Characteristics of Triple Seronegative Myasthenia Gravis: A Single Center Experience

Jonathan Morena D.O.[1], Benjamin Jiang M.D.[1], Miriam Freimer M.D.[1], J. Chad Hoyle M.D.[1], Bakri Elsheikh MBBS[1], W. David Arnold M.D.[1], Samantha LoRusso M.D.[1]

[1]The Ohio State University, Department of Neurology, Columbus, OH, USA

ABSTRACT

Background: There is variability in the literature regarding the characteristics of triple seronegative myasthenia gravis (SNMG) patients. Most studies were performed before LRP4 antibodies were discovered, and characterizations of triple seronegative patients are lacking in the literature.

Methods: We retrospectively investigated patients diagnosed with myasthenia gravis (MG) at Ohio State University from 2009 to 2019. Triple SNMG was defined by a history and examination that was consistent with MG and positive SFEMG, RNS or edrophonium testing, but negative serology for AChR, MUSK, and LRP4 antibodies.

Results: A total of 210 AChR+, 9 MuSK+, 6 LRP4+, 9 double SNMG, and 21 triple SNMG patients were reviewed. Triple SNMG patients required significantly fewer immunosuppressive agents compared with AChR+ patients (p=0.0001) and a trend towards a less frequent history of hospitalizations, myasthenic crises and intubations compared to all antibody positive groups. Triple SNMG patients had a significantly higher frequency of ocular disease (33%) compared to AChR+ patients (13%) (p=0.0250). One triple and one double SNMG patient had thymic hyperplasia and improved after thymectomy. 11 triple SNMG patients had negative genetic testing for CMS.

Conclusion: Our results further elucidate the clinical characteristics of triple SNMG, which include the predominance for ocular disease and a less severe disease course. Although likely rare, investigation for thymic pathology should be a consideration even in SNMG, and thymectomy should be considered when there are thymic abnormalities on imaging. We did not find alternate diagnoses in SNMG patients and thus ancillary testing should be considered in carefully selected patients for cost-effective care.

Introduction

Myasthenia Gravis (MG) is an autoimmune neuromuscular junction (NMJ) disorder affecting roughly 14-40 per 100,000 individuals in the United States. Acetylcholine receptor (AChR) autoantibodies were discovered in 1973 and are found in about 80% of MG patients [1,2]. In 2001, muscle-specific kinase (MuSK) antibodies were described, accounting for another 7-15% of generalized MG patients [3]. Lipoprotein receptor-related protein (LRP4) antibodies were found to be likely pathogenic in 2011 and recently became available for commercial testing [4]. The percentage of patients with double (i.e. negative testing for AChR and MuSK antibodies) seronegative myasthenia gravis (SNMG) with elevated titers of LRP4 antibodies has varied depending on the population studied, ranging from 2-50% [4-6]. The remaining population that is negative for AChR, MuSK and LRP4 autoantibodies is referred to as triple SNMG. For some of these triple SNMG patients, it may be that either the assay is not sensitive enough to identify the antibody or other disease-causing antibodies have not yet been identified [7-9].

In comparison to AChR or MuSK antibody positive patients, those with SNMG have been found to have overall less severe disease, sometimes making diagnosis more challenging [8,10]. Bulbar and respiratory muscle involvement are less frequent, and a thymoma is rarely found [11]. Electrophysiological abnormalities of NMJ transmission have been found to be more severe in seropositive compared to seronegative patients [12,13]. Further compounding the diagnostic challenge, congenital myasthenic syndromes (CMS) can also closely mimic SNMG [14,15].

The majority of studies that have evaluated the characteristics of patients with SNMG were performed prior to routine testing of LRP4 antibodies. Therefore, in this retrospective study, we sought to compare seropositive MG versus triple SNMG patients seen at The Ohio State University Wexner Medical Center (OSUWMC) in a ten-year span to advance the understanding of triple SNMG.

Methods

All patients seen at OSUWMC with an ICD-10 code diagnosis of MG from March 1, 2009 to June 30, 2019 were retrospectively reviewed. Cases were included if the patient was 18 years of age or older and had at least one of the following: positive antibodies (AChR, MuSK, or LRP4), a positive single fiber electromyogram (SFEMG),

a positive repetitive nerve stimulation (RNS), or a positive edrophonium test. Antibody positive MG was defined on the basis of elevated AChR, MuSK or LRP4 antibody titers. Triple SNMG was defined by a history and examination that was consistent with MG, positive NMJ testing (SFEMG, RNS or edrophonium testing), and negative testing for AChR, MUSK, and LRP4 antibodies. Data was collected through Research Electronic Data Capture (REDCap) and included patient demographics, clinical characteristics, and assessments. The clinical characteristics included age of symptom onset, time from onset to diagnosis, muscles affected, presenting symptoms, history of hospitalizations, history of myasthenic crisis, history of intubations, immunosuppressive agents used, history of thymectomy, and history of autoimmune disease. Results of physical examination, antibody testing, chest computed tomography (CT), electromyogram (EMG), RNS, SFEMG, and congenital myasthenia panel testing were reviewed. The congenital myasthenia panel included 26 mutations known to cause CMS. Ohio State's review board IRB approved this study. The review committee waived the requirement for written informed consent.

Graphpad Prism (ADD version) was used for all analyses. Descriptive statistics were calculated to summarize the groups (AChR, MuSK, LRP4, and SNMG). Continuous data were compared between groups using one-way ANOVA with Dunnett's multiple comparisons tests to compare the mean of the AChR, MuSK, and LRP4 versus the mean of the SNMG. To compare frequency data, Fisher's exact test was used to compare SNMG versus each seropositive group (AChR, MuSK, and LRP4).

Results

A total of 210 AChR+, 9 MuSK+, 6 LRP4+, 9 double SNMG, and 21 triple SNMG patients were identified and reviewed. A total of 16 patients were excluded due to negative antibody testing and either negative NMJ testing or having a clinical presentation that was atypical for a NMJ disorder. There were four patients with positive SFEMG testing that were excluded because the clinical presentation was either more consistent with an alternate diagnosis or was unclear. In these four patients, the alternate diagnoses included steroid myopathy, dropped head syndrome and CMS. One patient had an unclear diagnosis. Table 1 summarizes the demographics and characteristics of the included patient cohorts. The mean age of onset for triple SNMG patients was 52 and did not differ significantly from antibody positive patients. The average time from symptom onset to diagnosis was significantly longer in triple SNMG compared to AChR+ and MuSK+ patients, but did not differ from LRP4+ patients. Triple SNMG patients had

a similar female to male ratio compared with antibody positive patients. SNMG patients had a significantly higher percentage of ocular MG compared to AChR+ patients.

Triple SNMG patients required significantly fewer immunosuppressive agents compared with AChR+ patients. SNMG patients also showed a trend towards a less frequent history of hospitalizations, myasthenic crises and intubations compared to all antibody positive groups. MuSK+ patients had the highest rates of hospitalizations, myasthenic crises, and intubations. Bulbar weakness was seen most frequently in MuSK+ patients. Decrement on RNS was most commonly seen in MuSK+ patients, and was seen significantly more often than in patients with triple SNMG.

One triple and one double SNMG patient had thymic hyperplasia. No SNMG patients had a thymoma. One LRP4+ patient had a thymoma. The most commonly associated autoimmune disorder in all patients was Grave's disease (8/255 patients). Seven of these patients were AChR+ and one was double seronegative. Double and triple seronegative patients did not differ significantly in regards to any of the categories listed in Table 1.

Of the 21 triple SNMG patients, eight had AChR antibodies retested and were all negative on repeat testing. 11 triple SNMG patients had negative genetic testing for CMS. No patients had a family history of CMS and most responded well to immunotherapy if they were on immunosuppressive agents. Seven triple SNMG patients
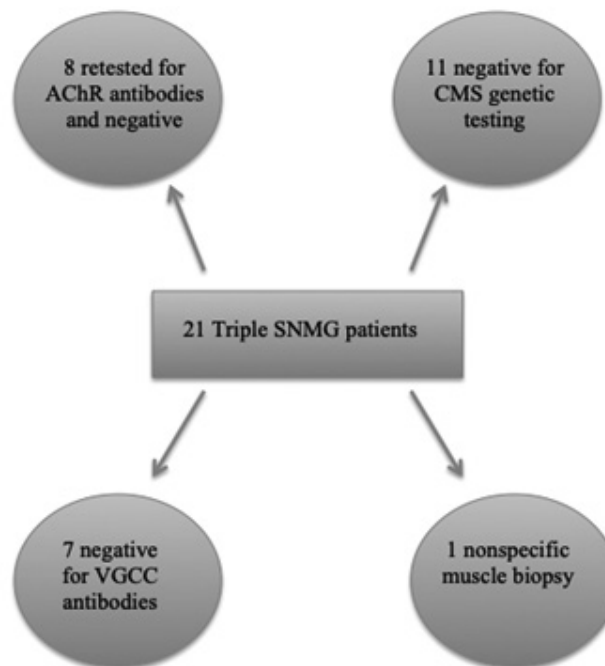

Figure 1. Diagnostic work-up performed in triple SNMG patients.

Table 1. Triple SNMG patients compared to antibody positive patients.

	SNMG (n=21)	AChR+ (n=210)	p-value	MuSK+ (n=9)	p-value	LRP4+ (n=6)	p-value	p-value (all groups)
Age of onset (years)	52	53	0.9743	45	0.7591	32	0.1519	0.1072
Time to diagnosis (years)	7.8	**2.1**	**0.0029***	**0.7**	**0.0433***	8.6	0.9933	**0.0026***
Female	11 (52%)	106 (51%)	0.9999	5 (56%)	0.9999	5 (83%)	0.3497	0.4598
Ocular MG	7 (33%)	**28 (13%)**	**0.0250***	1 (11%)	0.3742	0	0.1548	0.0666
History of MG related hospitalizations	7 (33%)	97 (46%)	0.3581	6 (67%)	0.1232	2 (33%)	0.9999	0.3507
History of myasthenic crisis	5 (24%)	69 (33%)	0.4702	5 (56%)	0.1155	2 (33%)	0.6334	0.4108
History of intubation	3 (14%)	42 (20%)	0.7730	2 (22%)	0.6220	1 (17%)	0.9999	0.9254
# of IS agents currently being used	0.24	**1.03**	**0.0001***	0.44	0.8444	0.83	0.2264	**0.0001***
MGFA classification at last evaluation	1.93	1.98	0.9931	2.29	0.6378	1.75	0.9549	0.6783
Bulbar weakness	2 (9.5%)	30 (14%)	0.7466	3 (33%)	0.1432	0	0.9999	0.2541
Decrement on RNS	7 (37%)	46 (54%)	0.2139	**8 (89%)**	**0.0157***	2 (40%)	0.9999	0.0722
Thymoma	0	28 (13%)	0.0854	0	0.9999	1 (17%)	0.2222	0.2011
Thymic hyperplasia	1 (4.8%)	16 (7.6%)	0.9999	0	0.9999	0	0.9999	0.6990
Race Caucasian African American Asian Hispanic	20 (95%) 1 (5%) 0 0	166 (82%) 28 (14%) 7 (3.5%) 1 (0.05%)		5 (56%) 3 (33%) 0 1 (11%)		4 (67%) 2 (33%) 0 0		

* p< 0.05

IS= Immunosuppressive, MGFA= Myasthenia Gravis Foundation of America

were negative for voltage gated calcium channel (VGCC) antibodies. One triple SNMG patient had a muscle biopsy for further evaluation, and this showed only nonspecific muscle fiber size variability. Figure 1 summarizes the diagnostic work up in our triple SNMG patients.

Discussion

It is important to better characterize and understand the SNMG population to prevent delay in diagnosis, misdiagnosis, and unnecessary testing. The average time from symptom onset to diagnosis in our triple SNMG population was 7.8 years, significantly longer than AChR+ and MuSK+ patients. This can lead to delay in treatment and potentially misguided treatments that can lead to side effects. There have been many theories on why the SNMG population is antibody negative. The leading hypotheses are that the sensitivity of the clinically available assays may be unable to detect the presence of AChR antibodies or that there are antibodies involved in the pathogenesis that are yet to be identified. Radioimmunoprecipitation (RIA) or enzyme-linked immunoassay (ELISA) is the predominant antibody test used to diagnose MG. However, these tests have limited sensitivity of antibody detection due to the antibodies binding poorly to recombinant or soluble antigens [7]. More recently, cell-based assays (CBA) have been developed and have an increased test sensitivity. However, compared to the RIA or ELISA, CBA can be costly and time consuming. CBA also requires access to

tissue culture facilities and staff with expertise in the assays, limiting their use to specialized research centers [7].

In addition to the currently available antibodies, it is likely that additional pathogenic antibodies will be discovered. Recently, antibodies to cortactin have been investigated for their potential role in MG and have been identified in 23.7% of patients with double SNMG [16]. Agrin antibodies inhibit MuSK phosphorylation and AChR clustering in vitro and have been found in both double and triple SNMG patients [6,17].

Our triple SNMG patients overall had less severe disease compared to seropositive patients, similar to what has been previously reported [6,8,10,18,19]. We found that SNMG patients required fewer immunosuppressive agents and trended towards a lower rate of hospitalizations, myasthenic crises, and intubations. Our MuSK+ population had the highest rates of hospitalizations, myasthenic crises, intubations, and highest mean MGFA classification, indicating they had the most severe disease course. This is consistent with prior reports [20].Our results indicate that triple SNMG patients more commonly had isolated ocular MG compared to seropositive patients. Previous literature has reported a wide range (16-55%) of ocular MG in seronegative patients, likely due to studies defining seronegative patients differently in terms of which antibodies were tested [10,21,22]. Our triple SNMG population fell within this range, having 33% ocular MG.

Our triple SNMG patients had a similar age of onset compared to AChR+ patients. There was a bimodal distribution in the AChR+ group but a normal distribution in the SNMG group. The SNMG mean age of onset was 52, similar to what has been reported [6,7,23]. There was a similar female to male ratio. Previous literature has reported an equal female to male ratio [19], while others have reported a slight female predominance [6,8,9].

One double and one triple SNMG patient had thymic hyperplasia. There is a wide range of reported prevalence of thymic hyperplasia in SNMG patients in the literature, ranging from 6-71% [11,24,25]. However, these studies defined SNMG as only AChR-. Despite this wide range, AChR+ patients have been shown to have similar rates of thymic hyperplasia to SNMG patients [24].

Both of our SNMG patients with thymic hyperplasia had improvement in symptoms and a reduction in MG medications after thymectomy. The triple SNMG patient had sustained improvement, whereas the double SNMG eventually had worsening symptoms years later and had to be restarted on pyridostigmine and prednisone.

Thymectomy in SNMG patients has generally resulted in similar results to seropositive patients, however, most studies have been performed on only AChR- patients (not tested for MuSK or LRP4) and there are no large studies on triple seronegative patients [11,24-26]. Our two cases emphasize the importance that thymic pathology can be present even in SNMG, and thymectomy can provide benefit at least when there are thymic abnormalities present on imaging and thymic hyperplasia found through biopsy.

Ancillary testing for patients with SNMG should be considered when other diagnoses are plausible. However, there should also be a balance between finding the correct diagnosis and cost-effective care in this patient population. CMS patients may be misdiagnosed as SNMG. Certain characteristics to prompt congenital myasthenia panels include: positive family history, early onset, slow progressive symptoms, and lack of response to immunotherapy. RNS characteristics may include afterdischarges or decrement that is brought out by prolonged exercise. In our population, the 11 triple SNMG patients tested for CMS all had negative genetic testing. However, these patients did not have the above-described characteristics to prompt testing. Our results indicate that if patients do not have the above-mentioned characteristics concerning for CMS, congenital myasthenia testing is low yield. When any of the aforementioned clinical characteristics favoring CMS are present though, including lack of response to immunotherapy, we would recommend pursuing CMS testing at this time.

Conclusion

Our results further elucidate the clinical characteristics of triple SNMG and the predominance for ocular disease and a less severe disease course. Although likely rare, investigation for thymic pathology should be a consideration in SNMG, and thymectomy should be considered when there are thymic abnormalities on imaging. In our population, we did not tend to find alternative diagnoses in SNMG patients and thus ancillary testing should be considered in carefully selected patients for cost-effective care.

Corresponding author:
Jonathan Morena
395 W 12th Ave #7
Columbus, OH 43210
Phone: 330-546-6168
Jonathan.Morena@osumc.edu

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