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REVIEW

Immunoserology Tests In Myastenia Gravis

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ABSTRACT

Myasthenia Gravis (MG) is an autoimmune neuromuscular disease that cause weakness due to the presence of autoantibodies that affect acetylcholine receptors (AChR) at the post synapse. Myasthenia gravis is a rare disease, but its prevalence is increasing. Classic clinical symptoms are characterized by fluctuating weakness. Diagnosis of myasthenia gravis requires a combination of clinical symptoms, physical examination and confirmatory tests in the form of bedside tests, serological tests, electrodiagnostics and imaging. Among the confirmatory tests available, serological tests has high sensitivity and specificity. In most cases of myasthenia gravis due to the presence of autoantibodies against AChR, other endplate proteins, such as muscle-specific receptor tyrosine kinase (MuSK) or lipoprotein-related protein 4 (LRP4) can be targets of autoantibodies. There are several examination methods for detecting autoantibodies in myasthenia gravis, several tests have been developed and commercialized such as radioimmunoprecipitation assay (RIPA),

INTRODUCTION

Myasthenia Gravis (MG) is an autoimmune disease of the neuromuscular junctions characterized by muscle weakness. Weakness is caused by autoantibodies that target acetylcholine receptors at the post-synapse. 1,2 This is a rare disease, but its prevalence is increasing. The peak incidence of this disease is found at the age of 20 to 40 years which is dominated by women with a ratio of 3:1.3,4 Diagnosis of myasthenia gravis requires a combination of clinical symptoms, physical examination and supporting examinations in the form of bedside tests, serological tests, electrodiagnostics and imaging.4,5

There is a distinct challenge in diagnosing cases with atypical clinical appearance such as the absence of ocular involvement or in

cases of seronegative myasthenia gravis which can occur in 7-34% of cases. Myasthenia gravis has a heterogeneous pathophysiology, depending on the autoantigen targets involved.[4.6]

MYASTENIA GRAVIS

Myasthenia gravis comes from the Greek "Myasthenia" which means muscle weakness, and the Latin "Gravis" which means weight. The term myasthenia gravis means severe muscle weakness. Myasthenia gravis is a rare disease, the prevalence varies with estimates ranging from 43 to 84 cases per 1 million people. The estimated number of new cases globally is 5.3 million people/year and positive findings of AChR autoantibodies are estimated to range from 4-18 per million people per year. 7,8 The peak incidence of this disease is bimodal, with the first peak occurring at the age of 20 to 40 years with a ratio of men and women of 1:3 and the second peak at the age of over 50 years with a ratio of men and women of 3:2.[2,7]

In MG, autoantibodies attack the nicotinic acetylcholine receptors (nAChR) on the postsynaptic membrane. The amount of ACh released is normal but the effect on the postsynaptic membrane is diminished. AChR antibodies affect neuromuscular transmission by activating complement in the NMJ, accelerating the degradation of AChR molecules and inhibiting AChR function. Antibodies destroy receptors faster than the body can replace them. Muscle weakness occurs when acetylcholine cannot activate receptors on the NMJ. [4,8,10]

In patients who do not have antibodies against AChR, there are other target antigens such as muscle-specific tyrosine kinase (MuSK), and margin-dependent proteins in the muscle membrane that are T cell-dependent. In addition to the explanation above, there is also an explanation regarding the possible role of the thymus gland in this disease.8,11 Classic clinical symptoms are characterized by fluctuating weakness or fatigue, either isolated in the ocular, bulbar, or generalized muscles. Ocular symptoms characterized by ptosis in one or both eyelids, diplopia of which 80% will develop into generalized MG. 2,8 Bulbar symptoms occur in 15% of cases. Severe symptoms of MG are characterized by symptoms of dysphagia, dyspnea with a vital capacity of <50% of normal, head drop, and weakness of the extremities both proximal and distal, which disrupts carrying out basic physical activities independently.[2.12]

Currently, there is no gold standard in diagnosing myasthenia gravis, information is needed from the history, physical examination, and confirmatory tests. 2,3 Bedside diagnostic tests include the warternberg, ice pack test, single breath counting test, prostigmine test, and tensilon test. 2,3,7,13,14 The next confirmation test is the Repetitive Nerve Stimulation (RNS) electrophysiological test which assesses the presence of decremental amplitude > 10% and Single-fiber electromyography (SFEMG) which in NMJ abnormalities will show jitter and blocking. [2,3,15]

Computer Tomography (CT) scan or Thoracic Magnetic Resonance Imaging (MRI) examination to ensure thymoma is also performed as a confirmation test. These tests have varying sensitivity and specificity. Among the confirmatory tests available, serological tests are the tests with high sensitivity and specificity. Serological tests are performed to detect anti-AChR antibodies. The sensitivity of this test is up to 96% for generalized MG but only 44% for ocular MG, and a high specificity for ocular 98% and 99% for generalized MG.3.5 The majority of patients (~85%) have anti-AChR antibodies. Anti-MuSK antibodies are found in approximately 6% of patients (approximately 50% of anti-AChR antibody negative), recently anti-lipoprotein-related protein 4 (LRP4) antibodies have been

found in approximately 2% of MG patients, and in some patients, no antibodies against these antigens are categorized as seronegative MG.[4.17]

Serological Tests as a Diagnostic Tool

In most cases, MG is caused by the presence of autoantibodies against AChR, but other endplate proteins, such as muscle-specific receptor tyrosine kinase (MuSK) or lipoprotein-related protein 4 (LRP4) can be autoantibody targets. Several tests have been developed and commercialized, such as the radioimmunoprecipitation assay (RIPA), and enzyme-linked immunosorbent assay (ELISA), there are also tests using antigen-expressing cells/cell-based assays (CBA) which are still being developed.

1. Acetylcholine receptor (AChR) antibodies

Pathophysiology

AChR antibodies are found in 85% of generalized MGs and 60% of ocular MGs. When it is found in ocular MG, it will increase the probability of becoming general MG. AChR antibody titer cannot be a prognostic marker of MG, because the number of antibody titers found does not correlate with MG severity. This inconsistent relationship may be due to some factors, including differences in anti-AChR specificity, immunoglobulin subclass, and ability to activate complement as well as differences in tissue and serum antibody concentrations. [20,21,22]

The acetylcholine receptor in the muscle is a transmembrane protein composed of 5 protein subunits: 2 identical α subunits, which contribute to the important structural elements of the attachment site for acetylcholine, and 3 homologous subunits β, γ, and δ.10 Antibodies to AChR consist of IgG 1 or 3, these antibodies primarily attack the main immunogenic region (MIR) of the acetylcholine receptor, namely the α subunit in the extracellular region.22 This antibody binding causes activation of the complement pathway to form a membrane attack complex (MAC). MAC causes the influx of calcium that causes local damage to the membranes, and the release of debris containing AChR into the synaptic cleft. Postsynaptic membrane damage will cause a loss of response to acetylcholine, which is confirmed with electrophysiological examination. MAC can also cause damage to sodium channels located in the secondary folds of the postsynaptic membrane, causing an increase in the excitability threshold required to trigger a muscle action potential.[24]

Three main types of AChR antibodies have been identified, namely binding, modulating, and blocking. Each has a different effector mechanism in influencing neuromuscular transmission.[24]

Anti-AChR binding

AChR-binding antibodies form binding to the acetylcholine receptor and activate complement at the NMJ. When an AChR-binding antibody forms a complex with AChR, the complex is recognized and attacked by the complement system and causes irreversible damage to the AChR and morphological deformation of the postsynaptic membrane resulting in impaired NMJ transmission. This mechanism also induces a decrease in sodium channels in the post-synaptic membrane. [10,24,25,26]

Anti-AChR modulating

AChR-modulating antibodies accelerate the degradation of the AChR molecule bound to the antibody (antigenic modulation). This antibody can cause cross-linked two receptor molecules. Receptors that bind to these modulating AChR antibodies are rendered useless, undergo

endocytosis, and are degraded. The speed of acetylcholine receptor endocytosis causes a decrease in the available acetylcholine receptors on the NMJ, causing muscle weakness. The presence of this type of antibody is usually associated with a more severe appearance of the disease.[10,24,25,26]

Anti-AChR blocking

AChR blocking antibodies, these antibodies have an affinity for binding to the binding site for acetylcholine, causing access blockade and disruption of acetylcholine signaling due to antibodies occupying the α subunit which should be the attachment site for acetylcholine to AChR, even at low concentrations, enough to cause access barriers, so neuro-muscular transmission is impaired and muscle activation does not occur.[24,25,26]

Methods of serological examination

There are several serological test methods for AChR antibodies, namely the radioimmunoprecipitation assay (RIPA), enzyme-linked immunosorbent assay (ELISA), flowcytometry and recently the cell-based assay (CBA) method. Each method has its sensitivity and specificity in detecting AChR antibodies.

Radioimmunoprecipitation Assay (RIPA)

The RIPA method uses a radioactive molecule 125I which will bind to laboratory-developed AChR and AChR. The method is carried out in 2 stages to increase sensitivity, first, the patient's serum will be purified and put into a container containing AChR which is developed in a laboratory and isolated, then radioactive molecule 125I α-bungarotoxin is added, if the serum contains anti-AChR antibodies then there will be competition between molecules radioactive with antibodies to bind to AChR. If a high radioactive signal is detected, it means that the radioactive molecule cannot bind to AChR, which indicates the number of receptors that have bound to antibodies from the serum.

AChR examination using the RIPA method has become the gold standard in the diagnosis of MG. Sensitivity is up to 85% for detecting generalized MG and about 65% for ocular MG and a very high specificity of up to 98%.17,21 The disadvantages of this method are the health risks for laboratory personnel and the impact of radioactive waste on the environment.[24]

Figure 1. AChR10 antibody effector mechanism

Enzyme-Linked Immunosorbent Assay (ELISA)

The next method is the ELISA examination method, this method relies on the ability of AChR antibodies in serum to bind to AChR competing with monoclonal antibodies such as MAb1 (which is in the ELISA container) and/or MAb2 and/or MAb3 (labeled as biotin). If there are no antibodies in the serum, a MAb1-AChR-MAb2/MAb3 bond complex will be formed which will be detected on the addition of streptavidin peroxidase at an optical density (OD) of 450 nm. The more antibodies present in the serum, the less OD will be detected.[24,27] The ELISA method has a sensitivity of up to 92% and a specificity of 100%, but in studies comparing RIPA and ELISA, 5% of false positives were found in the ELISA method. [21,25]

Cell-based Assay (CBA)

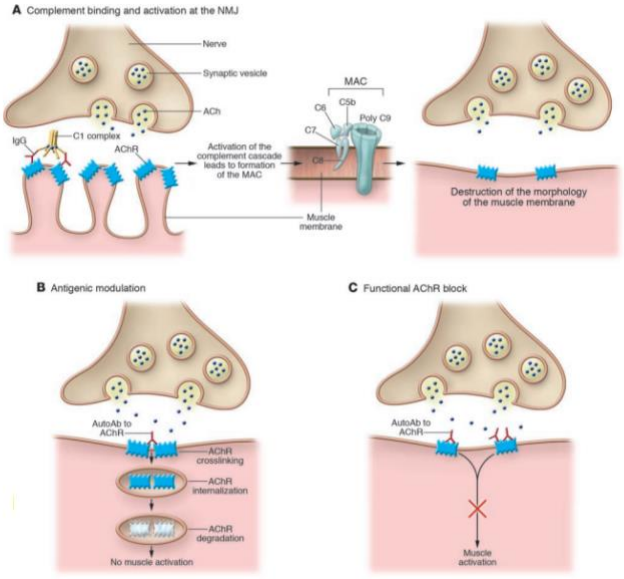
Examination of antibodies in MG has progressed rapidly but there are still 5-10% of patients who are seronegative for AChR as well as MuSK and LRP4, this may be related to the lack of RIPA sensitivity. The CBA assay for AChR antibodies has been developed. This method uses a human embryonic kidney (HEK) culture which is given AChR and rapsyn subunit DNA. Rapsyn is a key component in the subsynaptic cytoskeletal complex, added to obtain a dense AChR receptor group on the HEK membrane thereby facilitating the attachment of low-affinity antibodies.[20,28] This assay uses a fluorescent molecule α-bungarotoxin that passes through a laser to detect AChR antibodies.[20] Examination it has a sensitivity of 91% (better than RIPA) and a specificity of 99%.

The live-CBA (L-CBA) method can detect anti-AChR in up to 66% of patients who were previously MG seronegative, but this assay is still limited to laboratory studies. Recently a fixed-CBA (F-CBA) method has been developed for the commercial detection of AChR antibodies which has a higher sensitivity of 4% than RIPA, but this method still requires further comparative studies.

2. Muscle-specific receptor tyrosine kinase (MuSK) antibodies

Pathophysiology

Approximately 20% of MG patients do not have AChR antibodies and are referred to as seronegative MG patients. MuSK antibodies are found in



approximately 7-10% of all MG patients and up to 40% of general MG patients are seronegative for AChR antibodies. MuSK is an NMJ protein that is specifically expressed on the postsynaptic membrane, which is important for maintaining the functional integrity of the NMJ by concentrating AChRs in the NMJ. Inhibition of MuSK synthesis is known to cause AChR dispersion and end-plate disruption.[22] MuSK and NMJ protein and low-density lipoprotein receptor-related protein 4 (LRP4) together function as argin receptors, which are extracellular neural proteins. Argin, which is secreted from the presynapse, will interact with LRP4 causing the LRP4/MuSK complex, and with the help of the intracellular protein Dok7 which causes MuSK activation through phosphorylation. MuSK phosphorylation activates a signal to concentrate AChR at the NMJ.[22,25]

In contrast to AChR antibodies, the MuSK antibody titer correlates with disease MG severity.[19,25] MuSK antibodies belong to the IgG 4 subclass which does not cause complement activation. They are monovalent and cannot be cross-linked, so antigenic modulation does not occur. MuSK antibodies bind to the N-terminus of a portion of the extracellular domain of MuSK, causing inhibition of phosphorylation and interfering with argin-LRP4-MuSK-Dok7 signaling, thereby reducing the density of AChR on the postsynaptic membrane, causing progressive loss of AChR from the motor end plate and causing failure of synaptic transmission.[23,25,32]

Experimental studies have shown that passive transfer of MuSK IgG4 antibody binds to NMJ and causes a marked reduction in postsynaptic ACh sensitivity and decreased release of ACh from the presynaptic membrane during strenuous activity, leading to overt muscle fatigue. This experiment shows that the MuSK antibody does not only affect the postsynaptic membrane but also the presynaptic. In addition, MuSK antibodies are also known to interfere with the binding between MuSK and collagen tail (ColQ) of acetylcholinesterase. MuSK-ColQ interaction may be an explanation why acetylcholinesterase inhibitors are not effective in MG patients with MuSK antibody.[22]

Methods of serological examination

Radioimmunoprecipitation Assay (RIPA)

The RIPA method is becoming the standard test for MuSK antibodies. The procedure is the same as the RIPA test for AChR antibodies. MuSK antibodies in the sera of patients and controls will interact with radioactive ^{125}I-MuSK. Unbound radioactive ^{125}I-MuSK will be separated and the remaining radioactive level is proportional to the antibody level in the sample. The use of this method can detect MuSK antibodies in 13-41% of AChR seronegative patients. The specificity of this examination is estimated to be between 97% - 100%.[19,20,33]

Enzyme-Linked Immunosorbent Assay (ELISA)

In the ELISA method, the examination container has been coated with antigen, serum-containing antibodies will bind to the antigen and be detected by alkaline phosphate catalysis, causing a different color intensity that is proportional to the number of antibodies detected.[19,32] The sensitivity and specificity of the RIPA and ELISA methods are relatively the same as the sensitivity of 95% and specificity of 97% - 100%. Examination of the ELISA method is used more frequently due to the availability of examinations.[20,32]

Cell-based Assay (CBA)

Most patients with MuSK antibodies are detected positive by the RIPA method, but the CBA method is also useful because

MuSK is expressed strongly on human embryonic kidney (HEK) cells. This method uses human embryonic kidney (HEK) culture which is given MuSK DNA. Examination of the CBA method is more sensitive than RIPA or ELISA, and the specificity is estimated at 97-98%.[20,34] There is a cohort study that compared the accuracy of the ELISA examination with two other examination methods, namely CBA and RIPA which are in line with an accuracy of 80% and 90%.[22,32,33]

3. Lipoprotein-related protein 4 (LRP4) antibodies

Pathophysiology

Approximately 10% of MG patients do not have AChR or MuSK antibodies, these patients belong to the double seronegative MG group.[22,35,36] The percentage of positive double seronegative patients having LRP4 antibodies varies between 2-46% related to the examination method used and the ethnicity of the subject.[19]

Lipoprotein-related protein 4 is a transmembrane protein that contains several low-density lipoproteins. This protein has an important role in the development and maintenance of synapses. LRP4 forms a multiprotein complex with MuSK, acts as a muscle receptor for agrin and signals MuSK for AChR clustering in the NMJ via agrin/LRP4/MuSK/Dok signaling 7.[17,20,25]

LRP4 antibodies will inhibit the agrin-LRP4 interaction thereby disrupting AChR clustering in the postsynaptic membrane due to the absence of MuSK phosphorylation. The predominant LRP4 antibodies are of the IgG1 and IgG2 subtypes, as well as some of the IgG4 subtypes. These antibodies can bind complement and cause complement-mediated damage, as occurs with AChR-MG, then the IgG4 subtype can cause damage as MuSK antibodies do.[19,20,35]

Methods of serological examination

Enzyme-Linked Immunosorbent Assay (ELISA)

The examination of this method uses a plate coated with purified LRP4 recombinant, which is obtained from HEK added to the plasmid encoding LRP4. The examined serum is then added, followed by administration of secondary IgG alkaline phosphatase antibodies. Alkaline phosphatase immobilization activity was measured to estimate the amount of LRP4 antibody. This method found 9.2% of double seronegative positive patients had LRP4 antibodies. The specificity of the examination is estimated at 98%.[20]

Cell-based Assay (CBA)

HEK was added to the LRP4-GFP plasmid to bind to the LRP4 antibody. Then IgG fluorochrome was added to estimate LRP4 antibody levels semi-quantitatively. This test is more sensitive than ELISA, with a sensitivity approaching 98%.[20] A study using CBA in the double seronegative group found 19% of LRP4 antibody positive samples, or about 2% of all MG patients.[17]

The sensitivity of the LRP4 test is quite high.[37] Apart from NMJ, LRP4 is also found in motor neurons in the brain, and LRP4 antibodies have also been detected in 10-23% of cases of amyotrophic lateral sclerosis, several cases of other diseases such as optic neuromyelitis and multiple sclerosis and up to 20% MuSK antibody positive MG patients. LRP4 antibody detection is not directly a diagnostic guide for MG when compared to AChR or MuSK antibodies. Further validation is needed for the specificity of LRP4 antibody testing in MG and the interpretation of the test results must be based on clinical support.[17,20,38]

CONCLUSIONS

Serological tests in the population suspected of myasthenia gravis and selection of the right method are important for making an accurate diagnosis. Serological tests should be performed in patients are clinically suspected MG, because if a serological test is performed without clinical examination it will reduce the positive predictive value of the examination. In patients who are seronegative, the diagnosis should not rely solely on serological test results, because some markers can be found in other diseases (such as LRP4 antibodies in neuromyelitis optica, multiple sclerosis and amyotropic lateral sclerosis). Thus, a combination of positive serological tests (including anti-AChR, anti-MuSK, anti-LRP4) and highly supportive clinical manifestations form the basis for establishing the diagnosis of MG.

Selection of the appropriate serological examination should also consider clinical findings. AChR antibody detection is the first line of choice in relation to the majority of MG patients who are seropositive for AChR antibodies. MuSK antibody examination is performed if AChR antibody results are negative or clinically suspected of having MG associated with MuSK antibodies. If the patient belongs to the double seronegative category, the clinical assessment should be reviewed or other examinations considered. The RIPA examination method is still the examination of choice due to its high specificity and sensitivity. ELISA can be an alternative to RIPA examination. CBA is more sensitive than RIPA but its use is still limited.

REFERENCES

1. *Bird, S J. Diagnosis of Myasthenia Gravis. UpToDate .Wolters Kluwer. 2023. p1-6.*
2. *Hakim M, Gunadharma S, Basuki M. Pedoman Tatalaksana GBS, CIDP, MG Imunoterapi. PERDOSSI. Edisi 1. 2018; hal41-53.*
3. *Wijayanti. S. Aspek Klinis dan Penatalaksanaan Miastenia Gravis. FK UNUD. 2016; hal 1-13.*
4. *Jaber AL-Zwaini, I., & AL-Mayahi, A. (2019). Introductory Chapter: Myasthenia Gravis - An Overview. Selected Topics in Myasthenia Gravis. 2019; p 1-10. doi:10.5772/intechopen.85761*
5. *Benatar, M. A systematic review of diagnostic studies in myasthenia gravis. Neuromuscular Disorders. 2006; 16(7), 459–467. doi:10.1016/j.nmd.2006.05.006*
6. *Chan, K. H., Lachance, D. H., Harper, C. M., & Lennon, V. A. Frequency of seronegativity in adult-acquired generalized myasthenia gravis. Muscle & Nerve. 2007; 36(5), 651–658. doi:10.1002/mus.20854*
7. *Tugasworo, D. Myasthenia Gravis Diagnosis dan Tatalaksana. Undip Press Semarang. 2018; hal 1-29.*
8. *Miastenia Gravis and Related Disorders of the Neuromuscular Junction. In: Ropper A, Brown R, eds. Adam and Victor's : Principles of Neurology 11 th ed. McGraw Hill. 2019; p1469-77.*
9. *Muppidi, S.,Wolfe, G., Barohn, R. Diseases of the neuromuscular junction. Pediatric Neurology: Principles and Practice. 2012; 1549-1569.*
10. *Conti-Fine, B M., Milani, M., Kaminski, H J. Myasthenia gravis: past, present, and future". J. Clin. Invest. 2006; 116 (11): 2843–54.*
11. *Losen, M., Martínez-Martínez, P., Phernambucq, M., Schuurman, J., Parren, P W., D E Baets, M H. Treatment of myasthenia gravis by preventing acetylcholine receptor modulation". Annals of the New York Academy of Sciences. 2008; 1132: 174–9.*
12. *Khadilkar SV, Sahni AO, Patil SG. Miastenia gravis. JAPI. 2004 November; 52:897-903.*
13. *Information and Guidance for First Responders and Emergency Care. Emergency Management of Myasthenia Gravis. Myasthenia Gravis Foundation of America. 2019; p3.*
14. *Arie W, A A G A A., Adnyana, M O., Widyadharma, I P E. Diagnosis dan Tatalaksana Miastenia Gravis. Universitas Udayana*
15. *Neuromuscular Junction Disorders In: Shapiro, B E., Preston, B C. Electromyography and*

Neuromuscular Disorders Forth Edition. Elsevier. 2021;hal 654-61.
16. *Kang, S.-Y., Oh, J.-H., Song, S. K., Lee, J. S., Choi, J. C., & Kang, J.-H. Both binding and blocking antibodies correlate with disease severity in myasthenia gravis. Neurological Sciences. 2015; 36(7), 1167–1171. doi:10.1007/s10072-015-2236-8*
17. *Lazaridis, K., & Tzartos, S. J. Autoantibody Specificities in Myasthenia Gravis; Implications for Improved Diagnostics and Therapeutics. Frontiers in Immunology, 11. 2020;p1-13. doi:10.3389/fimmu.2020.00212*
18. *Andreetta, F., Rinaldi, E., Bartoccioni, E., Riviera, A. P., Bazzigaluppi, E., Fazio, R., … Baggi, F. Diagnostics of myasthenic syndromes: detection of anti-AChR and anti-MuSK antibodies. Neurological Sciences. 2017; 38(S2), 253–257. doi:10.1007/s10072-017-3026-2*
19. *Zisimopoulou P, et al, Serological diagnostics in myasthenia gravis based on novel assays and recently identified antigens, Autoimmun Rev (2013), http://dx.doi.org/10.1016/j.autrev.2013.03.002*
20. *Li, Y., Peng, Y., Yang, H. Serological Dagnosis of Myasthenia Gravis and Its Clinical Significance. Ann Transl Med 2023;11(7):290 | https://dx.doi.org/10.21037/atm-19-363*
21. *Oger, J., & Frykman, H. An update on laboratory diagnosis in myasthenia gravis. Clinica Chimica Acta. 2015; 444, 126–131. doi:10.1016/j.cca.2015.01.042*
22. *Meriggioli, M N., Sanders, D B. Muscle Antibodies in Myasthenia Gravis: Beyond Diagnosis?. Expert Rev Clin Immunol. 2012 July ; 8(5): 427–438. doi:10.1586/eci.12.34*
23. *Phillips, W D. Vincent, A. Pathogenesis of myasthenia gravis: update on disease types, models and mechanism. F1000Research. 2016; 5(F1000 Faculty Rev):1513 doi: 10.12688/f1000research.8206.1*
24. *Cromar, A. Development of An Assay for The Detection of Acetylcholine Receptor Blocking Antibodies by Flow Cytometry. Departement of Pathology. The University of Utah. 2014; p1-10.*
25. *Dresser, L., Wlodarski, R., Rezania, K., & Soliven, B. Myasthenia Gravis: Epidemiology, Pathophysiology and Clinical Manifestations. Journal of Clinical Medicine. 2021; 10(11), 2235. doi:10.3390/jcm10112235*
26. *Raquel C. Antunes. Autoimmune antibodies anti-AChR as biomarkers in myasthenia gravis. Universidade De Lisboa. 2014; p4-6.*
27. *Anti-AChR (Acetylcholine Receptor) ELISA Assay Kit. Eagle Biosciences, Inc. Catalog Number ACR31-K01*
28. *Cai Y, Han L, Zhu D, Peng J, Li J, Ding J, Luo J, Hong R, Wang K, Wan W, Xie C, Zhou X, Zhang Y, Hao Y and Guan Y (2021) A Stable Cell Line Expressing Clustered AChR: A Novel Cell-Based Assay for Anti-AChR Antibody Detection in Myasthenia Gravis. Front. Immunol. 12:666046. doi: 10.3389/fimmu.2021.666046*
29. *Cromar, A., Lozier, B. K., Haven, T. R., & Hill, H. R. Detection of Acetylcholine Receptor Blocking Antibodies by Flow Cytometry. American Journal of Clinical Pathology. 2016; 145(1), 81–85. doi:10.1093/ajcp/aqv009*
30. *Mirian, A., Nicolle, M W., Edmond, P., Budhram, A. Comparison of fixed cell-based assay to radioimmunoprecipitation assay for acetylcholine receptor antibody detection in myasthenia gravis. Journal of the Neurological Sciences. 2022; (432) 120084. https://doi.org/10.1016/j.jns.2021.120084*
31. *Spagni, G., Gastaldi, M., Businaro, P., Chemkhi, Z., Carrozza, C., Mascagna, G., et al. Comparison of fixed and live cell-based assay for detection of AChR and MuSK antibodies in myasthenia gravis. Neurol Neuroimmunol Neuroinflamm 2023;10:e200038. doi:10.1212/NXI.0000000000200038*
32. *Enzyme immunoassay for the qualitative and quantitative determination of autoantibodies against MuSK in human serum. Instruction for Use. IBL International. MuSK-Ab ELISA (RE51021)*
33. *Muscle Specific Tyrosine Kinase (MuSK) Autoantibody RIA Kit- Instructions for use. RSR Limited. 2021.*
34. *Vincent, A., Waters, P., Leite, M. I., Jacobson, L., Koneczny, I., Cossins, J., & Beeson, D. (2012). Antibodies identified by cell-based assays in myasthenia gravis and associated diseases. Annals of the New York Academy of Sciences, 1274(1), 92–98. doi:10.1111/j.1749-6632.2012.06789.x*
35. *Rivner, M. H., Quarles, B. M., Pan, J., Yu, Z., Howard, J. F., Corse, A., … Small, G. (2020). The Clinical Features of LRP4/Agrin Antibody Positive Myasthenia Gravis: A Multi-center Study. Muscle & Nerve. doi:10.1002/mus.26985*
36. *Kwon, Y N., Woodhall, M., Sung, J J., Kim, K K., Lim, Y M., Kim, H., et al. Clinical pitfalls and serological diagnostics of MuSK myasthenia gravis. Journal of Neurology. 2023: 270:1478–*

1486 https://doi.org/10.1007/s00415-022-11458-4

37. *Shen, C., Lu, Y., Zhang, B., Figueiredo, D., Bean, J., Jung, J. et.al. Antibodies against low-density lipoprotein receptor-related protein 4 induce myasthenia gravis. The Journal of Clinical Investigation. 2013; 123, 12: p 1-13.*

38. Rousseff, R.T. Diagnosis of Myasthenia Gravis. J. Clin. Med. 2021; 10, 1736. https://doi.org/10.3390/ jcm