An Algorithm for Acetylcholine Receptor Antibody Testing in Patients with Suspected Myasthenia Gravis

To the Editor:

Myasthenia gravis (MG)\(^1\) is a well-characterized autoimmune disease with an estimated prevalence of 1 in 5000 individuals (1). The clinical presentation varies from mild weakness of limited muscle groups (class I or ocular MG) to severe weakness of multiple muscle groups (class V or severe generalized MG). Detection of autoantibodies to the neuromuscular nicotinic acetylcholine receptor (ACHR) has proved useful in assisting in the diagnosis of MG; however, the complexity of this disease, combined with the variety of antibodies associated with MG, has led to multiple attempts to correlate disease severity with antibody detection and concentration. In general, the ACHR antibody concentration is directly proportional to disease severity, but neither the presence nor the absolute concentration of ACHR antibodies correlates with disease severity in any individual patient. ACHR antibodies are specific for MG because they are not detected in healthy individuals or in patients with other autoimmune or neuromuscular disorders; however, their absence does not rule out disease, because only about 85% of confirmed MG patients with generalized disease possess ACHR antibodies (2, 3). ACHR antibodies are less frequently detected in MG patients with mild disease or restricted muscle weakness (2, 3). MG patients without detectable ACHR antibodies often have antibodies to other neuromuscular junction proteins, such as muscle-specific kinase, which is detected in about 70% of seronegative MG patients (3).

The heterogeneous nature of the ACHR antibody response has led to the categorization of ACHR antibodies into 3 types: blocking, binding, and modulating. Assays of binding antibody, measure antibody binding to \(^{125}\text{I}-\alpha\)-bungarotoxin–labeled ACHR. Blocking antibodies interfere with receptor–ligand interaction and are measured by measuring the inhibition by patient serum of \(^{125}\text{I}-\alpha\)-bungarotoxin labeling of ACHR or by serum displacement of \(^{125}\text{I}-\alpha\)-bungarotoxin from bungarotoxin–receptor complexes. The dissociation constants reported for ACHR antibody \([K_D, \text{approximately } 2.35 \times 10^{-11} \text{ mol/L (4)}]\) and \(\alpha\)-bungarotoxin \([K_D, \text{approximately } 2.6 \times 10^{-10} \text{ mol/L (5)}]\) explain not only the ability of these antagonists to prevent the association of acetylcholine \([K_D, \text{6.2 } \times 10^{-6} \text{ mol/L (4)}]\) with its receptor but also the ability of blocking antibodies to displace \(\alpha\)-bungarotoxin from the ACHR. Modulating antibodies accelerate the rate of ACHR internalization by cross-linking adjacent receptors and are detected by measuring the amount of internalized, processed \(^{125}\text{I}-\alpha\)-bungarotoxin–labeled ACHR released from cultured cells (2, 3). In addition to being more technically demanding owing to the requirement of viable cell culture, modulating antibody assays cannot adequately distinguish between blocking antibody–released radioactivity and modulating antibody–released radioactivity and therefore cannot truly distinguish these 2 types of ACHR antibodies.

Different ACHR antibody–testing algorithms have been proposed to elucidate the relative importance of each ACHR antibody subtype with respect to diagnosis and disease severity, but a comparison of the various studies is complicated by the use of alternative methods in different patient populations. In this study, to determine the prevalence and frequency of each type of ACHR antibody, we retrospectively evaluated the presence and concentration of ACHR binding, blocking, and modulating antibodies in 39 380 samples of patient sera submitted from throughout the US to ARUP Laboratories for the assessment of all 3 ACHR antibodies.

Most samples \((n = 34 640, 88\%)\) did not possess detectable ACHR antibodies, whereas one or more ACHR antibody types were detected in 12% \((n = 4740)\) of the clinical samples tested. The clinical status of our patient population was not available to us; however, these data suggest that physicians most often use ACHR antibody testing to rule out rather than to confirm the diagnosis of MG (Fig. 1). In agreement with previous reports (2, 3), our most sensitive assay was the ACHR binding antibody assay, which was positive in 4178 (88%) of the 4740 ACHR antibody–positive serum samples. Modulating antibodies were detected in 70% \((n = 3297)\) of the samples, and blocking antibodies were least prevalent, detected in 65% \((n = 3074)\) of ACHR antibody–positive sera (Fig. 1). Combining binding and blocking ACHR antibody testing identified 97% of the patient population with detectable ACHR antibodies, whereas testing for binding and modulating ACHR antibodies identified only 93% of the ACHR antibody–possessing population. Of the 39 380 samples submitted, only 0.4% \((n = 160)\) tested positive for modulating antibodies in the absence of binding and blocking antibodies.

Approximately 15% of MG patients fail to demonstrate any ACHR antibodies; consequently, treatment does not change according to the type of ACHR autoanti-

---

1 Nonstandard abbreviations: MG, myasthenia gravis; ACHR, acetylcholine receptor.
body present. These data suggest that the most cost-effective algorithm in the diagnosis of MG is testing for ACHR binding and blocking antibodies with reflex testing for modulating antibodies only in the presence of one or both of these other ACHR antibodies.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References


Identification of the Hormone Kisspeptin in Amniotic Fluid

To the Editor:

Kisspeptin is the product of the KISS1 gene and is the ligand for the G-protein–coupled receptor, now known as the KISS1R. Both kisspeptin and KISS1R play a crucial role in the regulation of reproduction and puberty. The KISS1 gene encodes a precursor peptide of 145 amino acid residues, which undergoes proteolytic processing to generate kisspeptins 10, 13, 14, and 54. These peptides all share the common C-terminal decapeptide necessary for receptor activation. Inactivating mutations in the human KISS1R gene cause hypogonadotropic hypogonadism. During pregnancy, circulating plasma kisspeptin concentrations rise by

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures of Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoria: None declared.
Research Funding: All financial support was provided by the ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories (an enterprise of the University of Utah) and its Department of Pathology, or the University of Utah Department of Pathology.

Expert Testimony: None declared.

Thomas R. Haven2
Mark E. Astill2
Brian M. Pasi3
James B. Carper3
Lily L. Wu2,4
Anne E. Tebo2,4
Harry R. Hill2,4

2 ARUP Institute for Clinical and Experimental Pathology
Salt Lake City, UT
3 ARUP Laboratories, Inc.
Salt Lake City, UT
4 Department of Pathology
University of Utah
Salt Lake City, UT

*Address correspondence to this author at:
ARUP Institute for Clinical and Experimental Pathology
500 Chipeta Way
Salt Lake City, UT 84108-1221
Fax 1-801-584-5048
E-mail haventr@aruplab.com

Previously published online at DOI: 10.1373/clinchem.2009.140392