Annals of Neurology and Neuroscience
Case Report

Double Seronegative Myasthenia Gravis- Case Report of Two Patients and Review of Related Antibodies

Priyanka v Kashyap[1*], Vaibhav O P Pandey[2], Mayur K Bhat[2]

[1]Associate Professor & Incharge Neurology, AIIMS Bhopal, India

[2]Senior Resident (Academic) Neurology, AIIMS Bhopal, India

Citation: *Priyanka v Kashyap, Vaibhav O P Pandey, Mayur K Bhat. Double Seronegative Myasthenia Gravis- Case Report of Two Patients and Review of Related Antibodies. Anna Neur & Neurosci. 2026;1(2):1-7.*

Received Date: 14 November, 2026; **Accepted Date:** 17 November, 2026; **Published Date:** 18 November, 2026

[*]**Corresponding author:** Priyanka v Kashyap, Associate Professor & Incharge Neurology, AIIMS Bhopal, India

Copyright: © Priyanka v Kashyap, Open Access 2026. This article, published in Anna Neur & Neurosci (ANN) (Attribution 4.0 International), as described by http:// creativecommons.org/licenses/by/4.0/.

ABSTRACT

Myasthenia Gravis (MG) is an autoimmune neuromuscular junction disorder traditionally characterized by antibodies against the acetylcholine receptor (AChR) or muscle-specific kinase (MuSK). Approximately 10–15% of generalized and 50% of ocular MG cases remain "double-seronegative" (DSNMG), complicating diagnosis and management. We present two atypical cases of DSNMG. One presenting at sixth decade with respiratory failures with proximal muscle weakness along with facio-ocular bulbar weakness following sepsis, with notable positive family history for myasthenia gravis and rapid improvement with immunoglobulins (IVIG). Another case with same phenotype but without negative family history and refractory to standard steroids attained clinical remission with IVIG following rituximab therapy. A review of the "immune renaissance" in MG emerging pathogenic biomarkers is being discussed. Identifying novel autoantibodies through advanced cell-based assays is critical for personalizing treatment. Recognition of these subgroups allows for earlier intervention with targeted immunotherapies, reducing morbidity in patients who lack traditional serological markers. Hence presenting this case series.

Keywords: Myasthenia Gravis; Case Report; Antibodies

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disorder resulting from autoantibodies (Abs) against various functionally important components located at the neuromuscular junction (NMJ) in postsynaptic muscle membrane.[1] Autoantibodies directed against the nicotinic acetylcholine receptor (AChR) are found in majority of patients (80%) with generalized MG but in cases of ocular-restricted MG, they are found in upto half of the patients.[2] Approximately one-half of AChR antibody seronegative patients have autoantibodies against muscle-specific kinase (Musk) making it 5-10% of the total generalized MG patients.[3-5] Among the rest 10% of MG-patients, both AChR- and MuSK-antibodies are not found, making them difficult to diagnose with certainty. Over a period of three years, we saw two cases of ocular onset generalized MG who were negative both for

AChR antibodies and musk antibody and atypical presentation making it interesting to present. Here we present two cases of double seronegative myasthenia.

CASE 1

A 60 years old gentleman known diabetic, hypertensive and old ischemic stroke was admitted with high grade fever and sepsis followed by markedly fatigue, motor weakness in all limbs with dropping of both eyelids and change in voice and respiratory muscle with worsening.

Past history was non-contributory. There was no dysautonomia. On examination vitals were stable, GCS 15/15, no lymph nodes of thyromegaly, single breath count (SBC) was 5-6, conscious and oriented, dysarthria with hypophonia was noted with bilateral ptosis. Patient had bilateral gaze evoked nystagmus, bilateral facial muscles weakness, no bulbar or tongue weakness. In motor system examination no atrophy or wasting, Tone was normal in all 4 limbs, power assessment showed proximal quadriparesis (proximal shoulder 3/5, elbow 4-/5, wrist 5/5 & at hip 2/5, knee 3/5 and ankle jerk was 5/5, DTR BJ & TJ 2+, rest absent. Fatigability tests in form of arm abduction, sustained upgaze and SBC was positive. His son also suffers from asymmetric fatigable eye ptosis which worsens in evening without generalized weakness. A probable diagnosis of NMJ involvement with generalised MG in crisis was considered, admitted in neurology ICU for further management with IVIG with pyridostigmine and other supportive care. Serum AchR antibody and anti-Musk antibody was negative, CPK was 40, viral marker, TSH, neostigmine test & ANA was negative. CECT thorax, 2D echo and USG abdomen was normal. RNS was attempted however due to technical issues couldn't be done. NCV was normal (no evidence of demyelination). Patient improved symptomatically with IVIG steroids and other supportive care (MG ADL score on admission 23 on discharge 1). QMG was 23 on admission and on discharged 12 and MGFA 4.With above mentioned treatment patient improved symptomatically in MGADL scores and SBC (30-35). He gradually improved and discharged on immunosuppressants and pyridostigmine. Atypicality in form of first presentationat six decades, crisis as presentation with family history with seronegative.

CASE 2

57 years old right-handed gentleman presented with shortness of breath for last 1 year and drooping of eyelid and double vision for last 6 months. Exertional shortness of breath worsened to be persisting in lying down posture. After 5 months his near static shortness of breath, he developed progressive drooping of his left eyelid and double vision, maximal on lateral gaze and objects appear side by side. He also noticed dysarthria, neurogenic dysphagia and dropping of his head forward suggestive of neck flexor weakness. Both Anti AChR and Anti Musk antibody were negative. He needed BiPAP ventilation for type 2 respiratory failure and received steroids and mycophenolate. Later developed paradoxical breathing and dysarthria along with restricted extra ocular movements & ptosis (and proximal weakness) with normoreflexia. ANA was 1:320 titre positive (speckled and nucleolar pattern) and in

ENA profile was positive for PCNA. Paraneoplastic profile (NIMHANS) was negative. On electrophysiology, normal nerve conduction study of all four limbs with needle EMG in various muscle groups showed spontaneous activities suggestive of denervation along with early and complete recruitment. Repetitive nerve stimulation (RNS) study was nonconclusive as of technical issues. As the disease was poor responding to the above immunosuppressants, rituximab was initiated with protocol of 0, 2 weeks, 6 months interval. After 3 dosages of rituximab, patient had significant improvement in MRS, his respiration improved to an extent that he didn't require BiPAP support, ptosis and diplopia have improved and could hold his neck by himself for long duration.

DISCUSSION

Although the majority of the MG patients have either AChR or Musk antibodies, there are quite a few other antibodies associated with MG. AChR related MG present with classic features of proximal weakness with retained reflexes, whereas Musk antibodies related patients present with bulbar and other cranial muscle involvement, with a good response to immunotherapy, that includes Prednisone and also Rituximab. Of the initial first negative MG, among the patients seronegative for AChR-Ab MG, 15% of patients become seropositive after 12 months, and among the persistently seronegative patients, 38% are Musk antibody-positive, and seropositivity for miscellaneous, not muscle related antibodies are 43%.[6]

Seronegative MG is a heterogeneous group of autoimmune disorder. As the immune renaissance appeared, various antibodies are detected by use assays available making less percentage of seronegative MG. Among them, LRP4 can be present in 1-5% of MG patients with any phenotype, and among 7-33% of MG patients without both of AChR and Musk antibodies.[7] Among around 10% of MG patients, antibodies against Agrin has been detected, in both AchR and MUSK antibody positive patients, or LRP4 antibody positive patients and also among the patients with no other detectable antibodies. The finding that these antibodies have only been detected in MG patients suggests they are specific to Myasthenia Gravis. Although no directly pathogenic effect of agrin antibodies has been established, such antibodies inhibit Musk phosphorylation and AChR clustering in experimental mice models.[9-11] Anti-titin antibody is another antibody detected in 20-30% of MG patients with AChR antibodies, mostly in patients with thymoma associated disease or late-onset MG. Their presence indicates a more severe form of MG with mild myopathy. In 10-20% of patients with MG, antibodies against the α subunit of the voltage-gated K+ channel KV 1.4 in skeletal muscle are detected, but in vivo antibody binding to this channel has not been confirmed.[12] Cortactin antibodies have been found in 20% of MG patients negative for AChR and Musk antibodies, and in 5% of AChR antibody positive MG patients. Cortactin is a postsynaptic neuromuscular junction intracellular actin-binding protein in the skeletal muscle that promotes actin assembly and has been found in 19.7% of DSNMG.[14] Cortactin acts downstream from agrin/LRP4/ Musk, and promotes the clustering of AChR, thus playing an important role in neuromuscular transmission, and dysregulation in pathogenesis of MG. Antibodies directed against ryanodine receptor (RyR) are present in 70% of AChR positive MG patients with thymoma, and

in 14% of patients with late-onset AChR MG.[15] Antibodies against collagen Q were detected recently in the serum of 12 of 415 (3%) patients with MG but its pathogenic role is still unproven.[16] Since there are no commercial tests that are available to measure these antibodies, identification of these subgroups can be done in only a few centres.

Double-seronegative MG (DSNMG) refers to the patients with generalized MG in whom AChR and musk antibodies are absent, and occurs in around 15% of Generalised MG patients, and upto 50% of ocular MG patients. This seronegative MG group is also autoimmune MG mediated by autoantibodies lacking detectable threshold ith available measures. Lack of a detectable autoimmune biomarker (pathogenic antibody) in DSNMG leads to a hesitancy in formulating the diagnosis and management in these patients, especially in the ocular forms of the disease. A biomarker for restricted or dominant ocular MG phenotype is a must for proving it an autoimmune NMJ disease. This is because of the varied differential diagnosis of ptosis and diplopia in different decades of presentation, and electrophysiogical studies or pharmacological tests may not be conclusive in many of the patients. The association of DSNMG group includes patients in whom the threshold for detection has not been achieved by conventional testing, or the presence of antibodies that are yet to be defined and hence can't be tested, and the patients with symptoms of myasthenia which are not antibody mediated but rather due to late onset genetic forms due to rapsyn or other related muscle protein mutations. There is a role of repeated testing, which may lead to revision of diagnosis from seronegative MG group to one of the subgroups due to an increase in the test sensitivity, or mechanisms such as epitope spreading, or due to increased concentration of antibodies that now exceed the threshold limits.

Characterization of MG pathogenic antibody specificities helps in individualizing the most appropriate treatment for subset of MG. Low prevalence and lack of controlled studies with no appropriate guidelines add hurdle in treating this subset of seronegative MG patients. They are treated like antibody positive MG according to the first and second line immunomodulatory therapies. Thymectomy in seronegative patients is advised in only in patients who have revised diagnosis after detection of low-affinity anti-AChR antibodies on repeat testing. This urges for new and more sensitive biomarkers for DSN MG group. Different antibodies against several newly identified antigens at neuromuscular junction are being currently assessed. These autoantibodies presence confirms ongoing immune process which makes immunotherapy mandatory with promising results. Unidentified autoantibodies against the known and unknown and novel antigens, necessitates discovering of more sensitive assays which can be used in practice making MG diagnosis easier and less missed. Recent improved tests are being available like CBA (cell-based assay) for intracellular MG antigen including Musk, LRP4 and clustered AChR, RIPA for titin and two-step RIPA for AChR and Musk which have reduced the percentage of SNMG giving them a biomarker.[17] CBAs is unable to provide titre information and lacks commercial kits are, they are being suggested as second line tests in seronegative MG (by the standard RIPAs). Assays based on cytometric CBAs or immunosticks detect more than one antigen simultaneously and serve as a potentially useful alternative, if reliably and specifically produce quantitative

results. Immobilisation of several antigen expressing cells on different dots on a slide of immunosticks allows testing for all the major MG autoantibodies and reduce the time as are faster method. A retrospective study in Spain assessed the primary outcome of DSNMG patients with cortactin antibodies and inferred that presence of cortactin antibodies in DSNMG patients were younger, associated with milder forms of the disease, had more ocular MG at onset, and, and also had lesser bulbar signs at the time of maximal worsening, when compared with MG patients who were positive for Anti-AChR antibodies. Of a total of 17 patients with ocular DSNMG, 4 (23.5%) patients had antibodies to cortactin, and 2 patients with ocular MG went on to develop generalized MG. It may be inferred that the detection of cortactin antibodies in routine DSNMG diagnosis could potentially aid in identifying cases of ocular MG.[18] No association was observed between cortactin antibodies and MuSK, LRP4, and anti-striated muscle antibodies. Although a subset of individuals with MG and AChR antibodies exhibited cortactin antibodies, their prevalence was markedly lower than that observed in DSNMG cases (P = .02). Cortactin antibodies has not been found in healthy controls. Traditionally, double-seronegative MG has been regarded as clinically comparable to AChR-positive MG, both in presentation and therapeutic response. These patients more commonly presented with restricted ocular involvement at onset and required immunosuppressive therapy less frequently, had lesser rates of generalized disease development and presence of thymoma. Antibodies against low-density lipoprotein receptor—related protein 4 (LRP4) has been detected in 2-27% subgroups of patients with DSN MG14-16, LRP4 antibody subgroup exhibits a female predominance and is characterized primarily by ocular or mild generalized myasthenia gravis. Low-affinity IgG1 AChR antibodies binding clustered AChR in 38% of patients with DSNMG using human embryonic kidney cells based cellular assay.[19] Patients with low-affinity antibodies clustered to AChR typically present with early-onset, milder disease, characterized predominantly by isolated ocular symptoms and a low rate of generalization, and bulbar symptoms in 25% patients. Experts advocate for repeat antibody testing in seronegative MG patients within 6 to 18 months following the initial evaluation. Categorizing patients with MG into subgroups based on serological criteria has significant implications for diagnosis, prognosis, and treatment strategies. In one series, cortactin antibodies were present in upto 24% of patients with ocular DSNMG, and their detection proved to be a valuable biomarker, particularly within this patient group. Amongst the 9.5% of patients with MG and AChR antibodies, cortactin antibodies were identified. However, the clinical features of this subgroup were comparable to those of other patients. Furthermore, no correlation was observed between cortactin antibodies and MuSK, LRP4, or anti-striated muscle antibodies in this cohort. The absence of other autoantibodies in most patients indicates that the cortactin antibodies likely did not result from epitope spreading or neuromuscular junction damage. However, cortactin antibodies have been reported in other neuromuscular autoimmune disorders and in approximately 5% of healthy controls. Cortactin antibody has potential to serve as an important biomarker in seronegative MG especially in ocular restricted or ocular predominant form.[20]

CONCLUSIONS

Novel antibodies are been identified for double and triple seronegative MG narrowing the seronegative MG group. Among them LRP4, agrin, cortactin antibodies serve as a good biomarker based on identification on various assays and thus helping clinician in confidently deciding the use of

various immunosuppressants like rituximab in early period to decrease the morbidity and associated mortality with this subgroup. The future strategies include antigen- specific therapies, such as immunoadsorption or induction of immunological tolerance against AChR, Musk, and LRP4 requiring dedicated preclinical and clinical trials for implementing them into clinical practice.

REFERENCES

1. Gilhus NE, Skeie GO, Romi F, Lazaridis K, Zisimopoulou P, Tzartos S. Myasthenia gravis autoantibody characteristics and their implications for therapy. Nat Rev Neurol. 2016;12(5):259–68.
2. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. Lancet Neurol. 2015;14(10):1023–36.
3. Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. Nat Med. 2001;7(3):365–8.
4. Evoli A, Tonali PA, Padua L, Monaco M Lo, Scuderi F, Batocchi AP, et al. Clinical correlates with anti-MuSK antibodies in generalized seronegative myasthenia gravis. Brain. 2003;126 10):2304–11.
5. DeChiara TM, Bowen DC, Valenzuela DM, Simmons M V, Poueymirou WT, Thomas S, et al. The receptor tyrosine kinase MuSK is required for neuromuscular junction formation in vivo. Cell. 1996;85(4):501–12.
6. Chan KH, Lachance DH, Harper CM, Lennon VA: Frequency of seronegativity in adult-acquired generalized myasthenia gravis. Muscle Nerve. 2007;36:651–658.
7. Higuchi O, Hamuro J, MotomuraM, Yamanashi Y. Autoantibodies to low-density lipoprotein receptor-related protein 4 inmyasthenia gravis. Ann Neurol. 2011;69(2):418 422.
8. Lazaridis K, Tzartos SJ. Autoantibody Specificities in Myasthenia Gravis; Implications for Improved Diagnostics and Therapeutics. Front Immunol. 2020;11:212.
9. Zhang B, Shen C, Bealmear B, Ragheb S, Xiong W-C, Lewis RA, et al. Autoantibodies to agrin in myasthenia gravis patients. PLoS One. 2014;9(3):e91816.
10. Gasperi C, Melms A, Schoser B, Zhang Y, Meltoranta J, Risson V, et al. Anti-agrin autoantibodies in myasthenia gravis. Neurology. 2014;82(22):1976–83.
11. Witzemann V, Chevessier F, Pacifici PG, Yampolsky P. The neuromuscular junction: selective remodeling of synaptic regulators at the nerve/muscle interface. Mech Dev. 2013;130(6–8):402–11.
12. Romi F, Suzuki S, Suzuki N, Petzold A, Plant GT, Gilhus NE. Anti-voltage-gated potassium channel Kv1.4 antibodies in myasthenia gravis. J Neurol. 2012;259(7):1312–6.
13. Gallardo E, Martínez-Hernández E, Titulaer MJ, Huijbers MG, Martínez MA, Ramos A, et al. Cortactin autoantibodies in myasthenia gravis. Autoimmun Rev. 2014;13(10):1003–7.
14. Gallardo E, Martínez-Hernández E, Titulaer MJ, et al. Cortactin autoantibodies in myasthenia gravis. Autoimmun Rev. 2014;13(10):1003-1007.
15. Skeie GO, Apostolski S, Evoli A, Gilhus NE, Illa I, Harms L, et al. Guidelines for treatment of autoimmune neuromuscular transmission disorders. Eur J Neurol. 2010;17(7):893–902.

16. Zoltowska Katarzyna M, Belaya K, Leite M, Patrick W, Vincent A, Beeson D. Collagen Q—a potential target for autoantibodies in myasthenia gravis. J Neurol Sci. 2015;348(1–2):241–4.
17. Zisimopoulou P, Brenner T. Serological diagnostics in myasthenia gravis based on novel assays and recently identified antigens. Autoimmun Rev . 2013;12(9):924-30.
18. Vicente EC, Gallardo E. Clinical Characteristics of Patients With Double-Seronegative Myasthenia Gravis and Antibodies to Cortactin. JAMA Neurol. 2016;73(9):1099-1104.
19. Leite MI, Jacob S, Viegas S, et al. IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis. Brain. 2008;131(pt 7):1940-1952.
20. Jacob S, Viegas S, Leite MI, et al. Presence and pathogenic relevance of antibodies to clustered acetylcholine receptor in ocular and generalized myasthenia gravis. Arch Neurol. 2012;69(8): 994-1001.