsuo: Formal analysis; Methodology; Project administration; Writing – review & editing.

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Supplemental material

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