DO ACETYLCHOLINE RECEPTOR AND STRIATED MUSCLE ANTIBODIES PREDICT THE PRESENCE OF THYMOMA IN PATIENTS WITH MYASTHENIA GRAVIS?

EMILY CHOI DECROOS, MD, LISA D. HOBSON-WEBB, MD, VERN C. JUEL, MD, JANICE M. MASSEY, MD, and DONALD B. SANDERS, MD

Neuromuscular Section, Division of Neurology, Department of Medicine, Duke University Medical Center, DUMC 3403, Durham, North Carolina 27710, USA

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ABSTRACT: *Introduction:* Acetylcholine receptor (AChR) and striated muscle antibodies (StrAbs) are found frequently in myasthenia gravis (MG) patients with thymoma. In this study we aimed to determine the positive predictive value (PPV) and negative predictive value (NPV) of these antibodies for thymoma in patients with MG. *Methods:* Antibody findings, thymic histology, and onset age were reviewed for 1141 patients with MG. PPV and NPV of these antibodies for thymoma were determined. *Results:* The PPV of AChR binding antibodies plus StrAbs was highest (50.0%) with onset before the age of 40 years. The PPV of all antibodies was low (<9%) after age 40. Higher StrAb levels did not increase the PPV. The NPV of AChR binding antibodies was high (99.7%) for all ages. *Conclusions:* Patients without AChR binding antibody are not likely to have a thymoma. StrAbs and AChR binding antibodies are not diagnostic for thymoma, but in early-onset MG their presence should raise the clinical suspicion for thymoma.

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Myasthenia gravis (MG) is an autoimmune disorder characterized by weakness and fatigability. Antibodies to the nicotinic acetylcholine receptor (AChR) are found in about 85% of patients. Thymoma is present in approximately 15% of MG patients. Studies have demonstrated an increased frequency of AChR binding, blocking, and modulating antibodies in MG patients with thymoma.[1–3] Increased frequency of antibodies to components of striated muscle, including the ryanodine receptor and titin, have also been reported in MG associated with thymoma.[4–11]

Antibodies to AChR and striated muscle (StrAbs) are measured frequently in the diagnostic evaluation of MG, and it would be useful to know if the presence of these antibodies predicts the presence of thymoma. We calculated the predictive value of various combinations of AChR binding, blocking, and modulating antibodies and StrAbs for the presence of thymoma in a large population of MG patients. Because StrAbs are often present in late-onset MG without a thymoma, we calculated the predictive value of these antibodies in early- and late-onset disease. We also compared antibody levels in patients with and without thymoma.

METHODS

Data were obtained from the Duke Myasthenia Gravis Registry, which includes demographic, clinical, and laboratory data from all patients with MG seen in the Duke MG Clinic since 1980. The registry is approved by the institutional review board of Duke University. Data from the registry were cross-referenced with medical records to ensure accuracy of information.

Patients included in the study were those with acquired MG seen in the Duke MG clinic between July 1, 1980 and October 1, 2010. The diagnosis of MG was based on clinical presentation, response to treatment, antibody testing, and electrodiagnostic studies, including repetitive nerve stimulation and single-fiber electromyography (EMG). The diagnosis of thymoma was based on computed tomography (CT) chest imaging and histopathological examination after thymectomy. Patients were excluded if their antibody status or thymic histology was unknown.

Early-onset MG (EOMG) and late-onset MG (LOMG) were defined by symptom onset before or after the age of 40 years.

Antibody testing for most patients (94%) was performed at the Mayo Medical Laboratories (Rochester, Minnesota), using radioimmunoassay for detection of AChR antibodies and enzyme immunoassay for StrAbs.[12–14] The reference values for the testing laboratories were used to determine abnormality. When calculating predictive values, antibody results before thymectomy were used. Positive predictive value was calculated by dividing the number of patients with thymoma who had an antibody by the number of all patients who had the antibody. Negative predictive value was calculated by dividing the number of patients without thymoma who did not have an antibody by the number of all patients who did not have the antibody.

Median antibody levels of patients with and without thymoma were compared using the Mann–Whitney *U*-test.

Abbreviations: AChR, acetylcholine receptor; CT, computed tomography; EOMG, early-onset myasthenia gravis; LOMG, late-onset myasthenia gravis; MG, myasthenia gravis; NPV, negative predictive value; PPV, positive predictive value; StrAb, striated muscle antibody

Key words: acetylcholine receptor antibody; myasthenia gravis predictive value; striated muscle antibody; thymoma

Correspondence to: D.B. Sanders; e-mail: donald.sanders@duke.edu

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Table 1. Antibody findings in myasthenia gravis.

Antibody status	All			EOMG			LOMG		
	Number positive	Total number	Percent positive	Number positive	Total number	Percent positive	Number positive	Total number	Percent positive
Thymoma									
Binding	94	95	99%	31	31	100%	63	64	98%
Blocking	17	47	36%	4	15	27%	13	32	41%
Modulating	47	49	96%	16	16	100%	31	33	94%
StrAb	52	66	79%	13	21	62%	39	45	87%
No thymoma									
Binding	718	1046	69%	258	387	67%	460	659	70%
Blocking	119	483	25%	31	154	20%	88	329	27%
Modulating	370	591	63%	108	183	59%	262	408	64%
StrAb	242	678	36%	15	215	7%	227	463	49%

RESULTS

Data were obtained from 1141 patients with acquired MG, 95 of whom had a thymoma. AChR binding, blocking, and modulating antibodies and StrAbs were each found in a higher percentage of patients with thymoma than in those without thymoma. These percentages were calculated only for those who had the specific antibody tested.

Ninety-four of 95 patients with thymoma had elevated AChR binding antibodies at some point. Antibody results were only available after thymectomy for the 1 patient with thymoma who did not have elevated AChR binding antibodies. This patient was a woman who developed MG at age 59 years, approximately 3 years after thymomectomy. A 58-year-old man with thymoma tested negative for AChR binding antibodies before thymomectomy, but had elevated AChR binding antibodies when tested 3.5 years after surgery.

Almost all EOMG and LOMG patients with thymoma also had elevated AChR modulating antibodies. StrAbs were elevated in the majority of patients with thymoma in both age groups, and were found more often in the LOMG than EOMG patients.

The percentages of EOMG and LOMG without thymoma with AChR binding, blocking, and modulating antibodies were similar. However, the percentage of patients with StrAbs was much higher in LOMG without thymoma (49%) than in EOMG without thymoma (7%). A higher percentage of patients with EOMG with thymoma had StrAbs (62%) compared with EOMG without thymoma (7%) (Table 1).

In 1046 patients without thymoma, the percentage of patients with StrAbs increased with onset age, particularly after age 40. In patients without thymoma with onset before age 20, only 7% (4 of 61) had elevated StrAbs. In contrast, StrAbs were elevated in 74% (17 of 23) of patients with onset after age 80. The percentage of patients with AChR binding, blocking, and modulating antibodies did not increase with onset age (Fig. 1).

The PPV and NPV of various combinations of these antibodies for thymoma were calculated for EOMG and LOMG. In EOMG, the PPV was highest (50%) for the combination of AChR binding plus StrAbs. Among 150 EOMG patients in whom AChR binding and StrAbs were assayed, both were elevated in 8 patients; 4 of these patients had a thymoma. One EOMG patient with thymoma did not have StrAbs, but the thymoma was microscopic and detected only on histopathological examination. That patient had elevated AChR binding, blocking, and modulating antibodies. All 4 EOMG patients with AChR binding and StrAbs without thymoma were women; modulating antibodies were elevated in 2 of them. These patients were followed for an average of 11.8 years after the onset of their illness. None underwent thymectomy, so microscopic thymoma cannot be excluded in these patients.

The PPV for all antibodies and combinations was low in LOMG. The NPV was high for all antibodies in all age groups. In EOMG, the NPV of AChR

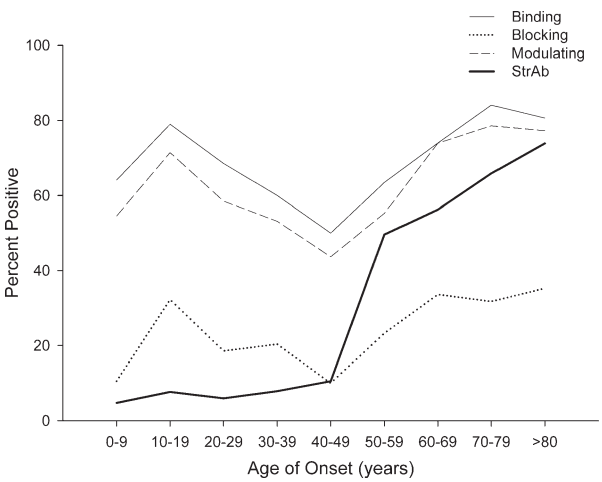

FIGURE 1. Antibody findings and age of onset in acquired MG without thymoma.

Table 2. Predictive value of antibodies for thymoma in myasthenia gravis.

Antibody status	Total number	Number with or without antibody	Number with or without thymoma	PPV	NPV
EOMG					
Binding	357	234/123	20/123	8.5	100.0
Blocking	119	25/94	1/92	4.0	97.9
Modulating	141	75/66	3/66	4.0	100.0
StrAb	151	12/139	4/138	33.3	99.3
Binding + StrAb	150	8/62	4/62	50.0	100.0
Binding + modulating + StrAb	125	4/49	2/49	50.0	100.0
LOMG					
Binding	685	490/195	41/194	8.4	99.5
Blocking	323	94/229	8/223	8.5	97.4
Modulating	396	261/135	11/133	4.2	98.5
StrAb	447	225/222	12/218	5.3	98.2
Binding + StrAb	447	222/139	12/138	5.4	99.3
Binding + modulating + StrAb	380	187/112	8/111	4.3	99.1

binding and modulating antibodies was 100%. In LOMG, the NPV of AChR binding antibody was 99.5%, and of modulating antibody was 98.5%. Other combinations of antibodies did not increase the NPV of AChR binding antibody (Table 2).

Antibody levels were calculated for EOMG and LOMG patients with and without thymoma. Among patients with elevated AChR binding antibodies, median antibody levels were marginally higher in those with thymoma in both age groups (Fig. 2).

In LOMG there was no difference in blocking and modulating antibody levels in thymoma versus non-thymoma patients. Mean and median StrAb levels were higher in patients without thymoma in this age group, although they were not statistically significant. A few patients without thymoma had very high StrAb levels (Fig. 3). There were too few data in EOMG patients to compare antibody levels.

Higher StrAb levels were not associated with a higher PPV in EOMG or LOMG. In EOMG, a StrAb titer >1:60 and >1:15,000 each had a PPV for thymoma of 33.3%; however, these calculations were limited by small numbers. In LOMG, StrAb levels >1:60 had a PPV of 4.9%, whereas, for a StrAb level >15,000, the PPV was 3.6%.

DISCUSSION

AChR binding, blocking, and modulating antibodies and StrAbs are frequently measured in the diagnostic evaluation of MG. Previous studies have

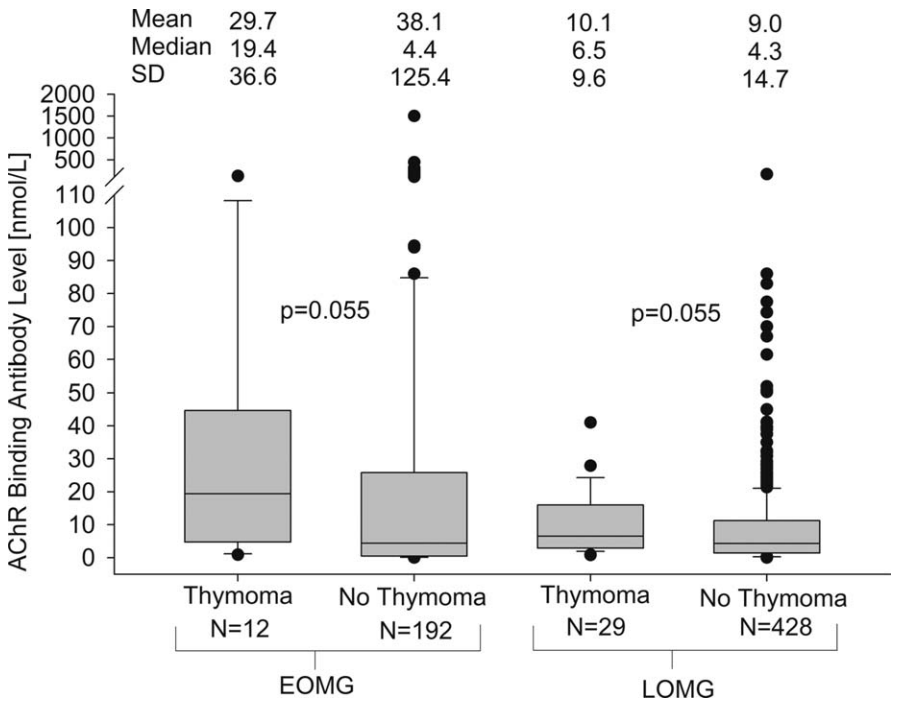

FIGURE 2. AChR binding antibody levels in EOMG and LOMG with and without thymoma.

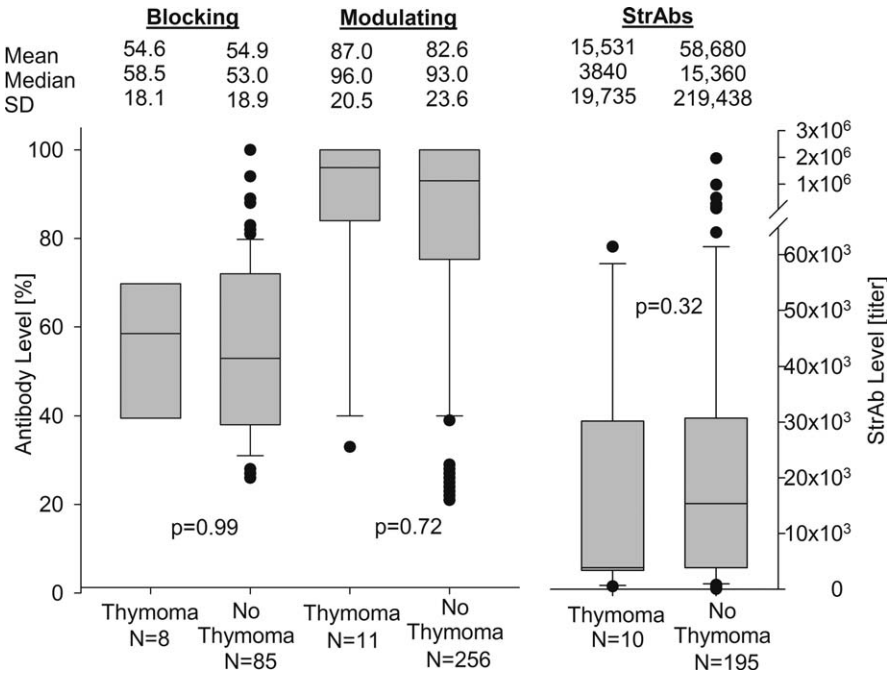

FIGURE 3. AChR blocking and modulating antibody and StrAb levels in LOMG with and without thymoma.

shown that these antibodies are more often present in MG patients with than without thymoma.[1–10,15]

In our study, myasthenic patients of all age groups without AChR binding antibody were very unlikely to have a thymoma: the calculated NPV of negative AChR binding antibody was 100% in EOMG and 99.5% in LOMG. Other antibodies also had high NPVs but did not increase the NPV of negative AChR binding antibody. This is consistent with previous studies, as only a few reports have been published on thymoma-associated MG occurring in the absence of AChR binding antibodies.[16,17] This raises the question of whether imaging studies for thymoma should be performed routinely if these antibodies are absent, particularly considering the cost and potential radiation risk of these tests.[18,19]

Our results are consistent with those of smaller studies of the predictive value of StrAbs in EOMG and LOMG. Similar to previous studies, we found that the percentage of MG patients with StrAbs increases with age, even in the absence of thymoma.[4,6,20] The PPV of StrAbs for thymoma was highest in patients with EOMG: thymoma was present in 50% of those with both StrAbs and AChR binding antibody, and StrAbs were rarely found in EOMG patients without thymoma. However, thymectomy was not performed in the other 50% of patients, so microscopic thymoma cannot be excluded.

AChR and StrAbs are less useful in predicting the presence of thymoma in LOMG, because StrAbs are commonly present in LOMG in the absence of thymoma and the PPV of StrAbs was low in this age group. Combinations of AChR antibodies and StrAbs did not increase significantly the PPV for thymoma in LOMG.

Most commercial laboratories perform only the binding AChR antibody assay, which by itself has a low PPV for thymoma. Our study has shown that AChR blocking and modulating antibodies do not increase the PPV or NPV for EOMG or LOMG.

We have also reported antibody levels in myasthenic patients with and without thymoma: AChR binding antibody levels were marginally higher in patients with thymoma in both age groups, whereas there was no difference in AChR blocking and modulating antibody levels in patients with and without thymoma with LOMG. Mean and median StrAb levels were higher in LOMG patients without thymoma due to a few LOMG patients who had very high antibody levels; however, these values were not significant statistically.

It has been reported recently that, among all patients with elevated StrAbs, the antibody levels are higher in those with thymic neoplasia; the PPV of thymoma for an antibody level >1:30,720 was 20% in that report, compared with 7% among all seropositive patients.[21] However, only 10% of that population had MG. This is an important distinction, because many patients with LOMG have StrAbs in the absence of thymoma. Thus, the PPV of StrAb for thymoma is lower in the LOMG population. Several of our LOMG patients without thymoma had very high StrAb levels (≥1:122,880). The results of the 2 studies taken together indicate

that higher StrAb levels predict an increased likelihood of thymoma or other malignancy in a population with a low prevalence of MG, but not in patients with LOMG.

In this study, most of the antibody testing was performed at 1 laboratory. Previous studies used different methods for antibody detection, which likely have varying sensitivities and specificities. Predictive values of antibodies detected through assays from different laboratories would be expected to vary as well.

The antibody tests in this study are available commercially and readily accessible to clinicians. This is in contrast to some reports in which antibody testing was performed in research laboratories; those results may be less applicable to clinical practice.

A limitation of our predictive value calculations is that, despite the large cohort size, there were relatively few patients with thymoma who had all antibodies measured before removal of the tumor. For example, among 151 patients with EOMG who had StrAbs measured before thymic surgery, only 4 had a thymoma. Our calculations also likely underestimate the predictive value of these antibodies for thymoma, because we excluded patients who did not have them measured before thymectomy.

The antibody status before initiation of immunosuppression was not available for many of our patients, and we cannot exclude that immunotherapy may have affected seropositivity rates and antibody levels.

Antibodies to components of striated muscle, including titin and the ryanodine receptor, are frequently found in patients with thymoma, and studies have suggested that these antibodies may be more specific for thymoma.[4,5,9,11,22,23] We did not evaluate these subtypes of StrAbs or their predictive value for thymoma.

In conclusion, although StrAbs and AChR binding antibodies are not diagnostic for thymoma, their presence in EOMG should raise the clinical suspicion and suggest the need for closer evaluation and follow-up studies.

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