Fixed cell-based assays for autoantibody detection in myasthenia gravis: a diagnostic breakthrough

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Accurate diagnosis of myasthenia gravis (MG), an autoimmune neuromuscular junction (NMJ) disease characterized by fluctuating muscle weakness, is essential to ensure prompt administration of potentially life-saving treatment. Autoantibodies against post-synaptic NMJ targets have been identified in patients with MG and serve as immensely useful diagnostic biomarkers. The most commonly detected autoantibodies in MG are those targeting the muscle-type nicotinic acetylcholine receptor (AChR), followed by muscle-specific tyrosine kinase (MuSK).[1] The gold standard of testing for these autoantibodies has historically been radioimmunoprecipitation assay (RIPA), although it has the disadvantage of requiring radioactive reagents. Enzyme-linked immunosorbent assay (ELISA) is an attractive alternative, but concerns have been raised surrounding false-positive results.[2] Live cell-based assay (CBA) has proven to be highly sensitive and specific, but its resource-intensive nature limits its use to specialized centres.[3–6] Recently, fixed CBA for autoantibody detection in MG, which is more easily implementable than live CBA in many laboratories, has become available. While initial retrospective studies of its diagnostic performance have been encouraging,[7,8] to date there has been no large, prospective study comparing fixed CBA to other assays for autoantibody detection in MG.

This prospective cohort study by Li and colleagues in *The Lancet Regional Health—Western Pacific*, which enrolled 2043 participants with clinically-diagnosed MG (generalized MG, 1110; ocular MG, 933) and 229 non-MG controls (MG mimics, 168; healthy individuals 61) from nine centres across China to compare autoantibody assay performance, addresses this research gap.[9] Serum from participants was aliquoted and submitted, without clinical information, to three independent clinical diagnostic laboratories for anti-AChR and MuSK testing by fixed CBA, RIPA and ELISA. The diagnostic accuracy of anti-MuSK fixed CBA was not significantly different from that of RIPA or ELISA; sensitivities across assays ranged from 2.4 to 2.9%, with perfect specificity except for two false-positives generated by ELISA. Meanwhile, diagnostic accuracy of anti-AChR fixed CBA was significantly higher than that of RIPA and ELISA; sensitivity of fixed CBA was 72.3% compared to only 62.7–64.1% for the other two methodologies, and specificity of fixed CBA and RIPA were similarly high at 97.8% while ELISA was comparatively lower at 94.8%.

Several conclusions can be drawn from this study that are of clear practical relevance to both the laboratorian and the clinician. Firstly, autoantibody testing by RIPA was confirmed to have reasonable sensitivity and high specificity for MG, as would be expected for a test that has historically been the gold standard for autoantibody detection in this disease. Secondly, autoantibody testing by ELISA was more liable to generate false-positives than fixed CBA and RIPA without any advantage to sensitivity, indicating that ELISA is less desirable as a screening assay for autoantibodies in MG. The third and most impactful finding was that anti-AChR fixed CBA had significantly higher diagnostic accuracy than the other two methodologies, with similarly high specificity and 8% higher sensitivity compared to RIPA. This study corroborates previous work indicating high diagnostic accuracy of fixed CBA and, by virtue of its prospective design and large sample size, is the strongest evidence to date that fixed CBA represents a first-line option for autoantibody testing in MG. Given its high diagnostic accuracy, relative ease of implementation, fast turn-around time and lack of need for radioactive reagents, it is plausible that fixed CBA may replace RIPA or ELISA for routine testing of anti-AChR and anti-MuSK in many clinical diagnostic laboratories worldwide.

Importantly, there are limitations to this study that should be considered. Firstly, use of a single assay to represent each test methodology as well as enrollment of only Chinese patients may limit generalizability, although findings are in keeping with previous smaller retrospective studies.[7,8] Secondly, specificity of both anti-AChR fixed CBA and RIPA was high but imperfect, underscoring the need to scrutinize positive autoantibody results in patients with atypical disease phenotypes like has been emphasized for autoimmune encephalitis.[10] Thirdly, while overall sensitivity of anti-AChR fixed CBA was higher than RIPA, 6% of those who were negative for anti-AChR by fixed CBA were positive by RIPA. Sensitivity was further increased by combining the two methodologies, suggesting benefit of a testing

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algorithm in suspected MG. Thirdly, and of relevance to the development of such an algorithm, live CBA was not compared in this study, which may have even higher sensitivity than fixed CBA while maintaining excellent specificity.[8] Future prospective studies comparing live CBA should take into consideration diagnostic accuracy, cost and turnaround time to help determine its place in a potential testing algorithm for suspected MG. One approach that may strike a balance among these three factors would be to screen for anti-AChR and anti-MuSK by fixed CBA as a first-line test, followed by live CBA for seronegative patients in whom there remains a high index of suspicion.[8] While further work is needed to the determine the optimal testing algorithm in clinical practice, the advent of accessible, highly accurate autoantibody testing by fixed CBA marks a diagnostic breakthrough in MG.

Declaration of interests

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