Idiopathic Autonomic Neuropathy

Comparison of Cases Seropositive and Seronegative for Ganglionic Acetylcholine Receptor Antibody

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Background: The clinical characteristics of autoimmune autonomic neuropathy are only partially defined. More than 50% of patients with high levels of ganglionic acetylcholine receptor (AChR) autoantibodies have a combination of sicca complex (marked dry eyes and dry mouth), abnormal pupillary light response, upper gastrointestinal tract symptoms, and neurogenic bladder.

Objective: To compare patients with idiopathic autonomic neuropathy who were seropositive (n=19) and seronegative (n=87) for ganglionic AChR antibodies.

Design: Retrospective review of autonomic programmatic database.

Setting: Autonomic Disorders Program Project at Mayo Clinic College of Medicine, Rochester, Minn.

Patients: We evaluated a cohort of 87 patients with idiopathic autonomic neuropathy who had undergone full autonomic testing and neurological evaluation and who had a complete panel of paraneoplastic and ganglionic AChR antibodies. We compared patients seropositive (n=19) and seronegative (n=87) for ganglionic AChR antibodies.

Results: The seropositive group had a significant overrepresentation of abnormal pupillary responses (12/18 [67%] vs 12/87 [14%]; P<.001), sicca complex (9/15 [60%] vs 11/47 [23%]; P=.01), and lower gastrointestinal tract dysautonomia (16/19 [84%] vs 48/85 [56%]; P=.02). A subacute mode of onset was more common in the seropositive group (12/19 [63%] vs 23/84 [27%]; P=.004). Results of quantitative autonomic function tests differed significantly in the 2 groups only in the cardiovascular domain. Because subacute onset was overrepresented in the seropositive group, we analyzed the data separately, controlling for temporal profile (ie, the relationship between antibody status and symptoms while controlling for rate of onset). The relationships between antibody status and clinical profile (eg, presence of sicca complex, pupillary abnormalities, and lower gastrointestinal tract symptoms) generally remained significant regardless of onset rate, indicating that the associations are not due to temporal profile.

Conclusions: These observations support the concept that ganglionic AChR antibodies are diagnostically and pathophysiologically important. Patients with orthostatic hypotension and prominent cholinergic dysautonomia are most likely to be seropositive for ganglionic AChR antibody.

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METHODS

Patients were identified from the database of the Mayo Autonomic Laboratory, Rochester,
Minn (January 1, 1997, through December 31, 2001). We reviewed all cases assigned a diagnosis of orthostatic hypotension, defined as a systolic blood pressure reduction of at least 30 mm Hg or a mean blood pressure reduction of at least 20 mm Hg within 3 minutes of head-up tilt. This definition is generally accepted and is supported by normative data. The medical record review and data analysis were conducted in accordance with Institutional Review Board regulations and with Institutional Review Board approval from Mayo Medical Center, Rochester. Only the medical charts of patients who agreed to have their records reviewed for research purposes were considered.

All medical records were reviewed carefully, and secondary forms of orthostatic hypotension (eg, diabetes-, multiple system atrophy–, and medication-induced hypotension) were excluded. A total of 819 charts were reviewed, and 288 cases were identified as idiopathic orthostatic hypotension. Of these, 106 had undergone testing for ganglionic AChR antibody. We considered these cases for further analysis and comparison. From these 106 cases, we extracted demographic, clinical, and the following laboratory variables: autonomic testing scores, thermoregulatory sweat test results, ganglionic AChR antibody levels, and plasma orthostatic catecholamine measurements. Subacute onset was defined as autonomic failure reaching a peak within 3 months. Chronic onset was defined as reaching a peak after 3 months (or having a progressive course).

Ganglionic AChR Autobody

Autoantibodies were detected by means of an immunoprecipitation assay in which AChR antigen is solubilized from a human neuroblastoma and complexed with iodine 1125–labeled epibatidine.

Autonomic Function Tests

Cardiovascular, adrenergic, and postganglionic sudomotor functions were evaluated as follows.7

The Quantitative Sudomotor Axon-Reflex Test, an evaluation of the postganglionic sympathetic sudomotor axon, is routinely recorded at 4 sites (forearm, proximal lateral leg, medial distal leg, and proximal foot). The stimulus is iontophoresed acetylcholine, and responses are recorded in a single compartment of a multicompartamental sweat cell that is separate from the stimulus compartment. The axon reflex is mediated by postganglionic sympathetic sudomotor fibers. Control values were derived from studies on 223 healthy subjects aged 10 to 83 years.8

Cardiovagal function evaluation is based on heart rate response to deep breathing (HRDB) and the Valsalva ratio.3 The HRDB is the range of heart rate in response to forced respiration of the maximal to minimal heart rate. Control values were derived from studies on 223 healthy subjects aged 10 to 83 years.3

The symptom of difficulty focusing was recorded only if confirmed by results of neurological examination of the pupil. We specifically sought reduced pupillary responses to light and/or accommodation, indicative of Adie pupil. Twelve (67%) of 18 patients in the Ab+ group had abnormal pupillary responses compared with 12 (14%) of 87 patients in the Ab– group (P < .001; Table 1).

Dry Eyes and/or Mouth

Because dry eyes or dry mouth in isolation is frequently reported in otherwise healthy subjects, we required both complaints to be unequivocal and consistent (ie, the full sicca complex) to record an abnormal finding. We ex-
The presence of neurogenic bladder and sexual dysfunction were recorded, with care to exclude symptoms attributable to nonautonomic causes (most commonly, post-surgical dysfunction and stress incontinence). Symptoms attributable to neurogenic bladder were present in 32 (41%) of 78 patients in the Ab− cohort and 10 (53%) of 19 in the Ab+ group (P=.36; Table 1). Because of difficulty attributing sexual dysfunction to dysautonomia in women, we could only analyze self-reported male erectile dysfunction, taking care to exclude preexistent conditions (mostly prostatectomy or medication effects). The small number of male patients in the Ab+ group did not allow for χ² analysis, but 4 (80%) of 5 male patients in the Ab+ group reported severe dysfunction (ie, complete inability to obtain an erection) vs 18 (45%) of 40 in the Ab− group. In the latter group, an additional 8 patients (20%) reported partial erectile dysfunction.

**THERMOREGULATORY FUNCTION**

A reduced ability to sweat and intolerance of heat were reported in 14 patients (74%) in the Ab+ group; 39 (57%) of 68 patients in the Ab− group reported similar problems. The difference did not reach statistical significance (P=.19; Table 1).

**ANTECEDENT ILLNESS**

More patients in the Ab+ group (8 [42%]) reported an antecedent event (presumed viral illness) than did those in the Ab− group (22 [25%]), but the difference was not statistically significant (P=.15; Table 1).

**TEMPORAL PROFILE**

In the Ab+ group, 12 patients (63%) had a subacute onset (<3 months), compared with 23 (27%) of 84 patients in the Ab− group, in which insidious onset and a chronic disease course was more common (P=.004; Table 1).

**AUTONOMIC FUNCTION TESTS**

Individual autonomic function test results were not significantly different between the Ab+ and Ab− groups (Table 2). The CASS and its subscores differed only for the cardiovagal index, which was higher (more abnormal) in the Ab+ group (P=.04). No significant difference was found in the thermoregulatory sweat test results for percentage of total body anhidrosis or for norepinephrine values.

**ANALYSIS BY TEMPORAL PROFILE**

We considered it possible that the difference in temporal profile between the 2 groups (overrepresentation of subacute onset in the Ab+ cohort) could account for at least some of the significant differences between the groups in symptom domains. For example, symptom complaints from patients with a subacute onset may be reported differently from those of patients whose symp-
toms develop insidiously. We therefore analyzed the data separately on the basis of the temporal profile, irrespective of the autoantibody status. We considered all cases with a time from onset to peak of 3 months or less as subacute and cases with onset to peak of greater than 3 months as chronic. The subacute onset group was characterized by significantly more frequent sicca syndrome (9/15 [60%] vs 11/47 [23%]; P = .02), lack of heat-induced sweating (20/28 [71%] vs 21/46 [46%]; P = .03), and pupillary abnormalities (15/33 [45%] vs 9/67 [13%]; P = .01). Although lower gastrointestinal tract symptoms were more frequent in patients with subacute onset (26/35 [74%] vs 38/67 [57%]), this difference did not reach statistical significance (P = .08).

Because mode of onset was significantly associated with several clinical features, we next repeated the analysis for variables that showed a significant bivariate relationship to autoantibody status (sicca complex, pupillary abnormalities, and lower gastrointestinal tract function), while controlling for symptom onset (subacute vs insidious). The significant relationships of autoantibody status to the sicca complex and pupillary abnormalities held (P = .03 and P < .001, respectively), but the relationship between autoantibody status and abnormal lower gastrointestinal tract function no longer reached significance (P = .08; adjusted χ² values [Table 1]). These findings suggest that the relationship between autoantibody status and these symptoms is not fully accounted for by rate of symptom onset.

**COMMENT**

Idiopathic neurogenic orthostatic hypotension has been traditionally classified by temporal profile and disease course. Cases with acute onset, often with antecedent viral infection, and monophasic course were considered the autonomic equivalent of Guillain-Barré syndrome and termed *acute pandysautonomia* or *panautonomic neuropathy*. The identification of a candidate pathogenic autoantibody and the significant correlation between autoantibody level and severity and distribution of autonomic failure supports an autoimmune basis for many patients with a subacute presentation. Anecdotal reports of improvement after immunomodulatory therapy further support this hypothesis. At the other end of the spectrum, cases with insidious onset and progressive course were considered to be degenerative and variously called pure autonomic failure or Bradbury-Eggleson syndrome. However, this classification is imprecise because some cases of AAN have insidious onset and slow progression.

The main findings of the present study are that, in patients with neurogenic orthostatic hypotension, the presence of ganglionic AChR antibody is associated with sicca complex, abnormal pupils, lower gastrointestinal tract dysfunction, and subacute onset of the neuropathy. Although subacute onset is more common in the seropositive cohort, our analysis demonstrated that the association of autoantibody status with cholinergic function, especially cholinergic secretomotor function, remained significant after controlling for temporal profile. This finding indicates that the time factor is not as critical as originally thought and cannot be the sole basis for diagnosis of AAN.

In an earlier publication, we reported that a combination of sicca complex, abnormal pupillary light response, upper gastrointestinal tract symptoms, and neurogenic bladder occurred in more than 50% of patients with high autoantibody levels. Higher autoantibody values correlated with greater autonomic dysfunction and a greater frequency of cholinergic dysautonomia. These observations support the concept that ganglionic AChR antibody is diagnostically and pathophysiologically important. Our previous observations were confined to patients who were seropositive for ganglionic AChR antibody. The results of the present study comparing seropositive and seronegative cohorts further support pathophysiological importance for this autoantibody. The only variables that significantly separated these cohorts were those previously identified, although lower gastrointestinal tract complaints (not analyzed in our first study) proved to be more significant than upper gastrointestinal tract complaints in this study. That the antibody is responsible for the generalized autonomic neuropathy in patients with AAN is suggested by the correlation between antibody levels and total autonomic deficits and by the development of experimental AAN in rabbits producing ganglionic AChR antibody in response to immunization with a fragment of this AChR protein, with the severity of dysautonomia paralleling serum autoantibody levels.

The ganglionic AChR autoantibody acts at the level of autonomic ganglia on postsynaptic adrenergic and cholinergic neurons. Therefore, the sympathetic and parasympathetic systems should be affected equally. Although deficits are present in both systems, an intriguingly robust relationship is observed between the degree of involvement of parasympathetic secretomotor functions and

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**Table 2. Comparison of Cases Seropositive and Seronegative for Ganglionic AChR Antibody by Autonomic Function Test Results**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patient Groups, Mean ± SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ab+ (n = 19)</td>
<td>Ab− (n = 87)</td>
</tr>
<tr>
<td>CASS-total</td>
<td>7.6 ± 0.43</td>
<td>6.9 ± 0.25</td>
</tr>
<tr>
<td>CASS-sudomotor</td>
<td>2.2 ± 0.22</td>
<td>2.1 ± 0.11</td>
</tr>
<tr>
<td>CASS-cardiovascular</td>
<td>2.1 ± 0.27</td>
<td>1.5 ± 0.13</td>
</tr>
<tr>
<td>CASS-adrenergic</td>
<td>3.5 ± 0.16</td>
<td>3.4 ± 0.09</td>
</tr>
<tr>
<td>% of anhidrosis</td>
<td>41.6 ± 7.4</td>
<td>49.3 ± 4.2</td>
</tr>
<tr>
<td>Supine norepinephrine level, pg/mL</td>
<td>129.2 ± 47.2</td>
<td>186.7 ± 24.4</td>
</tr>
<tr>
<td>Standing norepinephrine level, pg/mL</td>
<td>229.8 ± 88.6</td>
<td>338.2 ± 51.9</td>
</tr>
<tr>
<td>Change in norepinephrine level, pg/mL (supine to standing)</td>
<td>100.6 ± 64.7</td>
<td>137.6 ± 30.2</td>
</tr>
</tbody>
</table>

Abbreviations: Ab+, seropositive for the acetylcholine receptor (AChR) antibody; Ab−, seronegative for the AChR antibody; CASS, Composite Autonomic Severity Score. SI conversion factor: To convert norepinephrine to nanomoles per liter, multiply by 0.00591.

*Group sizes vary owing to lack of data.
†Calculated by means of the Mann-Whitney test.
‡Calculated by means of the 2-sample t test.

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the serum level of antibody, as evidenced by the correlation between the severity of autonomic failure and the overrepresentation of these symptoms in the seropositive cohort of this study. We do not have a clear explanation for this finding but can postulate a number of testable hypotheses. Perhaps the postganglionic cholinergic neuron is more sensitive to antibody than the postganglionic adrenergic neuron. Neurons in these 2 systems differ cytoarchitecturally. Parasympathetic cholinergic postganglionic neurons that affect tearing in the pterygopalatine ganglion, salivation in the submandibular ganglion, pupillary constriction in the ciliary ganglion, and smooth muscle contraction are unusually small compared with sympathetic ganglia. These parasympathetic ganglia are located closer to or within the target organs. The site of action of the ganglionic AChR antibody is at the proximal end of the postganglionic neuron. Sympathetic neurons residing in proximal ganglia of the sympathetic chain may have a more protective blood-ganglion barrier than parasympathetic ganglia at more peripheral locations. An alternative explanation for their greater susceptibility to functional impairment is that cholinergic neurons may present additional targets for the antibodies, including nicotinic autoreceptors on nerve terminals. (The quantitative sudomotor axon reflex response is evidence of nicotinic AChR on the distal sudomotor axon.) Another potential explanation is the dominance of parasympathetic tone over pupillary and secretomotor functions. The net effect is that the pathophysiological outcome of antibody binding is more prominent in affected parasympathetic ganglia.

Despite clear-cut clinical differences, we found a statistically significant abnormality only in the cardiovasculoregulatory score of autonomic tests performed in the seropositive and seronegative groups. There are a number of possible explanations for this. First, the clinical differences that were significant between groups (secretomotor, pupillary, and gastrointestinal tract function) were not quantitated. Salivation and tear production are not routinely tested. In individual cases, quantitative measurement of these secretomotor functions was markedly reduced in patients with sicca syndrome, but similar recordings were not performed routinely in most seropositive patients and not at all in seronegative patients. Only a limited number of patients had gastrointestinal motility studies. Second, there was a selection bias in that the two groups were balanced by severity of adrenergic failure (ie, all had documented orthostatic hypotension). Our routine autonomic tests define the severity of autonomic disturbance but are relatively insensitive in determining the site of pathophysiology (central nervous system vs ganglia vs nerve). More detailed autonomic studies, including pharmacological dissection of autonomic dysfunction, would likely be more sensitive in differentiating autonomic neuropathy due to ganglionopathy.

CONCLUSIONS

Although a positive result in ganglionic AChR antibody testing is useful diagnostically and guides specific treatment strategies, some seronegative patients have a clinical course and features that are compatible with AAN. Furthermore, some individual seronegative patients respond to immunotherapy (P.A.L. unpublished observations). The frequency of idiopathic autonomic neuropathy will likely shrink as other autoantibody specificities and neuronal antigen-specific effector T lymphocytes are discovered.

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REFERENCES