

AUTOANTIBODIES TO GANGLIONIC ACETYLCHOLINE RECEPTORS
IN AUTOIMMUNE AUTONOMIC NEUROPATHIES

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ABSTRACT

Background Idiopathic autonomic neuropathy is a severe, subacute disorder with a presumed autoimmune basis. It is indistinguishable from the subacute autonomic neuropathy that may accompany lung cancer or other tumors. Autoantibodies specific for nicotinic acetylcholine receptors in the autonomic ganglia are potentially pathogenic and may serve as serologic markers of various forms of autoimmune autonomic neuropathy.

Methods We tested serum from 157 patients with a variety of types of dysautonomia. Immunoprecipitation assays with iodine-125-labeled epibatidine and solubilized human neuroblastoma acetylcholine receptors were used to detect autoantibodies that bound to or blocked ganglionic receptors.

Results Ganglionic-receptor-binding antibodies were found in 19 of 46 patients with idiopathic or paraneoplastic autonomic neuropathy (41 percent), in 6 of 67 patients with postural tachycardia syndrome, idiopathic gastrointestinal dysmotility, or diabetic autonomic neuropathy (9 percent), and in none of 44 patients with other autonomic disorders. High levels of the binding antibodies correlated with more severe autonomic dysfunction (including the presence of tonic pupils). Levels of these antibodies decreased in patients who had clinical improvement. All seven patients with ganglionic-receptor-blocking antibodies had ganglionic-receptor-binding antibodies and had idiopathic or paraneoplastic autonomic neuropathy.

Conclusions Seropositivity for antibodies that bind to or block ganglionic acetylcholine receptors identifies patients with various forms of autoimmune autonomic neuropathy and distinguishes these disorders from other types of dysautonomia. The positive correlation between high levels of ganglionic-receptor antibodies and the severity of autonomic dysfunction suggests that the antibodies have a pathogenic role in these types of neuropathy. (N Engl J Med 2000;343:847-55.)

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IN patients who have myasthenia gravis, antibodies that react with nicotinic acetylcholine receptors in muscle disrupt transmission at the neuromuscular junction.¹ Normally, nicotinic acetylcholine receptors also mediate fast synaptic transmission through autonomic ganglia in both the sympathetic and the parasympathetic nervous systems.^{2,3} As in myasthenia gravis, autoantibodies specific for neuronal nicotinic acetylcholine receptors in autonomic ganglia (ganglionic receptors) may disrupt cho-

linergic synaptic transmission and lead to autonomic failure. We developed an assay to detect antibodies that bind to ganglionic receptors. These antibodies are proving to be useful serologic markers of neurologic disorders that have an autoimmune basis, including paraneoplastic disorders of the central or peripheral nervous system and certain types of acquired autonomic neuropathy.⁴

Dysfunction of the autonomic nervous system is prominent in several neurologic disorders. Dysautonomia may occur in association with a degenerative disorder of the central nervous system, as in multiple-system atrophy (also known as the Shy-Drager syndrome),⁵ or may be due to dysfunction of the peripheral autonomic nervous system (comprising the autonomic nerves and ganglia). A slowly progressive disorder of the autonomic nerves, presumed to be degenerative, was first described by Bradbury and Eggleston⁶ and is now known as pure autonomic failure. Chronic acquired autonomic neuropathy may also occur in patients with amyloidosis or diabetes, and autonomic dysfunction is a feature of some inherited neuropathies.

In patients with idiopathic autonomic neuropathy, severe panautonomic failure develops in a subacute manner (over a period of days or weeks).^{7,8} The course of their disease is generally monophasic, with slow and incomplete recovery.⁸ Sympathetic failure is manifested as severe orthostatic hypotension and anhidrosis, and parasympathetic failure as dry mouth, sexual dysfunction, an impaired pupillary response to light and accommodation, and a fixed heart rate. Many patients have gastrointestinal dysmotility and present with anorexia, early satiety, abdominal pain and vomiting after eating, constipation, or diarrhea. Motor-nerve and sensory-nerve abnormalities are minimal or absent. An identical syndrome of subacute autonomic failure may occur as an indirect immunologic effect of cancer, especially small-cell carcinoma of the lung.⁹

The concept that idiopathic and paraneoplastic forms of autonomic neuropathy are immune-mediated (and thus may be described as forms of auto-

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immune autonomic neuropathy) is based in part on the associations of these syndromes with lung cancer,⁹ myasthenia gravis, and thymoma^{10,11} and on their clinical similarity to the acute autonomic disturbances that may accompany Guillain-Barré syndrome.¹² The onset of idiopathic autonomic neuropathy frequently follows the resolution of a viral illness. The level of protein in the spinal fluid may be high, and perivascular lymphocytic infiltrates may be seen in biopsy specimens of the sural nerve.¹² There have been individual case reports of benefit from intravenous immune globulin or plasma exchange.¹³⁻¹⁵ Our previous identification of ganglionic-receptor antibodies in five patients with idiopathic or paraneoplastic autonomic neuropathy supports the classification of these disorders as forms of autoimmune autonomic neuropathy.⁴

The clinical features of autoimmune autonomic neuropathy, with or without cancer, are usually distinct from those of degenerative autonomic disorders. For example, in patients with pure autonomic failure, the onset of orthostatic hypotension is usually insidious, and there is no prominent gastrointestinal dysmotility, dryness of the mouth, or pupillary dysfunction.¹² Nevertheless, early differentiation among these disorders is sometimes difficult. The accuracy of the diagnosis has important prognostic and therapeutic implications, because pure autonomic failure is a slowly progressive disorder, whereas idiopathic or paraneoplastic autonomic neuropathy is a life-threatening disorder that may respond to immunomodulatory therapy or to therapy for cancer. In some cases of idiopathic autonomic neuropathy, the condition improves spontaneously.

Our preliminary observations suggested that antibodies that bind to ganglionic receptors may be useful diagnostic markers of various forms of autoimmune autonomic neuropathy and that they may be pathogenic.⁴ In the current study, which involved a large number of patients with a variety of autonomic disorders, we investigated whether antibodies that bind to or block ganglionic receptors are markers that may help to distinguish autoimmune from degenerative forms of dysautonomia. We measured the serum levels of ganglionic-receptor-binding antibodies and assessed ganglionic-receptor-blocking activity in these patients, and we analyzed the serologic findings in relation to the results of quantitative tests of autonomic function.

METHODS

Patients

This study was approved by the institutional review board of the Mayo Clinic in Rochester, Minnesota, and did not require written informed consent. Serum samples from patients with autonomic dysfunction were submitted to the neuroimmunology laboratory of the clinic by the neurologists or gastroenterologists who were caring for these patients. Patients with a diagnosis of the Lambert-Eaton syndrome were excluded. To assign a clinical

diagnosis, two or more investigators (at least one of whom was unaware of the results of the antibody assays) reviewed the patients' histories, notes from their physical examinations, and the results of autonomic-function tests, gastrointestinal-motility studies, and other clinical tests. Forty-seven patients were excluded from the study for one of the following reasons: a lack of objective evidence of autonomic impairment at the time of serologic testing (the most common reason for exclusion), insufficient information to establish the type of dysautonomia, or a lack of consensus among the reviewers with respect to the diagnosis.

A total of 157 patients were included in the study and were classified as having one of the following diagnoses: idiopathic autonomic neuropathy, paraneoplastic autonomic neuropathy, postural tachycardia syndrome, idiopathic gastrointestinal dysmotility, diabetic autonomic neuropathy, pure autonomic failure, multiple-system atrophy, or nondiabetic sensorimotor and autonomic neuropathy. The last category included Guillain-Barré syndrome, chronic inflammatory demyelinating polyradiculoneuropathy, hereditary sensory and autonomic neuropathy, and idiopathic small-fiber peripheral neuropathy with objective autonomic involvement. Fifty-one of the 157 patients had participated in our preliminary study of ganglionic-receptor-binding antibodies⁴ (9 of whom had idiopathic autonomic neuropathy, 3 paraneoplastic autonomic neuropathy, and 39 other dysautonomias).

Autonomic-Function Studies

A validated set of autonomic-function tests was used to assess the distribution and severity of sudomotor, cardiac vagal, and adrenergic failure.¹⁶ The Quantitative Sudomotor-Axon Reflex Test¹⁷ was used to evaluate postganglionic sympathetic function. Recordings were made from one site on an arm and three sites on a leg. Cardiac vagal function was evaluated on the basis of the heart-rate response to deep breathing and the Valsalva maneuver. Adrenergic function was evaluated on the basis of beat-to-beat responses in blood pressure during head-up tilting and the Valsalva maneuver. The results were compared with control values derived from studies of 357 healthy subjects 10 to 83 years old.¹⁷

The Composite Autonomic Severity Scale combines the results of these three sets of autonomic-function tests; sudomotor function is assigned a score of 0 to 3 points, cardiac vagal function 0 to 3 points, and adrenergic function 0 to 4 points (higher scores indicate a greater deficit). The semiquantitative composite score for autonomic failure thus ranges from a total value of 0 (normal function) to 10 (maximal deficit); a score of 7 or more indicates severe autonomic failure.¹⁸ The score is adjusted for the effects of sex and age.

The thermoregulatory sweat test was used to measure the amount and pattern of sweat output over the entire anterior surface of the body. Sweating was detected by the change in color of alizarin red, an indicator powder that was applied to the skin. The patient was then subjected to a standardized thermal stimulus and photographed; results were expressed as the percentage of body-surface area that did not sweat (100 percent indicates complete anhidrosis).¹⁹

Gastrointestinal-motility studies were performed in patients with symptoms of gastrointestinal dysmotility. These studies included scintigraphic testing of gastric and intestinal transit and endoscopy with esophagogastroduodenal manometry to investigate the patterns of contractions before and after food intake.

Antibody Assays

To test serum for antibodies that bind to neuronal ganglionic receptors,⁴ antigen was solubilized from a human neuroblastoma cell line (IMR-32) and bound to epibatidine (final concentration, 2 nmol per liter),²⁰ a high-affinity agonist, which was radiolabeled with iodine-125. Five microliters of serum was added to each of two identical 40-fmol aliquots of the ¹²⁵I-epibatidine-ganglionic-receptor complex. After incubation for 16 hours at 4°C, human IgG and IgM were precipitated by the addition of goat antiserum, and radioactivity was then measured (in terms of counts per min-

ute) in washed pellets that were obtained by repeated centrifugation. Positive and negative control samples of human serum were included in each assay. Serum samples that yielded precipitates with more radioactivity than the mean value +1 SD obtained for four normal control samples run in the same assay were retested at least once and were diluted 10-fold if needed to attain a linear range of antigen binding. The antigen-binding capacity of each serum sample was expressed in terms of picomoles of ¹²⁵I-epibatidine-ganglionic-receptor complex precipitated per liter of serum. Studies to date have validated a value of 50 pmol per liter as the upper limit of normal for serum from healthy, nonimmunosuppressed subjects.

To detect antibodies that block the ganglionic receptor (i.e., that bind to or near to the agonist-binding site on the receptor), we modified the assay established for detecting antibodies that block the binding of α-bungarotoxin to skeletal-muscle acetylcholine receptors.²¹ Twenty-five microliters of serum was added to 80-fmol aliquots of solubilized ganglionic receptor, and a total volume of 175 μl was incubated for 16 hours at 4°C before the addition of ¹²⁵I-epibatidine (to a final concentration of 2 nmol per liter). After a 15-minute incubation at 22°C, the receptors were precipitated by sequential addition of a standard rat monoclonal antibody (Mab35, which binds to acetylcholine receptors)⁴ and goat antiserum that reacts with rat and human immunoglobulins. The mean radioactivity of precipitates obtained when acetylcholine receptor was incubated with normal human serum was defined as 100 percent binding of ligand (i.e., 0 percent blockade). Antibodies that interfere with the binding of ¹²⁵I-epibatidine to ganglionic receptors decrease the amount of radioactivity precipitated. Results were expressed as the percentage blockade of ligand binding; the normal value established in studies of healthy subjects is less than 25 percent blockade.

Paraneoplastic autoantibodies specific for neuronal nuclear and cytoplasmic antigens were detected with a standardized indirect immunofluorescence assay.²²

RESULTS

Frequency of Ganglionic-Receptor Antibodies in Patients with Dysautonomia

Ganglionic-receptor antibodies were not detected in 133 healthy control subjects or in 13 control patients who were referred because they had gastrointestinal symptoms and who had normal results on motility studies. The antibody assays had good reliability (coefficient of variation, 23.0 with samples stored and tested repeatedly for six months), and the results were consistent when successive serum samples from individual subjects were tested.

Ganglionic-receptor-binding antibodies were detected in the serum samples from 25 of the 157 patients with various autonomic disorders (Tables 1 and 2). Nineteen of these 25 patients (76 percent) had a clinical diagnosis of either idiopathic or paraneoplastic autonomic neuropathy. Of the five seropositive patients with paraneoplastic autonomic neuropathy, one had small-cell lung carcinoma, two had thymoma, one had bladder carcinoma, and one had rectal carcinoma. The 14 patients without evidence of cancer constituted 50 percent of the 28 patients classified as having idiopathic autonomic neuropathy.

A high serum level of ganglionic-receptor-binding antibody (Fig. 1) predicted the presence of idiopathic or paraneoplastic autonomic neuropathy (median value among seropositive patients; 370 pmol per liter)

TABLE 1. FREQUENCY OF GANGLIONIC-RECEPTOR-BINDING AND GANGLIONIC-RECEPTOR-BLOCKING ANTIBODIES IN PATIENTS WITH DYSAUTONOMIA.*

DIAGNOSTIC CATEGORY	NO. OF PATIENTS	GANGLIONIC-RECEPTOR-BINDING ANTIBODIES	GANGLIONIC-RECEPTOR-BLOCKING ANTIBODIES
Idiopathic autonomic neuropathy	28	14 (50)	4 (14)
Paraneoplastic autonomic neuropathy	18†	5 (28)	3 (17)
Postural tachycardia syndrome	15	1 (7)	0
Idiopathic gastrointestinal dysmotility	34	3 (9)	0
Diabetic autonomic neuropathy	18	2 (11)	0
Nondiabetic sensorimotor and autonomic neuropathy	10	0	0
Pure autonomic failure	24	0	0
Multiple-system atrophy	10	0	0
Total	157	25 (16)	7 (4)

*None of 133 healthy control subjects and none of 13 control patients with gastrointestinal symptoms and normal results on motility studies were seropositive for ganglionic-receptor-binding antibodies.

†Fifteen of these 18 patients (83 percent) had one or more antibody markers of neurologic autoimmunity. Twelve of the 18 patients had small-cell lung carcinoma (10 with the type 1 antineuronal nuclear autoantibody),²³ 4 had thymoma, 1 had bladder carcinoma, and 1 had rectal carcinoma.

as compared with other types of dysautonomia. Only six patients with another type of dysautonomia had detectable levels of ganglionic-receptor-binding antibodies (highest level, 106 pmol per liter): one was classified as having postural tachycardia syndrome, three as having idiopathic gastrointestinal dysmotility, and two as having diabetic autonomic neuropathy. It is noteworthy that ganglionic-receptor-binding antibodies were not found in any of the 34 patients with a degenerative form of dysautonomia (pure autonomic failure or multiple-system atrophy) or in any of the 10 patients with nondiabetic sensorimotor and autonomic neuropathy.

Ganglionic-receptor-blocking antibodies were not found in the absence of ganglionic-receptor-binding antibodies. The seven patients with ganglionic-receptor-blocking antibodies had values ranging from 31 to 75 percent receptor blockade (Table 2), and all seven had autoimmune autonomic neuropathy (four idiopathic and three paraneoplastic). Thus, the presence of ganglionic-receptor-blocking antibodies or high levels of ganglionic-receptor-binding antibodies was a specific predictor of idiopathic or paraneoplastic autonomic neuropathy.

Correlation of Levels of Ganglionic-Receptor-Binding Antibody with the Severity of Autonomic Dysfunction

Among the 19 patients with dysautonomia who were seropositive for ganglionic-receptor-binding an-

TABLE 2. CLINICAL CHARACTERISTICS OF THE 25 PATIENTS WITH DYSAUTONOMIA WHO WERE SEROPOSITIVE FOR GANGLIONIC-RECEPTOR-BINDING ANTIBODY.*

PATIENT NO.	AGE (YR)/SEX	KNOWN ANTECEDENT FACTORS	GANGLIONIC-RECEPTOR-BINDING ANTIBODIES†	GANGLIONIC-RECEPTOR-BLOCKING ANTIBODIES‡	PUPILLARY REFLEX TO LIGHT	GASTROINTESTINAL SYMPTOMS	SECRETOMOTOR SYMPTOMS
			pmol/liter	% blockade			
1	41/F	Thymoma††	17,180	0	Absent	Constipation	Dry eyes and mouth
2	39/F	Minor surgery	14,770	13	Absent	Early satiety, constipation	Dry eyes and mouth
3	75/F	None	9,700	0	Absent	Constipation	Dry eyes and mouth
4	68/M	None	6,730	50	Absent	None	Dry eyes and mouth
5	53/M	Gastroenteritis	4,400	0	Impaired	Early satiety, constipation	Dry mouth
6	52/M	None	3,910	33	Normal	Gastroparesis, constipation	Dry eyes and mouth
7	51/F	Rectal cancer	3,760	31	Normal	None	Dry mouth
8	55/F	Upper respiratory tract infection	2,250	57	Impaired	Early satiety, constipation	Dry mouth
9	66/F	Small-cell lung cancer††	1,540	45	Impaired	Constipation	Dry eyes and mouth
10	60/F	Laparotomy	370	16	Normal	Constipation	Dry mouth
11	66/F	None	271	8	Normal	Constipation	Dry mouth
12	64/M	Thymoma††	255	2	Absent	Intestinal pseudo-obstruction	None
13	40/M	None	239	0	Absent	None	Dry eyes, hypersalivation
14	58/F	Upper respiratory tract infection	171	7	Normal	Early satiety	None
15	63/M	Bladder cancer	94	75	Normal	Gastroparesis	None
16	67/F	None	91	7	Normal	Anorexia	None
17	65/M	None	85	0	Normal	None	None
18	66/F	None	60	67	Normal	Constipation	Dry eyes and mouth
19	73/F	None	54	15	Normal	None	Dry mouth
20	35/F	None	106	0	Normal	None	None
21	46/F	None	97	6	Normal	Gastroparesis, constipation	None
22	43/F	None	94	5	Normal	Gastroparesis	None
23	31/F	None	71	7	Normal	Slow transit, constipation	None
24	76/M	Diabetes	94	0	Normal	None	Dry mouth
25	50/F	Diabetes	90	1	ND	Gastroparesis	None

*ND denotes not done.

†The upper limit of normal is 50 pmol per liter.

‡The upper limit of normal is 25 percent blockade.

§The Composite Autonomic Severity Scale¹⁸ combines the results of tests of sudomotor function (0 to 3 points), cardiac vagal function (0 to 3 points), and adrenergic function (0 to 4 points), with adjustment for sex and age. A score of 0 indicates normal function, and a score of 10 maximal autonomic deficit. Dashes indicate that there were insufficient data to calculate the composite score.

¶The change in systolic blood pressure during a five-minute head-up tilt was assessed. A decrease of 30 mm Hg was considered to be evidence of orthostatic hypotension. If tilt-table testing was not performed, orthostatic symptoms are listed (in parentheses).

||Cardiac vagal function was assessed by evaluating heart-rate responses to deep breathing, the Valsalva maneuver, and head-up tilting.

**The amount of sweat output was determined by the thermoregulatory sweat test¹⁹; 100 percent indicates complete anhidrosis.

††The tumor was diagnosed during the evaluation of dysautonomia.

‡‡Patient 20 had postural tachycardia.

TABLE 2. CONTINUED.

GENITOURINARY SYMPTOMS	SCORE ON COMPOSITE AUTONOMIC SEVERITY SCALES	ORTHOSTATIC CHANGE IN SYSTOLIC BLOOD PRESSURE¶	CARDIAC VAGAL FUNCTION	ANHIDROSIS**
		mm Hg		% of body surface
None	—	ND (syncope)	ND	ND
Urinary retention	9	-36	Absent	58
Urinary retention, dyspareunia	10	-96	Absent	62
Erectile dysfunction	6	-136	Normal	97
Erectile dysfunction	7	-34	Impaired	7
Urinary retention, erectile dysfunction	9	-70	Impaired	ND
None	10	-83	Impaired	ND
None	10	-114	Impaired	40
None	10	-96	Absent	84
None	10	-76	Impaired	48
None	8	-142	Impaired	58
None	—	ND (light-headedness)	ND	ND
Urinary retention	4	0	Normal	100
None	7	-70	Impaired	61
None	3	-16	Impaired	97
None	7	-64	Impaired	ND
Erectile dysfunction	8	-30	Impaired	7
Urinary retention	7	-52	Impaired	40
None	—	0	ND	50
None	0	+10‡‡	Normal	40
None	2	-4	Normal	12
None	—	ND	ND	ND
None	—	(no symptoms)	ND	ND
None	—	(no symptoms)	ND	ND
None	2	+2	Normal	34
None	—	ND	ND	ND
None	—	(no symptoms)	ND	ND

tibody and who underwent detailed quantitative autonomic testing, high levels of antibodies were significantly correlated with more severe autonomic failure, as assessed by the score on the Composite Autonomic Severity Scale (Fig. 2). Tonic pupils (an impaired pupillary response to light and to accommodation) were observed in 11 patients (8 with idiopathic and 3 with paraneoplastic autonomic neuropathy), 9 of whom had ganglionic-receptor-binding antibodies.

Six of the 25 patients with dysautonomia who were seropositive for ganglionic-receptor-binding antibody had follow-up antibody tests and autonomic-function tests five months to six years and four months after their initial evaluation (Table 3 and Fig. 3). In three

of these six patients, symptoms improved, whereas three patients continued to have severe autonomic failure. In all six patients, the change or lack of change in the values for ganglionic-receptor-binding and ganglionic-receptor-blocking antibodies correlated with the change or lack of change in autonomic function. Three patients had persistent autonomic failure and stable or increased levels of ganglionic-receptor-binding antibodies. By contrast, there was a reduction in the levels of ganglionic-receptor-binding antibodies in the three patients who had an improvement in autonomic function, and two of them had no detectable ganglionic-receptor-blocking antibodies after clinical recovery. In two of the three patients with clinical recovery, the autonomic symptoms improved spontaneously, without specific treatment; in the third, who had paraneoplastic autonomic neuropathy, the symptoms improved dramatically after radiation and chemotherapy for small-cell lung carcinoma.

Frequency of Paraneoplastic Neuronal Nuclear and Cytoplasmic Antibodies in Patients with Dysautonomia

Five of the 18 patients with paraneoplastic autonomic neuropathy were seropositive for ganglionic-receptor-binding antibody. Twelve of the 18 patients had small-cell lung carcinoma, and 10 of these 12, and no others, had type 1 antineuronal nuclear autoantibody (also called anti-Hu). Type 1 antineuronal nuclear autoantibody has been reported to be a marker of paraneoplastic autonomic neuropathy and gastrointestinal dysmotility related to small-cell lung carcinoma.²³⁻²⁵

DISCUSSION

The results of this study justify the classification of idiopathic and paraneoplastic forms of autonomic neuropathy as autoimmune autonomic neuropathy. Ganglionic-receptor autoantibodies with specific binding or blocking actions were highly associated with both of these diagnoses, and positive results on serologic tests distinguished these disorders from degenerative forms of dysautonomia. High levels of ganglionic-receptor-binding antibody correlated significantly with the severity of autonomic dysfunction, and the level of such antibodies decreased or became undetectable with improvement of autonomic function. These findings suggest that the autoantibodies contribute to the pathogenesis of subacute autonomic failure. Although seropositivity for the ganglionic-receptor-binding or ganglionic-receptor-blocking antibodies unambiguously identified patients with autoimmune autonomic neuropathy, a negative result did not rule out this diagnosis.

Clinical features that are characteristic of autoimmune autonomic neuropathy are a subacute onset, prominent symptoms of gastrointestinal dysmotility, and an abnormal pupillary response to light and to accommodation. An impaired pupillary response in pa-

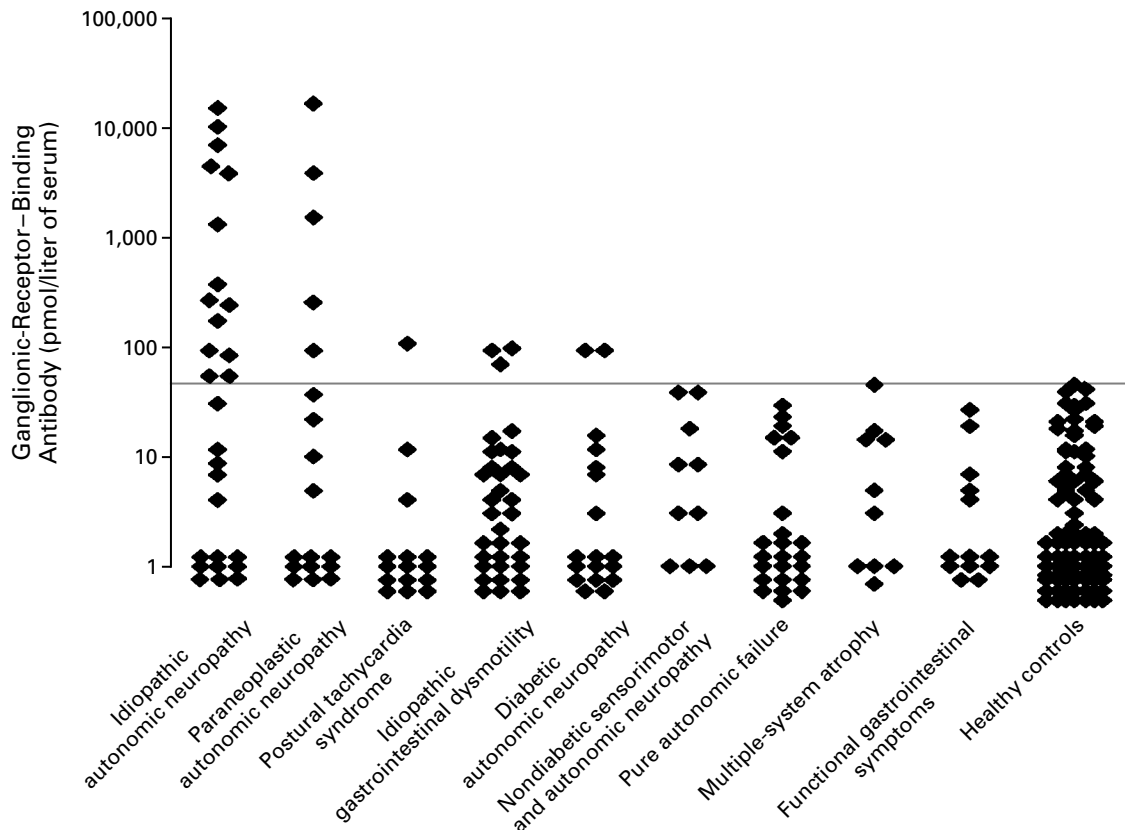


Figure 1. Serum Concentrations of Ganglionic-Receptor–Binding Antibody in Patients with Acquired Dysautonomia and in Control Subjects.

Values for healthy control subjects and control patients with functional gastrointestinal symptoms were less than or equal to 46 pmol per liter, a value consistent with the previously established upper limit of normal for nonimmunosuppressed subjects (50 pmol per liter [horizontal line]).⁴ Values are plotted for individual patients in each diagnostic category on a logarithmic scale. Seropositivity was identified in patients in five of the diagnostic categories. Ganglionic-receptor–binding antibodies were most prevalent and were present at the highest levels in patients with idiopathic or paraneoplastic autonomic neuropathy. Antibody values were slightly greater than normal in a minority of patients with postural tachycardia syndrome, idiopathic gastrointestinal dysmotility, or diabetic autonomic neuropathy. Patients with nondiabetic sensorimotor and autonomic neuropathy, pure autonomic failure, or multiple-system atrophy (the Shy–Drager syndrome) were seronegative. Points clustered at 1 pmol per liter of serum represent patients with no detectable antibody.

tients with severe parasympathetic dysfunction was the clinical feature that most reliably predicted seropositivity for the ganglionic-receptor–binding antibody. This observation is consistent with the striking mydriasis reported in mutant mice lacking the $\alpha 3$ subunit of the ganglionic acetylcholine receptor.²⁶

Because the various forms of acquired dysautonomia may be difficult to distinguish on the basis of the clinical history and clinical examination alone, ancillary testing remains important for establishing a diagnosis of autoimmune autonomic neuropathy. For example, a case of isolated gastrointestinal dysmotility would be difficult to distinguish from autonomic neuropathy without the results of specific laboratory tests

of cardiovascular and sudomotor autonomic functions. Serologic testing for ganglionic-receptor–binding antibodies early in the clinical evaluation of patients with autonomic disorders should improve diagnostic accuracy and may help identify patients who are likely to benefit from immunomodulatory therapy. The clinical characteristics of the seropositive patients with autonomic neuropathy in our study were indistinguishable from those of patients with autonomic neuropathy whose symptoms were reported to have improved in response to intravenous immune globulin therapy,^{13,14} probably as a result of accelerated catabolism of pathogenic IgG.²⁷

Six of the patients in this study who were seropos-

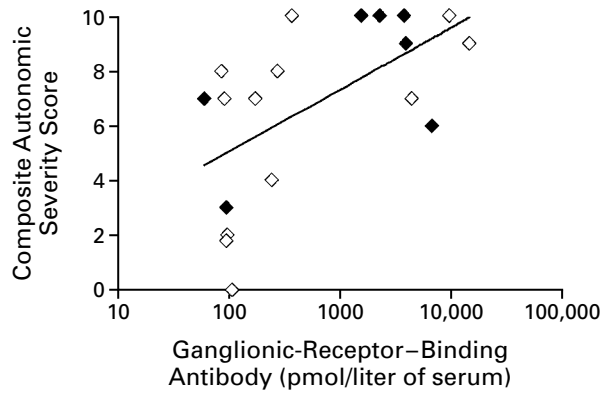


Figure 2. Correlation of Serum Levels of Ganglionic-Receptor-Binding Antibody and the Severity of Autonomic Dysfunction in 19 Patients with Dysautonomia and Ganglionic-Receptor-Binding Antibodies.

Antibody values for individual patients are plotted on a logarithmic scale. According to linear regression analysis, higher levels of antibody were significantly associated with greater severity of autonomic dysfunction ($r=0.59$, $P=0.007$). Of these 19 patients, 7 also had ganglionic-receptor-blocking antibodies (solid symbols) and 12 did not (open symbols).

itive for the ganglionic-receptor-binding antibody did not have a clinical diagnosis of idiopathic or paraneoplastic autonomic neuropathy. Of these six patients, three had a diagnosis of idiopathic gastrointestinal dysmotility, two had diabetic autonomic neuropathy, and one had postural tachycardia syndrome. Their serum levels of ganglionic-receptor-binding antibodies were lower than those in most of the other seropositive patients. Formal testing of cardiovascular and sudomotor autonomic function was not performed in three of them (two with idiopathic gastrointestinal dysmotility and one with diabetic autonomic neuropathy). Thus, signs of a more diffuse but mild dysautonomia may not have been recognized because of the prominent gastrointestinal symptoms and signs.²⁸ The fact that these patients were seropositive for the ganglionic-receptor-binding antibody supports the concept that in some cases, isolated gastrointestinal dysmotility, postural tachycardia syndrome, and autonomic neuropathy in diabetic patients represents variant presentations of autoimmune autonomic neuropathy.²⁸⁻³⁰ In our study, there were no overt clinical differences between seropositive patients and seronegative patients with these diagnoses.

TABLE 3. SEROLOGIC AND AUTONOMIC FINDINGS AT THE INITIAL EVALUATION AND AT FOLLOW-UP IN SIX PATIENTS WITH AUTOIMMUNE AUTONOMIC NEUROPATHY.

PATIENT NO.	MONTH AND YEAR	CLINICAL AUTONOMIC STATUS	GANGLIONIC-RECEPTOR-BINDING ANTIBODIES	GANGLIONIC-RECEPTOR-BLOCKING ANTIBODIES	SCORE ON COMPOSITE AUTONOMIC SEVERITY SCALE†	GASTROINTESTINAL SYMPTOMS	ANHIDROSIS‡
			pmol/liter*	% blockade†			
9	6/96	Improved	1540	45	10	Constipation	84
	7/99		236	0	2	None	3
10	1/93	Not improved	370	16	10	Constipation	48
	4/99		447	0	8	Constipation	89
13	1/99	Worse	239	0	—	None	100
	7/99		251	0	4	None	100
15	11/97	Improved	94	75	3	Gastroparesis	97
	8/98		66	0	0	None	4
17	4/99	Not improved	85	0	8	None	7
	10/99		127	0	8	None	4
19	12/98	Improved	54	15	—	None	50
	4/99		0	0	—	None	5

*The upper limit of normal is 50 pmol per liter.

†The upper limit of normal is 25 percent blockade.

‡The Composite Autonomic Severity Scale¹⁸ combines the results of tests of sudomotor function (0 to 3 points), cardiac vagal function (0 to 3 points), and adrenergic function (0 to 4 points). A score of 0 indicates normal function, and a score of 10 maximal autonomic deficit. Dashes indicate that there were insufficient data to calculate the composite score.

§The amount of sweat output was determined by the thermoregulatory sweat test¹⁹; 100 percent indicates complete anhidrosis.

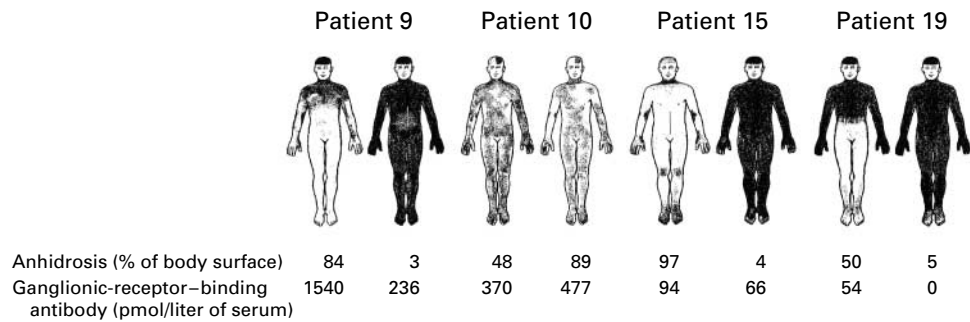


Figure 3. Recovery of Sweating Function in Patients with Subacute Autonomic Neuropathy.

The results of thermoregulatory sweat tests at the first evaluation (each left-hand figure) and at the follow-up evaluation (each right-hand figure) are shown. Shading represents areas of sweating; the face was not tested. Patients 9, 15, and 19 had clinical autonomic recovery; in these patients, recovery of sweating correlated with a decrease in the level of ganglionic-receptor-binding antibody and with the disappearance of ganglionic-receptor-binding antibody, if present initially. Patient 10 had persistent anhidrosis and an increased level of ganglionic-receptor-binding antibody at follow-up.

The sensitivity of the assay for ganglionic-receptor-binding antibody in patients with idiopathic autonomic neuropathy (50 percent of whom were seropositive) is lower than the seropositivity rates of more than 90 percent for assays of the muscle-receptor-binding antibody or modulating antibody in adult patients with generalized myasthenia gravis³¹ and for the assay of the P/Q-type calcium-channel-binding antibody in patients with the Lambert-Eaton syndrome.³² However, unlike autoimmune disorders of the neuromuscular junction, autoimmune autonomic neuropathy is often a monophasic illness. For many of the patients in this study, serologic testing was not performed until several months after the onset of symptoms. Thus, transient seropositivity during the acute phase of the illness may have been missed. Another possibility is that the neuronal antigens that are the targets of pathogenic autoantibodies may differ among individual patients with autonomic neuropathy. This appears to be the case for patients with autoimmune disorders of neuromuscular hyperexcitability, in whom both voltage-gated potassium channels and presynaptic neuronal nicotinic acetylcholine receptors have been implicated as autoantigens.^{33,34} It is also possible that nonimmune mechanisms account for some of the seronegative findings in patients with subacute autonomic failure.

In addition to suggesting a useful serologic marker for establishing a diagnosis of autoimmune autonomic neuropathy, our data indicate that ganglionic-receptor antibodies may be effectors of autonomic dysfunction in patients with idiopathic or paraneoplastic autonomic neuropathy. First, we documented a significant correlation between the serum level of ganglionic-receptor-binding antibody and the severity of autonomic dysfunction. Second, decreases in the level of this antibody were accompanied by improvement in clinical

and laboratory measures of autonomic function. Third, ganglionic-receptor antibodies with blocking specificity, which have the greatest potential to impair ganglionic synaptic transmission *in vivo*, disappeared from the serum in parallel with clinical improvement. Similarly, in patients with myasthenia gravis, muscle-receptor-blocking antibodies are less prevalent than muscle-receptor-binding antibodies,³⁵ and the levels of both antibodies decrease with clinical improvement.³⁶ Currently, we have only indirect evidence that ganglionic-receptor antibodies disrupt autonomic function. Definitive proof will require demonstration that an IgG antibody that is specific for the ganglionic receptor interferes with synaptic transmission when administered to laboratory animals or applied to autonomic ganglia *in vitro*.

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