Original Article

Anti-titin antibodies in a cohort of myasthenia gravis patients

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Background: Anti-titin antibodies have been previously associated with thymoma-associated myasthenia gravis (MG) and a more clinically severe form of MG. While currently only serving as a disease biomarker, its possible utility as an indicator of underlying thymus malignancy may be of value in clinical practice.

Methods: Data was retrospectively collected and analyzed from 2013 to 2022 using an institutional record of MG patients. Anti-titin antibodies were assessed using Line Blot immunoassay.

Results: From 130 MG cases, 32 (24.6%) were anti-titin positive. Anti-titin positive cases were associated with older age of disease onset [median (IQR): 63.0 (44.3–70.8) *vs.* 35.5 (24.8–60.8) years] (P<0.01). Thymectomy was performed in 46 (35.4%) MG patients, 12 of which anti-titin positive (26.1%). Thymectomy samples from anti-titin positive patients comprised 10 (83.3%) cases of thymoma and 2 (16.7%) cases of thymus hyperplasia. There was a tendency towards anti-titin positive patients having more thymoma while anti-titin negative displayed more hyperplasia (P<0.01). Anti-titin positivity correlated with thymoma in patients with age of onset bellow 50 years (P=0.028). Anti-titin positivity was significantly associated with generalized MG in the late-onset group (P=0.005).

Conclusions: The presence of anti-titin antibodies appears to correlate with underlying thymoma in early-onset MG cases and with generalized MG in late-onset cases. Prospective studies are needed to further study this association.

Keywords: Myasthenia gravis (MG); thymoma; anti-titin; neuroimmunology

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Introduction

Myasthenia gravis (MG) is characterized by fatigable weakness of voluntary muscles ranging from mild ocular symptoms to generalized dysfunction with bulbar and respiratory involvement (1,2). The point prevalence of MG in northern Portugal is 111.7 patients per million inhabitants, similar to that reported in other European countries (3). This immune-mediated disorder is characterized by the production of autoantibodies that

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target receptors in the postsynaptic membrane of the neuromuscular junction of skeletal muscles, resulting in weakness (4). Antibodies against acetylcholine receptors (anti-AchR) are present in the serum of most patients and are directly pathogenic. Muscle-specific kinase and lipoprotein receptor-related protein 4 (LRP4) are also targeted by antibodies and define specific MG subgroups (2).

Additionally, antibodies directed at skeletal muscle components are present in a subset of MG cases, and their direct pathogenic role is not as clearly established (1). One of these components is titin, an intramuscular protein important for regulating muscle elasticity (5). Anti-titin antibodies were first described by Gautel *et al.* [1993] and associated with the presence of underlying thymoma (6). Thymomas are present in approximately 10–15% of all patients with MG (2). A previous study suggested an association between anti-titin antibodies and thymoma-associated MG (7). This probably results from T cell autosensitization due to overexpression of muscle-like epitopes by the thymoma microenvironment (8). Furthermore, in patients without thymoma, anti-titin positivity correlates with late-onset MG with intermediate titers of anti-AchR antibodies (9). Some studies suggest that anti-titin positive antibodies are associated with a more clinically severe form of MG (10). However, these results may reflect other autoantibodies that tend to co-occur with anti-titin in some cases (11).

As a result of the lack of direct pathogenicity and inconsistency in correlation with clinical severity, anti-titin is not included in the current classification of MG subgroups (11,12). Still, its utility as a biomarker of underlying thymus malignancy may be of value in clinical practice.

This study aims to characterize a population of MG patients according to anti-titin serostatus. We present this article in accordance with the STROBE reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-94/rc).

Methods

Study setting

This study included all MG patients followed in the neuroimmunology outpatient clinic of Centro Hospitalar Universitário de Santo António. Data were retrospectively collected and analyzed from 2013 to 2022.

Clinical data

Patient information was retrieved from institutional records. Retrieved information included demographic and clinical data, namely date of birth, gender, age of onset, age of diagnosis and follow-up time. MG was diagnosed based on a combination of clinical features and at least one of the following: (I) specific autoantibodies [anti-AchR or anti-muscle-specific kinase (MuSK)]; (II) electrophysiologic testing with repetitive nerve stimulation and/or single-fiber electromyography; (III) improvement of symptoms with either pyridostigmine or immunosuppressive therapy (1,13).

Disease severity at the time of diagnosis was graded according to the Myasthenia Gravis Foundation of America (MGFA) classification, and the patients were classified as Grade I, Grade II, Grade III, Grade IV, and Grade V. For further analysis, we separated patients with ocular MG (Grade I) from patients with generalized MG (Grade II to V). The histological pattern of thymectomy samples was collected from clinical records of MG patients submitted to surgery.

Antibody assays

Anti-titin antibody determination was performed in Centro Hospitalar Universitário de Santo António using the Line Blot immunoassay Paraneoplastic Neurological Syndromes 12 Ag (Euroimmun, Lübeck, Germany), according to the manufacturer's instructions. The samples were collected several years after the thymectomy in most patients.

Statistical analysis

For descriptive statistics, qualitative variables were studied using absolute and relative frequencies. For the quantitative variables, the mean and standard deviation, or median and interquartile range (IQR), were calculated according to the normality of the distribution. Chi-square and Fisher's exact tests were used for correlation between categorical variables. The group of patients with positive anti-titin antibodies at some point during disease history was compared with the seronegative group.

IBM SPSS Statistics, version 27.0, was used for the analysis. A P value inferior to 0.05 was considered statistically significant.

Table 1 Comparison MG patients based on anti-titin serostatus

Variables	Anti-titin negative (n=98, 75.4%)	Anti-titin positive (n=32, 24.6%)	P
Demographic data			
Female sex, n (%)	69 (70.4)	18 (56.3)	0.20
Age of onset (years), median (IQR)	35.5 (24.3–60.8)	63.0 (44.3–70.8)	<0.01
Diagnostic data, n (%)			
Generalized MG	73 (74.5)	23 (71.9)	0.81
Supportive EMG	64 (65.3)	24 (75.0)	0.58
Positive anti-AchR	69 (70.4)	26 (81.3)	0.32
Thymectomy, n (%)	34 (34.7)	12 (37.5)	0.83
Thymoma	11 (11.2)	10 (31.3)	<0.01
Thymus hyperplasia	17 (17.3)	2 (6.3)	

From the total sample, 130 patients (56.5%) were tested for anti-titin. MG, myasthenia gravis; IQR, interquartile range; EMG, electromyography; AchR, acetylcholine receptor.

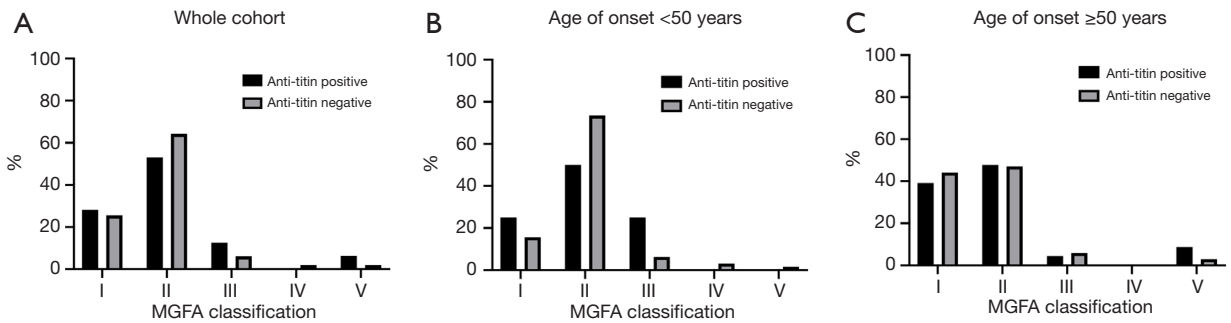

Figure 1 Distribution of MGFA classification grades according to the anti-titin serostatus for the whole cohort (A) and for patients with age of disease onset below (B) and above 50 years (C). MGFA, Myasthenia Gravis Foundation of America.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was authorized by the CHUdSA/ICBAS Ethics Committee. Individual consent for this retrospective analysis was waived.

Results

From 230 MG patients followed in the outpatient clinic, anti-titin antibodies were tested in 130 patients (56.5%), of which 32 were positive (24.6%). *Table 1* summarizes the features of MG cases with respect to anti-titin serostatus.

The distribution of patients by age of onset was the following: 73 (56.2%) early-onset (<50 years) and 57 (43.8%) late-onset (≥50 years). The median age of onset for anti-titin positive cases was 63.0 years (IQR, 44.3–70.8 years), which is significantly higher than that of seronegative cases (median, 35.5 years; IQR, 24.8–60.8 years) (P<0.001). There was no association between anti-titin and anti-AchR or anti-MuSK. The frequency of generalized MG was similar between the anti-titin positive and negative groups (71.9% and 74.5%, respectively) (P=0.81). A subanalysis of patients with age of onset ≥50 years showed that anti-titin positivity was significantly associated with generalized MG in this age group (P=0.005). The same was not replicated in patients with early-onset MG (P=0.341). *Figure 1* shows the distribution through the MGFA classes for this subset of patients, for comparison with the whole cohort. The positivity of anti-AchR antibodies or EMG features supportive of MG diagnosis did not differ significantly according to anti-titin serostatus.

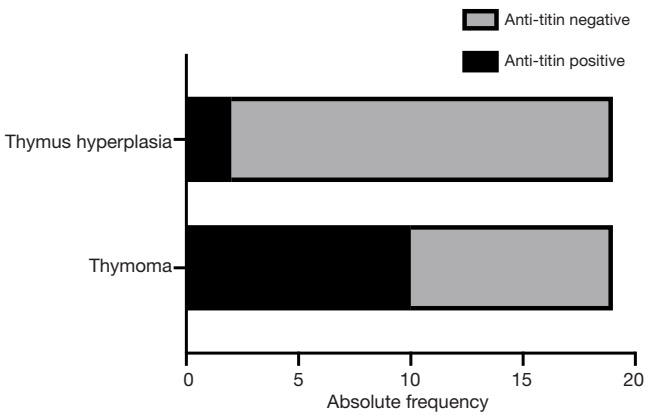

Figure 2 Distribution of histological patterns of thymectomy specimens according to the anti-titin serostatus.

Thymectomy was performed in 46 (35.4%) MG patients, 12 of which belonging to the anti-titin positive group (26.1%). There was no significant difference in the frequency of thymectomy between groups. The histological pattern of thymectomy samples for anti-titin positive patients comprised 10 (83.3%) cases of thymoma and 2 (16.7%) cases of thymus hyperplasia. None of the thymectomized anti-titin positive cases had normal thymus histology. For comparison, there were 11 (32.4%) thymomas and 17 (50.0%) cases of thymus hyperplasia in the anti-titin negative group, which was significantly different (P<0.01). There was a tendency toward anti-titin positive patients having more thymoma, as shown in *Figure 2*. A subanalysis of patients with onset below 50 years showed that 50% of the thymectomized anti-titin positive patients had thymoma, compared to 9.4% of the anti-titin negative group, while thymus hyperplasia was significantly more represented in the second (2 *vs.* 17, respectively) (P=0.007).

Discussion

In our study, the prevalence of thymoma was higher in the anti-titin-positive group, which represents a small proportion of MG cases. In fact, anti-titin antibodies were detected in less than a third of tested subjects, in a similar proportion to that obtained by previous studies (10,14). An interesting finding in our study was that all anti-titin positive patients had abnormal thymus histology (either thymoma or thymus hyperplasia).

We found that 83.3% of the thymectomized anti-titin positive patients corresponded to cases of histologically proven thymoma, which recapitulates findings from previous studies (10,12). A study by Voltz *et al.* found that anti-titin antibodies predicted thymoma with a specificity of 100% in a sample of 44 thymectomized MG patients (15). However, besides the relatively small sample size, the exclusive consideration of thymectomized patients may skew the analysis towards early-onset patients, since operated patients tend to be younger. Another issue to consider in this regard is that thymoma-associated MG cases are generally younger at onset, which appears to reflect thymoma epidemiology, with a mean age at presentation below 50 years (16,17). In fact, our subanalysis of patients with early age of onset showed the same positive correlation between anti-titin positivity and thymoma. Anti-titin positivity was still high in the older-onset group, which translates to reduced clinical utility in indicating underlying thymoma in this age group. Since the early-onset group corresponded to 56.2% of our sample, it might be that these are the drivers of the statistical correlation. In agreement, anti-titin antibody positivity in nonthymomatous patients has been shown to occur mostly in older patients (10,12).

Besides the presence of thymoma, anti-titin associated MG has been described to be associated with a more severe clinical course (14,18,19). This association is still debatable, with relevant studies not demonstrating a correlation with clinical severity and only showing an association with the older onset of MG, which our findings recapitulate (20).

The published literature mostly focuses on generalized MG cohorts when considering the impact of anti-titin antibody serostatus (10). Our study does show a correlation between anti-titin antibody positivity and generalized MG for the late-onset MG subgroup, which translates to higher MGFA scores.

One important issue to consider is that thymoma-associated MG cases were already diagnosed and thymectomized before anti-titin was measured, in most cases. This means that anti-titin antibodies persist in circulation for several years or even indefinitely after the removal of the thymus. It has been previously suggested that anti-AchR-producing B cell clones mature in the thymus before migrating to the circulation where they persist after thymectomy (21). Titin epitopes are expressed on the neoplastic epithelial cells in cortical-type thymomas (9,22). This might induce the autosensitization of T cells that further induce B cell clones that continuously produce this marker of autoimmunity.

This study has several limitations that should be considered. Its retrospective design and the variable time

interval between relevant events, namely the thymectomy and anti-titin antibody testing dates, prevent us from systematically analysing the data, since the serological dynamics of anti-titin antibodies is still not completely understood. We opted not to analyse the clinical progression and treatment response, and so we were unable to extrapolate the effects of anti-titin serostatus in terms of disease severity. Since there is no correlation between the thymoma histological pattern and anti-titin positivity (23), this data was not included in our analysis.

In the future, it would be interesting to prospectively study a cohort of MG patients in order to establish if (I) anti-titin is a suitable marker to select patients for thymectomy, specifically to address cases where imaging features of malignancy are still not present in the screening computed tomography scans; and (II) if the clinical course of MG is associated with the presence of these antibodies.

Conclusions

In our cohort, the presence of anti-titin antibodies correlated with underlying thymoma. We found that late-onset MG patients tended to have more generalized MG if the anti-titin antibody was present. Anti-titin antibody determination should be included in the baseline study of suspected MG patients. Prospective studies are needed to further elucidate the clinical utility of anti-titin and other antibodies directed at muscle components that develop as a consequence of thymomas.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-94/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-94/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-94/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was authorized by the CHUdSA/ICBAS Ethics Committee. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Individual consent for this retrospective analysis was waived.

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