Peer Review File

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Reviewer A

Comment 1: In the introduction, it would be interesting to mention authors who described the presence of titin antibodies in myasthenia gravis for the first time (Ref. Neurology. 1993 Aug;43(8):1581-5. doi: 10.1212/wnl.43.8.1581.)
Reply 1: We have added this information.
Changes in text: lines 72-74

Comment 2: The Methods section (Antibody assays) could specify the range of concentrations detected by the kit used, and which titer values were considered positive.
Reply 2: The line blot used gives the results in a binary fashion into positive and negative, so we don't have quantitative values associated with the results.

Comment 3: In the Results section, how many of the 320 patients seronegative for anti-AChR/anti-MuSK were anti-titin positive? How many of the patients with ocular onset MG (oMG) with titin antibodies worsened to gMG during the follow-up period?
Reply 3: From the 230 MG cases, 130 were tested for anti-titin and this is the sample we are referring to in the publication. Most patients (73.1%) were anti-AchR positive. We don't have the data concerning the generalisation of ocular MG patients because we are considering the disease state at diagnosis for the purpose of the analysis.

Comment 4: In the Discussion section, do the authors believe that titin antibodies are a diagnostic/prognostic biomarker for MG, and if so, do they think that the titin antibodies determination should be included in the baseline study of all suspected MG patients regardless of their age?
Reply 4: We believe that the anti-titin serostatus is informative towards the clinical phenotype (generalized MG) of the underlying disease and associated with the presence of thymoma. In this sense, it should indeed be tested in all MG cases. The interpretation of the results is different in the early and late-onset MG subgroups, as shown by our study.
Changes in text: We have added this information in lines 228-230.

Reviewer B

I applaud the authors for this work. The authors state that there is an association between anti-Titin antibodies and myasthenia gravis complicated thymoma. However, there are various problems and questions in reaching such a conclusion.

Comment 1: Anti-titin antibodies were measured after thymoma surgery, but postoperatively would not be appropriate to evaluate the association with thymoma. In that case, it would be

necessary to evaluate the results of the preoperative and postoperative measurements. If the association with thymoma is to be evaluated, the preoperative period may be more useful.
Reply 1: Thank you for your comments. We share with the reviewer the same concern and have addressed this issue in the discussion (lines 199-208). Anti-titin antibodies are present even after thymectomy due to continuous production of the antibody by circulating B cells. This is a qualitative analysis that could represent the previous contact with thymus neoplasia.

Comment 2. The clinical significance of the anti-titin antibody is not clear from the present study. If myasthenia gravis were present, a CT scan would be performed as a screening for the presence of thymoma.
Reply 2: The mediastinal CT scan can be poor in distinguishing a thymoma from thymus hyperplasia. Our study suggests that anti-titin could be helpful in distinguishing between these conditions. Furthermore, it is also associated with disease severity.

Comment 3. The authors stated a conclusion about correlation between anti-titin antibody and MG with thymoma, however, no correlation test has been performed. I believe that a correlation test should be performed.
Reply 3: We used a Pearson Chi-Square to show that anti-titin positivity was associated with thymoma while anti-titin negativity was associated with thymus hyperplasia ($p=0.007$).

Comment 4. I would like to know if there is an association between anti-Musk antibodies and anti-titin antibodies in this cohort.
Reply 4: We found no association between anti-titin and anti-Musk.
Changes in text: We have added this information in line 140

Comment 5: In summary, no novelty can be found in this study in thoracic disease. This study is not in the thoracic disease area but in the neurological area. I believe that a journal specializing in that area would be more appropriate.
Reply 5: We chose to submit this work to this journal since our results concern a potential marker of underlying thymoma in MG patients. This is a relevant area of collaboration between Neurologists and Thoracic Surgeons and this work provides information that could be useful for both.

Reviewer C

Comment 1: This was very well written and brings awareness of a little-known potential biomarker. I think future studies involving trending Ab titers could be useful to evaluate potential MG treatment response but also could investigate if this could be used for tumor recurrence surveillance, potentially reducing the need for serial chest CTs with patients s/p thymectomy for thymoma.
Reply 1: Thank you for your comments.