Laboratory Testing of Myasthenia Gravis: New Treatments Drive Change

Hans Frykman* **and Pankaj Kumar**

Myasthenia gravis (MG) is a neuromuscular disorder, mediated largely by autoantibodies (Abs), that targets functionally important proteins at the neuromuscular junction in the postsynaptic muscle membrane (1). MG is a heterogeneous disorder with regard to autoimmune profile, immunopathology, and the multifaceted immune response. Antibody testing plays a central role in confirming MG diagnosis and directing the management of MG patients. The majority (approximately 80%) of patients with generalized MG (GMG) develop antibodies against the acetylcholine receptor (AChR), whereas muscle-specific kinase (MuSK) Abs are detected in 1%–10% of patients (1–3). However, despite the progress made in serology, none of these antibodies are detected in 1%–15% of GMG patients, (i.e., sera are negative for AChR and MuSK Abs with current gold standard tests) (1–3). It is generally believed that patients with seronegative MG are most likely similar in their immunopathology to patients who are antibody positive, except that either the current testing methodologies are not sensitive enough to detect low-affinity or low-titer antibodies or the target proteins in the neuromuscular junction have not been fully identified. Substantial efforts have been made toward finding novel antibodies and developing improved detection techniques. In recent years, new antibody targets have been identified in some patients with MG against lipoprotein-receptor–related protein 4 (LRP4), agrin, collagen, antistriational muscle (Kv1.4, titin, and ryanodine receptors) and cortactin (1–3). These antibodies mostly coexist with AChR and MuSK Abs; therefore, studies are required to establish their pathogenic role in patients with MG. Antititin and anticortactin Abs are of particular interest because they are associated with more severe symptoms (1–3). Furthermore, some are not exclusively MG specific. For instance, Abs against LRP4 are reported in 1%–2% of patients with seronegative MG; however, LRP4 is also frequently detected among patients with amyotrophic lateral sclerosis (10%–23%) (4).

With the introduction of new, highly effective, antigen-specific treatment options (e.g., eculizumab in patients with refractory GMG who are positive for AChR Abs and rituximab in patients who are positive for MuSK Abs), it is increasingly important to evaluate levels of the specific antibody responsible for pathogenesis in individual patients. A major advancement in the field has been the recent and ongoing development of highly sensitive

[a]University of British Columbia, Vancouver, BC, Canada.
*Address correspondence to this author at: Department of Medicine, University of British Columbia, Vancouver, British Columbia, V6T 2B5 Canada. Fax 604-8220758; e-mail hans.frykman@ubc.ca.
Received August 7, 2020; accepted October 6, 2020.
DOI: 10.1093/jalm/jfaa199

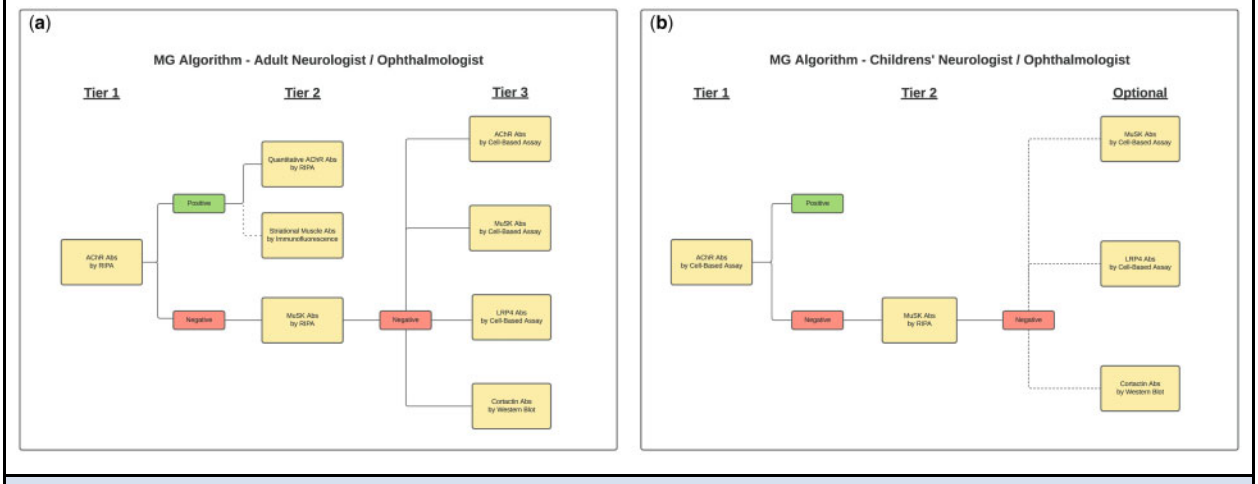

Fig. 1. MG algorithm for adults' and children's Ab testing. Dotted lines indicate optional testing (made with http://Lucidchart.com).

and specific live cell–based assays (CBAs) for clustered AChR, MuSK, and LRP4 Abs (4). Regarding detection of AChR Abs, although the radioimmunoprecipitation assay (RIPA) has been the gold standard test for the past few years, not all clinically relevant antibodies bind well to ^{125}I-α-bungarotoxin–labeled AChR antigens in solution (4). Typically, rapsyn-clustered AChR antigens (clustered AChR) are expressed on a HEK293 cell surface at a density similar to that at the neuromuscular junction. Clustered AChR Abs that have low affinity for soluble antigens (in standard RIPA assay) bind well to clustered AChR in its native form. In routine diagnostic settings, the clustered AChR Abs are detected in approximately 20% of patients with seronegative MG (4). The sensitivity of the clustered live CBA is increased when both the adult and fetal forms of the AChR antigens are used (2, 4). Interestingly, although the clustered CBA detects the same subclass of antibodies (IgG1 and IgG3) as standard RIPA, the patients with clustered AChR Abs have higher prevalence of ocular MG, milder disease severity, and better treatment response (2, 4). In addition, in the pediatric population, the importance of distinguishing between acquired and congenital MG makes the high-sensitivity CBA test a first-line option. In a recent study conducted at our laboratory, 16% (7/44) of children with seronegative MG tested positive for clustered live AChR Abs with a CBA (5). These 7 children with positive results have been verified as having acquired MG (5).

Anti-AChR and MuSK Abs are also detected by ELISA, fluorescence immunoprecipitation assay, and dot-blot methods; however, overall sensitivity and specificity are generally lower than the gold standard RIPA assay or the live CBA (1–3).

With advancements in improved diagnostics and, more important, early treatment of patients with GMG including intravenous immunoglobulin and plasma exchange, there has been a recent proposed change to MG testing algorithms. Because many treatments confound the test results (e.g., false-negative results are possible if patients have received intravenous immunoglobulin and plasma exchange within 6 weeks of their antibody test or rituximab or eculizumab within 24 weeks of their test), if the patient is under the care of an adult neurologist or ophthalmologist,

we now recommend a complete reflex testing algorithm on the first pretreatment sample from a patient with suspected MG, starting with the binding and blocking assays for AChR Abs by RIPA (Fig. 1, A). The simultaneous presence of striational Abs should be tested in AChR Abs–positive sample with suspected thymoma-associated MG (optional). If AChR Abs are negative, then reflex to MuSK Abs by RIPA. If MuSK Abs tests are negative, then use concurrent testing with high-sensitivity clustered AChR Abs, MuSK Abs, and LRP4 Abs by CBA and anticortactin Abs by western blots. In the case of children, the sample should be tested directly for clustered AChR Abs by CBAs (Fig. 1, B). If AChR Abs are negative, then reflex to MuSK Abs by RIPA. If both the tests are negative, then MuSK Abs, LRP4 Abs, and anticortactin Abs tests are optional. Importantly, the algorithm-based approach does not affect the test turnaround time because the samples are simultaneously tested and reported.

We foresee that in the near future, the specificity of these CBAs will convince clinicians that the assays should be part of systematic testing in the presence of seronegative generalized MG, thus stimulating laboratories to make these tests available. Availability remains an issue at the present time.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 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; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:* **Employment or Leadership:** H. Frykman, BC Neuroimmunology Lab. **Consultant or Advisory Role:** None declared. **Stock Ownership:** H. Frykman, BC Neuroimmunology Lab. **Honoraria:** H. Frykman, Alexion Pharmaceuticals. **Research Funding:** None declared. **Expert Testimony:** H. Frykman, Alexion Pharmaceuticals. **Patents:** None declared.

Acknowledgments: Authors would like to thank Alan Cashman for creating the figure.

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