Ganglionic Acetylcholine Receptor Autoantibody

Oncological, Neurological, and Serological Accompaniments

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Objective: To describe the clinical utility of the nicotinic ganglionic acetylcholine receptor (α3-AChR) autoantibody as a marker of neurological autoimmunity and cancer.

Design: Case-control study.

Setting: Mayo Clinic, Rochester, Minnesota.

Patients: A total of 15,000 patients seen at Mayo Clinic (2005-2007) and evaluated on a service basis for paraneoplastic neurological autoimmunity for whom clinical information was obtained retrospectively by medical record review as well as 457 neurologically asymptomatic patients or control subjects of whom 173 were healthy, 245 had lung cancer, and 39 had systemic lupus erythematosus or Sjögren syndrome.

Outcome Measures: Neurological, oncological, and serological associations of α3-AChR autoantibody seropositivity.

Results: Of 15,000 patients tested on a service basis, 1% were seropositive (median, 0.12 nmol/L; range, 0.03-18.8 nmol/L; normal, ≤0.02 nmol/L), 55% were male, and the median age was 65 years. Cancer was found (new or by history) in 24 of 78 patients evaluated for cancer while at Mayo Clinic (30%); 43% had adenocarcinoma (more patients had breast cancer than prostate, lung, and gastrointestinal cancers; each of the latter groups had about the same number of patients). Of 12 patients with high antibody values (≥1.00 nmol/L), 83% had pandysautonomia. Of 85 patients with medium antibody values (0.10-0.99 nmol/L), neurological presentations were more diverse and included peripheral neuropathies (36%), dysautonomia (20%, usually limited), and encephalopathy (13%). Of 58 patients with low antibody values (0.03-0.09 nmol/L), 54% had a nonautoimmune neurological disorder or no neurological disorder. Of 245 control patients with lung cancer, 7.8% were seropositive. Only 1 of 212 control patients without cancer (0.5%) was seropositive (P<.001).

Conclusion: The detection of α3-AChR autoantibody aids the diagnosis of neurological autoimmunity and cancer.

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Cancer. Triangles, cancer only; and diamonds, controls without neurological disease or cancer. SLE indicates systemic lupus erythematosus; circles, patients referred for paraneoplastic evaluation; triangles, cancer only; and diamonds, controls without neurological disease or cancer.

Figure 1. Nicotinic gangionic acetylcholine receptor antibody (α3-ACHR Ab) values detected in 155 patients with neurological disease (those referred for paraneoplastic evaluation) and 457 controls (those with cancer only or those without neurological disease or cancer). SLE indicates systemic lupus erythematosus; circles, patients referred for paraneoplastic evaluation; triangles, cancer only; and diamonds, controls without neurological disease or cancer.

Spectrum of oncological, neurological, and serological accommodations during a 27-month period. We also determined the frequency of α3-ACHR Ab in the sera of the following 3 control groups: (1) healthy persons age- and sex-matched to seropositive patients, (2) neurologically asymptomatic patients with lung cancer, and (3) neurologically asymptomatic patients with systemic lupus erythematosus (SLE) or Sjögren syndrome.

METHODS

PATIENTS

The study was approved by the institutional review board of Mayo Clinic, Rochester, Minnesota (IRB 06-005457). From January 1, 2005, to March 31, 2007, service paraneoplastic autoantibody evaluations were performed on the serum samples of approximately 15,000 patients evaluated clinically at Mayo Clinic’s 3 sites (Rochester, Minnesota; Scottsdale, Arizona; and Jacksonville, Florida).

NORMAL VALUES

The normal value range for α3-ACHR Ab was established by testing the serum of 173 healthy control subjects matched in sex and age to the study patients. Only 1 (0.6%) had a value exceeding 0.02 nmol/L (Figure 1). We therefore analyzed study patients’ data according to the serum α3-ACHR Ab value ranges high (≥1.00 nmol/L), medium (0.10-0.99 nmol/L), and low (0.03-0.09 nmol/L).

PATIENTS

Of 15,000 patients evaluated at the Mayo Clinic for paraneoplastic autoantibodies in a 27-month period, α3-ACHR Ab values exceeded 0.02 nmol/L in 155 (1%); median, 0.12 nmol/L; range, 0.03-18.8 nmol/L. Oncological, neurological, and serological data are summarized in Tables 1, 2, and 3. Six patients (4%)
were not white, 86 (55%) were male, and 69 (45%) were female. The median age at neurological symptom onset was 65 years (range, 17-103 years). The median follow-up period was 2 months (range, 0-96 months). Informative follow-up data were available for 70 patients (45%; median, 6 months).

**ONCOLOGICAL ASSOCIATIONS**

Of 78 seropositive patients (50%) who had an evaluation for cancer at Mayo Clinic, 24 (30%) were confirmed to have cancer, either active or by history; 6 had multiple malignant neoplasms. The cancer incidence or type did not correlate with antibody level. Adenocarcinomas accounted for 43% of all identified cancers. Three of 16 with a history of cancer were receiving active treatment when diagnosed with neurological disease and 13 were in apparent remission (2-14 years). Oncological investigation prompted by detection of α3-AChR Ab revealed cancer in another 9 patients (Table 1); 2 had lung carcinomas (1 adenocarcinoma, 1 histology unknown), 2 thyroid carcinomas (1 papillary, 1 histology unknown), 2 bladder carcinomas, 1 renal cell carcinoma, 1 melanoma, and 1 multiple myeloma. Of note, 2 of these 9 patients lacked evidence of neurological autoimmunity. One had genetically confirmed Huntington disease and was found to have bladder carcinoma, and 1 with multifactorial gait disorder was found to have bronchogenic carcinoma. Cancers were detected by whole-body computed tomography (5 patients), whole-body positron emission tomography (3 patients, all with negative computed tomographic scans; Figure 2), and bone marrow aspirate biopsy (1 patient).

Of 77 patients who did not undergo oncological evaluation at Mayo Clinic after identification of α3-AChR, 8 had a history of cancer (10%). These cancers were lung, 2; prostate, 2; breast, 1; melanoma, 1; epidermoid brainstem tumor, 1; and multifocal recurrent meningiomas, 1.

**NEUROLOGICAL MANIFESTATIONS**

Neurological symptoms and signs were multifocal in 29% of patients. Symptom onset was subacute in 46% and insidious in 54%.

**Patients With High α3-AChR Ab Values**

High autoantibody values were those 1.00 nmol/L or higher. Twelve patients (8%) had a median serum antibody value of 2.03 nmol/L (range, 1.02-18.8 nmol/L). Eleven of those (92%) were considered to have neurological autoimmunity (a neurological disorder with an autoimmune pathogenesis; Table 2). Ten had dysautonomia (84%), 7 severe pandysautonomia, and 3 limited dysautonomia (1 gastrointestinal dysmotility, 1 mild somatomotor impairment, and 1 orthostatism). One patient presented with subacute autoimmune encephalopathy and recovered completely after 5 days of treatment with intravenous methylprednisolone (Table 4). The twelfth patient had orthostatic headache attributed to a dural cerebrospinal fluid leak.

**Table 1. Cancer Associations in 78 α3-AChR Ab–Seropositive Patients Who Underwent Oncological Evaluation by Titer**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Total</th>
<th>High</th>
<th>Medium</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancers</td>
<td>30</td>
<td>2</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>13 (43)</td>
<td>1</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Breast</td>
<td>4</td>
<td>0</td>
<td>4b</td>
<td>0</td>
</tr>
<tr>
<td>Prostate</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Lung</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>GI tract</td>
<td>2</td>
<td>0</td>
<td>2b</td>
<td>0</td>
</tr>
<tr>
<td>Thyroid, papillary</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Urterine</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>2 (6)</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lymphoid</td>
<td>5 (17)</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>B cell lymphoma</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>1c</td>
</tr>
<tr>
<td>CLL</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myeloma</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>10 (34)</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Melanoma</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2b</td>
</tr>
<tr>
<td>Bladder carcinoma</td>
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<td>0</td>
<td>1</td>
<td>1c</td>
</tr>
<tr>
<td>Small cell carcinoma, lung</td>
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<td>0</td>
<td>0</td>
<td>1c,d</td>
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<tr>
<td>Lung carcinoma</td>
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<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Thymoma</td>
<td>1</td>
<td>0</td>
<td>1b</td>
<td>0</td>
</tr>
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<td>Thyroid carcinoma</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
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<td>Tonsilar carcinoma, squamous</td>
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<td>0</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Ovarian carcinoma</td>
<td>1</td>
<td>0</td>
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</tr>
</tbody>
</table>

Abbreviations: α3-AChR Ab, nicotinic ganglionic acetylcholine receptor autoantibody; CLL, chronic lymphocytic leukemia; GI, gastrointestinal.

a A total of 30 cancers were identified in 24 of 78 patients. See “Oncological Associations” subsection of the “Results” section for cancer histories in 77 patients who did not undergo testing for cancer.
b Muscle AChR Ab was detected in 2 patients with breast carcinoma and 1 each with GI carcinoma, melanoma, and thymoma; 2 had myasthenia gravis.
c Coexisting glutamic acid decarboxylase 65-isofrom antibody.
d Coexisting antineuronal nuclear antibody 1 (1:30 720) predicted small cell lung carcinoma in a patient with limbic encephalitis, neuropathy, and GI dysmotility.
e Unknown histology.

**Patients With Medium α3-AChR Ab Values**

Medium values were those between 0.10 and 0.99 nmol/L. The median serum antibody value for these 85 patients (55% of the total) was 0.14 nmol/L. The predominant neurological presentation in 31 patients (36%) was peripheral neuropathy (Table 2). Another 5 patients had neuropsychologic evidence of distal small fiber neuropathy, 2 distal anhidrosis (thermoregulatory sweat test), 2 elevated thresholds for cold sensation, and 1 abnormality in both tests. Seventeen patients (20%) had objective evidence of dysautonomia, 3 pandysautonomia, 14 limited dysautonomia (8 gastrointestinal dysmotility, 3 orthostatism, 2 sicca syndrome [xerostomia and xerophthalmia], and 1 partial anhidrosis), and 6 had coexisting sensorimotor neuropathy.

Eleven patients had acute or subacute onset of cognitive and psychiatric symptoms and signs such as depression, psychosis, executive dysfunction, personality change, and amnestic mild cognitive impairment. One patient (Table 4, patient 12) had a frontotemporal syn-
drome characterized by depression and dysexecutive symptoms; his condition improved after oral prednisone therapy (Table 4). Two patients had a subacute extrapyramidal syndrome characterized by akinetic rigid parkinsonism. Cerebrospinal fluid was inflammatory in one, and symptoms and signs in the other improved following intravenous methylprednisolone therapy. Three patients had indeterminate ataxia.

The diagnosis in 1 patient was stiff-man syndrome (GAD65 Ab, 4.63 nmol/L) and in another was myasthenia gravis (muscle AChR–binding Ab, 28.7 nmol/L); normal values for both antibodies are 0.02 nmol/L or less.

The diagnosis in 3 patients was multiple sclerosis and in 16 (19%) was either a non–immune-mediated neurological disorder (11 patients) or a nonneurological disorder (5 patients); 5 patients (30%) had polyclonal hypergammaglobulinemia.

Patients With Low α3-AChR Ab Values

Low values were those between 0.03 and 0.09 nmol/L. The median antibody value in this group of 58 patients (37% of the total) was 0.06 nmol/L. Thirty-one (54%) had either a non–immune-mediated neurological disorder (22 patients) or a nonneurological disorder (9 patients). Twelve (39%) had monoclonal (2 patients) or polyclonal hypergammaglobulinemia (10 patients).

Peripheral neuropathy was the predominant neurological manifestation in 13 patients (22%). Six (10%) had dysautonomia, 3 with pandysautonomia and 3 with limited dysautonomia. Subacute neuropsychiatric presentations were documented in 3 patients, 1 with limbic encephalitis with coexisting ANNA-1 autoantibody and 2 with subacute memory loss and depression. Three patients had myasthenia gravis and were seropositive for muscle AChR–binding Ab (range, 2.56-38.9 nmol/L). Two patients had an inflammatory demyelinating central nervous system disorder, 1 with neuromyelitis optica and 1 with multiple sclerosis.

**ACCOMPANYING AUTOANTIBODIES**

One or more additional neuronal or muscle autoantibodies were detected in 40 of the α3-AChR Ab–positive patients (26%) in the following descending frequency: GAD65, muscle AChR, neuronal voltage-gated cation channel (N-type calcium channel was more common than potassium channel, which was more common than P/Q-type calcium channel), striational, ANNA-1, and CRMP-5 IgG. Six patients had multiple coexisting antibodies (Table 3).
**CEREBROSPINAL FLUID**

Abnormalities were detected in 22 of 35 patients tested (63%); 2 had elevated white blood cell counts (100/mL and 9/mL, respectively; normal value, 0-5/mL); 22 increased protein levels (median, 63 mg/dL; range, 48-181 mg/dL; normal range, 14-45 mg/dL); and 4 had 4 or more oligoclonal bands (normal value, <4).

**MRI IMAGING**

Only 4 of 80 patients tested had remarkable findings. One patient (medium antibody value) presented with an akinetic rigid parkinsonian state and inflammatory cerebrospinal fluid and had bilateral increased signal on T2-weighted and fluid inversion recovery imaging in the putamen and caudate nuclei consistent with basal ganglionitis; CRMP-5 IgG was negative. Three patients had radiologic findings compatible with a diagnosis of multiple sclerosis.

**THERAPIES AND OUTCOMES**

Table 4 summarizes the outcomes for 16 patients who received immunotherapy; 2 of these patients also received tumor-directed therapy. Neurological improvement was described in 12 patients (75%) who received immunotherapy and in 2 patients who received cancer-directed therapy (chemotherapy in one and tumor resection in the other). The delay from symptom onset to initiation of treatment was not significantly shorter for those reported as improved (P = .76). Follow-up of more than 1 month (median, 9 months; range, 4-20 months) was available for 5 patients with neurological disorders (peripheral neuropathy, 3; cognitive disorder, 1; ataxia, 1) who did not undergo immunotherapy. All but 1 had no change documented; 1 patient with a cognitive disorder continued to decline. Four deaths were reported (mean survival, 28 months after neurological symptom onset; none had a diagnosis of cancer).

**CONTROLS**

Presence of α3-AChR Ab was more common among neurologically asymptomatic patients with lung cancer (19 of 245) than other controls (1 of 212; 1 of 173 healthy; 0 of 39 with SLE or Sjögren syndrome; P < .001) (Figure 1).

Small cell lung carcinoma was not documented in any seropositive patient with lung cancer; 8 had squamous cell carcinoma (median, 0.13 nmol/L; range, 0.07-0.53 nmol/L), 7 had adenocarcinoma (median, 0.11 nmol/L; range, 0.04-0.15 nmol/L), and 4 had other lung cancer types (2 non–small cell lung carcinoma not otherwise specified, 1 adenosquamous carcinoma, 1 bronchoalveolar carcinoma; median, 0.20 nmol/L; range, 0.12-0.23 nmol/L). No α3-AChR Ab was detected in any control patient with SLE or Sjögren syndrome.

Detection of α3-AChR Ab is common in patients referred for a paraneoplastic serological evaluation (1% vs 0.4% for both ANNA-1 and CRMP-5 IgG) and those with cancer (7.5% in patients with lung cancer vs 0.5% for healthy controls). Studies to date have emphasized the clinical association of α3-AChR Ab with idiopathic paraneoplastic pandysautonomia (typical values exceed 1.0 nmol/L) and cancer associations restricted to thymoma and small cell lung carcinoma. Our present study has identified diverse cancer types, the most common being adenocarcinomas. Serum levels in most patients were lower than 1.0 nmol/L and neurological presentations were more diverse, including peripheral neuropathy and central nervous system disorders.
While 30% of patients who had an oncological evaluation had cancer or a history of cancer, a new cancer was detected in 12% of patients evaluated subsequent to antibody detection. The median duration of follow-up was only 6 months, and only half of patients had an extensive oncologic evaluation. While the rate of cancer detection among seronegative patients during the same follow-up period is unknown, the 15-fold higher prevalence of 3-AChR Ab in the principal study cohort was directly proportional to the antibody titer. Patients with high (>1.0 nmol/L) and medium (0.10-0.99 nmol/L) values for 3-AChR Ab were considered to have an autoimmune disorder in 92% and 82% of cases, respectively. Of patients with low 3-AChR Ab values, 54% had nonspecific symptoms without a neurological diagnosis and hypergammaglobulinemia was common. In addition to cancer, a monoclonal or polyclonal hypergammaglobulinemia should be considered as a potential cause of falsely positive low antibody values.

It is noteworthy that 21% of seropositive patients had symptoms of dysautonomia. Patients with high 3-AChR Ab values were more likely to have multiple autonomic deficits, and patients with medium or low values were more likely to have limited dysautonomia. This laboratory previously observed that 14.6% of patients with postural orthostatic tachycardia syndrome had low 3-AChR Ab values.

Peripheral neuropathy was the most common accompaniment of 3-AChR Ab, documented in 28% of patients. Most had length-dependent sensory or sensorimotor polyneuropathies, but demyelinating neuropathies and disorders of nerve root, dorsal root ganglion, and cranial nerves were also encountered. Central nervous system disorders of subacute onset were encountered in 20% of seropositive patients and mainly affected cortical and subcortical structures. Most of these patients had neuropsychiatric manifestations or extrapyramidal disorders. Immuno-
therapies were beneficial in 75% of treated patients, but follow-up was limited.

It is unlikely that the AChR α3-subunit is the primary autoantibody target in all neurological presentations described in this article. The α-2-AChR-binding Ab that we detected may reflect an antibody primarily directed at 1 of numerous antigenerically related AChR α subunits.\(^{18}\) We have described α-3-AChR Ab both as a marker of neuronal AChR antibody mediated channelopathy\(^4\) and as an accompaniment of a cytotoxic T-cell mediated neuropathy.\(^{11}\) The α-3-AChR subunit is not confined to autonomic ganglia. It is found throughout the nervous system including the sensory dorsal root ganglia, trigeminal ganglia, and in rat\(^{19,20}\) and mouse brains.\(^{19}\)

We detected 1 or more coexisting antibodies specific for neuronal nuclear or neuronal or muscle cytoplasmic antigens in 26% of seropositive patients. Muscle AChR antibody was frequently encountered, including in 2 patients who had myasthenia gravis in the setting of cancer. Myasthenia gravis coexisted with subacute autoimmune failure in 1 patient (in the context of thymoma, as reported previously\(^{21}\)), and another patient had dual muscle and neuronal autoimmune channelopathies in the context of metastatic renal carcinoma. The most common autoantibody detected in the company of GAD65 neuronal AChR Ab was GAD65-specific (7% of patients). The GAD65 Ab level in 1 patient approached 20 nmol/L, a value generally distinguishing patients with GAD65 neurologic autoimmunity from patients with uncomplicated type 1 diabetes.\(^{13}\) That patient had stiff-man syndrome without evidence of cancer.

In summary, α-3-AChR Ab-seropositive patients have diverse neurological presentations encountered and cancer types detected (most commonly adenocarcinoma). The 30% rate of cancer detected is quite high and should prompt consideration of a cancer search in seropositive patients.

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