Clinical Utility of Acetylcholine Receptor Antibody Testing in Ocular Myasthenia Gravis

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IMPORTANCE The sensitivity of acetylcholine receptor (AChR) antibody testing is thought to be lower in ocular myasthenia gravis (OMG) compared with generalized disease, although estimates in small-scale studies vary. There is little information in the literature about the implications of AChR antibody levels and progression from OMG to generalized myasthenia gravis.

OBJECTIVES To test the hypothesis that serum AChR antibody testing is more sensitive in OMG than previously reported and to examine the association between AChR antibody levels and progression from OMG to generalized myasthenia gravis.

DESIGN, SETTING, AND PARTICIPANTS A retrospective, observational cohort study was conducted of 223 patients (mean [SD] age, 59.2 [16.4] years; 139 [62.3%] male) diagnosed with OMG between July 1, 1986, and May 31, 2013, at 2 large, academic medical centers.

MAIN OUTCOMES AND MEASURES Baseline characteristics, OMG symptoms, results of AChR antibody testing, and progression time to generalized myasthenia gravis (if this occurred) were recorded for each patient. Multiple logistic regression was used to measure the association between all clinical variables and antibody result. Kaplan-Meier survival analysis was performed to examine time to generalization.

RESULTS Among the 223 participants, AChR antibody testing results were positive in 158 participants (70.9%). In an adjusted model, increased age at diagnosis (odds ratio [OR], 1.03; 95% CI, 1.01-1.04; \( P = .007 \)) and progression to generalized myasthenia gravis (OR, 2.92; 95% CI, 1.18-7.26; \( P = .02 \)) were significantly associated with positive antibody test results. Women were less likely to have a positive antibody test result (OR, 0.36; 95% CI, 0.19-0.68; \( P = .002 \)). Patients who developed symptoms of generalized myasthenia gravis had a significantly higher mean (SD) antibody level than those who did not develop symptoms of generalized myasthenia gravis (12.7 [16.5] nmol/L vs 4.2 [7.9] nmol/L; \( P = .002 \)).

CONCLUSIONS AND RELEVANCE We demonstrate a higher sensitivity of AChR antibody testing than previously reported in the largest cohort of patients with OMG available to date. Older age, male sex, and progression to generalized myasthenia gravis were significantly associated with a positive antibody test result. In addition, to our knowledge, this is the first report of an association between high AChR antibody levels and progression from OMG to generalized disease.

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Myasthenia gravis (MG) can cause weakness of the eyelids and extraocular muscles in up to 90% of patients; approximately half of such patients will present with isolated ocular symptoms (ie, ptosis and/or diplopia only). The diagnosis of ocular MG (OMG) is not always clinically evident, as the pattern of deficits can mimic a cranial nerve palsies, internuclear ophthalmoplegia, or thyroid eye disease. In addition, some patients with suspected OMG do not respond to first-line treatment with pyridostigmine and are candidates for immunosuppressive therapy, which carries a risk of harmful adverse effects. For these reasons, ancillary testing is often used to confirm the diagnosis of OMG.

Confirmatory tests for OMG include edrophonium challenge, single-fiber electromyography (SFEMG), and serum acetylcholine receptor (AChR) antibody. Edrophonium testing lacks specificity and may be complicated by bradycardia and bronchial constriction, while SFEMG of the orbicularis oculi and frontalis muscles is sensitive and specific for OMG but is technically challenging to perform and not widely available. AChR antibody testing is highly specific but is traditionally thought to be less sensitive in OMG (approximately 50%) compared with generalized MG (85%-90%).

Our clinical experience suggests that AChR antibody testing is more sensitive in OMG than previously reported. We have also noted an association between high AChR antibody levels and progression from OMG to generalized disease. To test these hypotheses, we examined the results of AChR antibody testing, as well as the clinical implications of antibody values, in a large, multicenter cohort of patients presenting with OMG.

Methods

A retrospective, observational cohort design was used. Following approval by the University of Michigan and Michigan State University institutional review boards, medical records were searched to identify patients diagnosed with OMG between July 1, 1986, and May 31, 2013, at the 2 study sites. This study was deemed exempt from requiring patient consent as it was a retrospective collection of existing, deidentified data. Analysis was conducted from July 1, 2013, to May 15, 2015.

Data abstracted for each patient included age at symptom onset, sex, ocular symptoms (ptosis and/or diplopia), duration of follow-up, and progression time to generalized MG (if this occurred). All patients underwent serum testing for the AChR binding antibody (muscle AChR complexed with 125I-labeled-α-bungarotoxin; Mayo Medical Laboratories and Quest Diagnostics), with values greater than 0.02 nmol/L considered a positive result.

Descriptive statistics for patient characteristics were calculated and differences by antibody result were assessed using bivariate logistic regression for continuous and categorical variables. Two-sample t tests evaluated the association between age and likelihood of having a positive antibody test result, as well as between mean antibody titer and development of generalized MG. Multiple logistic regression was used to measure the association between all measured clinical variables and antibody result. Because studies of the incidence of MG have reported a bimodal age distribution, with younger women and older men most often affected, an interaction variable involving sex and dichotomized age (older or younger than the mean age of the cohort) was also examined. The model was evaluated using conventional criteria: goodness-of-fit tests, calibration plots, and area under the receiver operating characteristic curve.

Progression to generalized MG was compared by age and sex as well as antibody status with Kaplan-Meier estimates and log-rank tests. All analyses were performed in Stata, version 12 (StataCorp).

Results

We identified 223 patients with OMG, 84 (37.7%) of whom were female, with a mean (SD) age at diagnosis of 59.2 (16.4) years. Presenting symptoms included ptosis in 25 patients (11.2%), diplopia in 76 patients (34.1%), and both ptosis and diplopia in 122 patients (54.7%). Acetylcholine receptor antibody test results were positive in 158 patients (70.9%). Data on antibody binding capacity were available in 87 of these patients (55.1%), with a mean (SD) value of 6.13 (11.03) nmol/L (range, 0.02-64.3 nmol/L). Forty-five patients (20.2%) developed generalized MG during a mean follow-up interval of 60 months, with 162 (72.6%) of the cohort followed up for at least 2 years (Table 1).

The mean (SD) age of patients with a positive AChR antibody test result was significantly higher than those with a negative test result (61.1 [16.2] years vs 54.7 [15.9] years; \( P = .008 \)). Women were less likely to have a positive AChR test result (odds ratio [OR], 0.42; 95% CI, 0.23-0.76; \( P = .004 \)). A combined variable looking at the interaction of age and sex
was not significant (OR, 0.59; 95% CI, 0.163-2.210; P = .42) and was therefore excluded from the final model. Patients who developed generalized symptoms were more likely to have a positive AChR antibody test result (OR, 2.62; 95% CI, 1.106-6.23; P = .03) and had a significantly higher mean (SD) antibody binding capacity (12.7 [16.5] nmol/L vs 4.2 [7.9] nmol/L in the group that did not develop generalized MG; P = .002). Presentation with diplopia, ptosis, or both did not significantly predict antibody status (P = .55, P = .07, and P = .18, respectively).

After adjusting for the presence of ptosis or diplopia, age at diagnosis (OR, 1.03; 95% CI, 1.01-1.04; P = .007) and development of generalized symptoms (OR, 2.92; 95% CI, 1.18-7.26; P = .02) continued to significantly predict positive antibody status. In addition, women continued to have decreased odds of positive AChR antibody test results (OR, 0.36; 95% CI, 0.19-0.68; P = .002) (Table 2). The area under the receiver operating characteristic curve was 0.71, and the Hosmer-Lemeshow test was not significant (P = .27), suggesting that the model fit the data.

Kaplan-Meier curves for progression to generalized MG based on age and sex as well as antibody status are illustrated in Figure 1 and Figure 2. The log-rank test was significant for antibody status (P = .04) but not for age (P = .43) or sex (P = .83).

**Discussion**

In this study, we report the sensitivity of serum AChR antibody testing in the largest cohort of patients with OMG available to date. Previous estimates of the rate of antibody positivity in patients with OMG vary from 40% to 70% in studies of 15 to 86 patients. Our result of 71% sensitivity falls at the upper limit of this range and suggests increased utility of AChR antibody testing as a diagnostic tool.

There are several possible explanations for the higher sensitivity seen in our study compared with that in previous reports. One potential reason is that the reference standard used to define cases of OMG differs between studies. In our cohort, the reference standard was the clinical impression of 1 of 4 experienced neuro-ophthalmologists (including E.R.E. and W.T.C.) who followed up each patient longitudinally for a mean of 60 months. With extended follow-up, equivocal cases could be confirmed based on the natural history of disease and response to treatment. In contrast, studies reporting a lower yield for AChR antibody testing often use less-specific testing modalities as a reference standard. For example, Nicholson et al. found an AChR antibody sensitivity of 48%, but this cohort of patients also underwent edrophonium testing, a much less specific test, that likely led to the inclusion of patients without OMG. In these instances, an antibody test result considered a false-negative would actually be a true-negative test result.

Another possible explanation for our higher rate of AChR antibody sensitivity lies in the association between antibody levels and the duration of MG symptoms. Vincent and Newsom-Davis suggested that the high affinity of antibodies for end-plate AChRs at the neuromuscular junction could explain why some patients with OMG have negative serum test results early in the course of the disease but later seroconvert. By this logic, it is not until an individual reaches a certain level of receptor-binding saturation that antibodies begin to freely circulate in the peripheral blood. Because many patients in our cohort were referred to neuro-ophthalmology following evaluation by another physician, it is possible that they had a longer average symptom duration and thus higher levels of circulating antibodies for detection.

Finally, our higher rate of AChR antibody sensitivity may reflect changes in the testing assay compared with those used in prior studies. For example, a lowering of the titer cutoff required for a positive test result has occurred during the past few decades following reports that detectable levels of AChR antibodies in patients without MG are quite rare. We also report an increased likelihood of detecting AChR antibodies in older male patients with OMG. Although Lindstrom et al. reported no association between AChR antibody titers and age in generalized MG, Somnier did find a similar age-related trend in patients with OMG, with the sensitivity of AChR antibody testing increasing by 17% in patients presenting after the age of 50 years. Limburg et al. found no significant association between sex and antibody titer, although this cohort of patients with OMG was much smaller (n = 20). There did not appear to be a significant effect of age and sex on the risk of progression to generalized MG in our cohort based on the Kaplan-Meier estimate (Figure 1) and log-rank tests.

Another important finding is that a higher AChR antibody level at presentation was associated with an increased risk of developing generalized MG. Although antibody levels have widely been reported to be lower in OMG compared with generalized disease, this is the first report to our knowledge linking antibody levels in OMG with the risk of progression to generalized MG. Kupersmith et al. noted a significant association between positive AChR antibody test results in OMG and the risk of progression to generalized MG, a result supported by our Kaplan-Meier estimate (Figure 2) and log-rank test, but found no predictive value in antibody levels. In our cohort, the mean antibody level in patients who developed generalized symptoms (12.7 nmol/L) was significantly higher than in those who did not develop symptoms (4.2 nmol/L) (P = .002). Although antibody levels were available only for a subset of our cohort, we believe that this finding adds clinical value to

**Table 2. Multiple Logistic Regression Predicting Acetylcholine Receptor Antibody Testing in Ocular Myasthenia Gravis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.03 (1.01-1.04)</td>
<td>.007</td>
</tr>
<tr>
<td>Female sex</td>
<td>0.36 (0.19-0.68)</td>
<td>.002</td>
</tr>
<tr>
<td>Diplopia</td>
<td>0.79 (0.27-2.32)</td>
<td>.67</td>
</tr>
<tr>
<td>Ptosis</td>
<td>1.80 (0.93-3.47)</td>
<td>.08</td>
</tr>
<tr>
<td>Conversion to generalized myasthenia gravis</td>
<td>2.92 (1.18-7.26)</td>
<td>.02</td>
</tr>
</tbody>
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antibody testing, suggesting that patients with OMG and unusually high antibody levels may require closer follow-up with consideration for early immunomodulatory therapy.17

As mentioned previously, one limitation of our study is that the patients were drawn from a select population referred for subspecialty evaluation. This selection likely resulted in longer symptom duration and may have affected the outcome of AChR antibody testing. Also, we may have been able to capture more cases of generalization with longer patient follow-up. Although 72.6% of our patients were followed up for at least 2 years, our rate of conversion from OMG to generalized disease (20.2%) is lower than that typically quoted in the literature (approximately 50%).16 Finally, it is possible that the reference standard for diagnosing OMG in our study—specifically, the overall clinical impression of the treating neuro-ophthalmologist—may have been affected by a positive AChR antibody result, resulting in a skewed calculation of AChR sensitivity in OMG. However, 29.1% of the cohort received a diagnosis of OMG despite negative AChR antibody test results based on clinical features, SFEMG results, and response to treatment.

Conclusions

We demonstrate a higher sensitivity of AChR antibody testing (70.9%) than previously reported in a large cohort of patients with OMG. Older age, male sex, and progression to generalized disease were significantly associated with a positive antibody result. In addition, this is the first report to our knowledge of an association between high AChR antibody levels and progression from OMG to generalized disease. We hope that a better understanding of the implications of serum testing for OMG will improve its utility as a diagnostic tool.
ARTICLE INFORMATION

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Author Contributions: Dr Peeler had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Peeler, Eggenberger, Cornblath.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Peeler.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: De Lott.
Administrative, technical, or material support: Peeler, Lemos, Eggenberger.
Study supervision: De Lott, Eggenberger, Cornblath.

Conflict of Interest Disclosures: None reported.

REFERENCES


