Peer Review File

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Reviewer A

In this retrospective study of 83 patients who underwent surgical resection of a thymic epithelial tumor (TET) during a 10-year period, the relationship between location of calcifications and clinicopathological features was studied. The patients were divided into 3 groups: inner calcification (n=9), marginal calcification (n=5), and no calcifications (n=69). The authors conclude that marginal calcification predicts low invasiveness as well as low-risk pathology, which is also indicated in the title.

Reply: I really appreciate for your review of our manuscript.

Comment 1:
-this is a retrospective study only including operated patients which induces a selection bias; no information is provided on TETs with calcifications that were not operated upon for a variety of reasons (higher stage, comorbidity, older age,…)

Reply 1: I agree with this opinion. We did not include non-operated patients. Such patients were not referred to our department of thoracic surgery. In addition, we think accurate pathological diagnosis is needed in the present study. For accurate diagnosis, surgically resected samples are superior to samples obtained by needle biopsies. We added some sentences in the text as shown below in patients and methods.

Changes in the text: We included only operated cases pathologically diagnosed with thymoma based on surgically resected samples. Patients without surgical resection were not included in the present study. (P6, L9-11).

Comment 2: Overall, the study population is quite heterogeneous, and no details are given on staging, comorbidity, severity of myasthenia and multimodality therapy

Reply 2: I agree with the opinion of the heterogeneity of the patients. Heterogeneity is a kind of characteristics of thymomas. This time we focus the calcification of thymoma. In heterogenous thymomas, we would like to identify characteristics of thymomas with marginal calcification. Including heterogenous thymomas can identify the characteristics of thymomas with marginal calcification. This time, we have added data of other autoimmune diseases. Multiple modality therapy before surgical resection was not performed in any cases. Adjuvant therapy also was not added in any cases before recurrence. We added a sentence in the text as shown below in results.

Changes in the text:

As with other autoimmune diseases, pure red cell aplasia, SLE, Issacs syndrome, and chronic thyroiditis were diagnosed in one non-MG patient each. Polymyositis and carditis were observed as complications in an MG patient. (P10, L4-6).

Comment 3: There were only 5 patients in the marginal calcification group, which is too low to make any meaningful conclusions that can be generalized

Reply 3: I agree with your opinion of low number of patients. We think this is a big limitation. However, we could follow up for long time in 2 patients. We have added details of the two patients in case presentation. We believe this case presentation can support our data.

Changes in the text: Case presentation (P8, L10-P9, L16)

Comment 4: Average observation period is 38 months which is quite low for TETs

Reply 4: I agree with this opinion of short observation period. This is a big limitation. We have updated data of the latest visit of patients and the observation period prolonged to 42.9 months.

Changes in the text: The average observation period was 42.9 (0.1-133.0) months. **(P11, L1).**

Comment 5: Due to a low number of events no multivariate analysis could be performed to determine whether calcification is an independent prognostic factor

Reply 5: We have data of prognostic factors. Only Masaoka stage and TNM classification were the prognostic factor of recurrence. However, independent prognostic factor was not suggested in multivariate analysis.

Changes in the text: Only the Masaoka stage and TNM stage classification were prognostic factors for recurrence. There was no significant difference in the recurrence-free duration between the groups with and without calcification (p=0.667) (Figure 3A). There were also no significant differences in the recurrence-free duration between groups I, M, and N (p=0.759) (Figure 3B). (P11, L2-6)

Comment 6: As the authors indicate themselves, a multicenter study is required to obtain more valid data to determine whether calcifications are related to clinical behavior

Reply 6: Thank you for your comments.

Changes in the text: We are planning our next study to involve multiple institutions with a large number of thymoma patients. (P14, L7-8)

Comment 7: In summary, the conclusions are too strong and not fully substantiated by the presented data.

Reply 7: I agree with your opinion. We have added details of the two patients in case presentation. We believe this case presentation can support our data. We have changed the expression of the title and conclusions.

Changes in the text: Conclusions
We categorized thymoma cases based on the location of calcification and indicated details of thymoma cases with calcifications. Differences in the location of calcification indicated differences in the characteristics of thymomas. Inner calcifications seemed to indicate more invasive characteristics than marginal calcifications. The location of calcification should therefore receive focus when evaluating thymomas. (P14, L11-16)

Reviewer B

Comment 1: In this study, Imazu et al aim to evaluate the clinical importance of different patterns of calcification in thymic epithelial tumors. To this end, they analyzed 83 thymic tumors from a single institution and distinguish between marginal and inner calcification on preoperative CT imaging. As calcifications in thymic malignancies are not too frequent, they were able to include only very low numbers of calcified tumors – leaving only 5 marginally calcified tumors for analysis.
In the study population, they describe that marginal calcification was more frequent in low-risk thymomas (type A, AB and B1) compared to high-risk lesions (type B2 and B3). Indeed, this marginal pattern of calcification was only described in AB and B1 thymomas in the study. Furthermore, marginal calcification was only found in tumors with Masaoka-Koga stage I and II. From these observations, the authors claim that marginal calcification of thymic epithelial tumors indicates noninvasive behavior and the presence of a low-grade histologic subtype. However, it is hardly possible to make this generalization given the small number of cases analyzed in this monocentric study. The data presented here merely represents a case series.
Authors are strongly encouraged to (I) either change the manuscript to provide a more descriptive case series or (II) greatly enhance the numbers of patients included in the analysis.

Reply: I really appreciate for your review of our manuscript and suggestion to improve our manuscript. According to the suggestion, we have added a part of case presentation.

Case presentation
Case 1 (Figures 1E, 2A and 2B). A 71-year-old woman with dilated cardiomyopathy and chronic kidney disease requiring hemodialysis. Although an anterior mediastinal tumor had been noted 20 years ago, she had been followed up without surgical resection due to comorbidities. Anti-AChR antibody was negative. Anemia appeared and progressed, and transfusion was repeated. She was diagnosed with pure red cell aplasia and referred to the Department of Thoracic Surgery. The maximum diameter of the tumor increased from 2.5 cm to 8.4 cm over 20 years. Marginal calcification was recognized on CT taken two years ago (Figure 2A), and the calcifications became clearer over two years (Figure 2B). Thoracoscopic thymectomy using the subxiphoid approach (14) was performed with an operation time of 190 minutes and blood loss of 10 g. The postoperative condition was excellent, and she was discharged on the fifth postoperative day. The pathological

diagnosis was type AB thymoma with Masaoka-Koga stage I and T1aN0M0 TNM classification. She is alive without recurrence of thymoma at 17 months after the operation.
Case 2 (Figures 1F, 2C and 2D). A 65-year-old man underwent open abdominal surgery due to traffic trauma. At that time, an anterior mediastinal tumor had been pointed out incidentally by CT (Figure 2C). Following a seven-year follow-up period without enlargement of the tumor, he was referred to the Department of Thoracic Surgery (Figure 2D). The maximum diameter of the anterior mediastinal tumor was 3.0 cm. During this period, ring formation of calcification became more continuous, and calcification thickened partially. Anti-AChR antibody (3.6 nmol/dL) was positive, but symptoms of myasthenia gravis were not recognized. Thoracoscopic thymectomy using the subxiphoid approach was performed with an operation time of 104 minutes and 5 g of blood loss. The postoperative condition was excellent, and he was discharged on the third postoperative day. The pathological diagnosis was type AB thymoma with Masaoka-Koga stage I and T1aN0M0 TNM classification. He is alive without recurrence of thymoma at 16 months after the operation.

Changes in the text: Case presentation (P8, L10-P9, L16)

Reviewer C

Calcification was recognized in 14 tumors (16.9%) that were categorized into groups I (n=9) and M (n=5). In group I, stippled calcification was recognized in two cases, nodular calcification in six cases and large calcification in one case. In group M, focal or multifocal calcification was recognized in three cases dot lined calcification in one case ring calcification in one case. I liked the study and presentation. However, the meaningful outcome from this limited number of patients creates concerns.

Comment 1: Please write a paragraph of limitations.

Reply: I really appreciate for your review of our manuscript. Limitations were added in discussion.

Changes in the text: While the results of this study are encouraging, any conclusions should be tempered by the limitations. The single-institution setting, small number of cases, short observation period, and inclusion of only operated cases may limit the validity of the present results. (P14, L6-8)

Comment 2: Edit with a native speaker.

Reply: This article has been carefully reviewed by Mr. Brian Quinn. He is an official English editor of Japan Surgical Society Association. He is an experienced medical editor whose first language is English and who is specialized in the editing of papers written by physicians and scientists whose native language is not English.

Comment 3: Need to demonstrate the relationship with MG or other autoimmune diseases.

Reply: The data of non-MG autoimmune diseases were added.

Changes in the text: As with other autoimmune diseases, pure red cell aplasia, SLE, Issacs syndrome, and chronic thyroiditis were diagnosed in one non-MG patient each. Polymyositis and carditis were observed as complications in an MG patient. (**P10,L4-6 and tables 1-2)**

Reviewer D

Imazu and al aimed to better categorise the calicifications of TETs and to try to correlate them with tumour aggressiveness and disease prognosis. The initial idea was good but the article has several weaknesses. My questions and suggestions are as follows:
- one of the main concerns of the study is that it involves few patients with a disproportion between thymoma and thymic carcinoma, we cannot therefore conclude anything on the basis of this study alone.

Reply: I really appreciate for your review of our manuscript. We have excluded thymic carcinoma cases.

Comment 1: Authors are encouraged to conduct a larger study.

Reply: I really appreciate for your review of our manuscript. We are planning multiple institutional study of thymoma calcifications.

Changes in the text: While the results of this study are encouraging, any conclusions should be tempered by the limitations. The single-institution setting, small number of cases, short observation period, and inclusion of only operated cases may limit the validity of the present results. We are planning our next study to involve multiple institutions with a large number of thymoma patients. (P14, L6-10).

Comment 2: If a study was carried out on a larger number of patients, it would be interesting to see how patients with both types of calcifications behave and to make a single group out of them.

Reply: Thank you for your suggestive comments.

Comment 3: In my opinion, it would be necessary to specify the median follow-up time with a minimum and a maximum. it is also stated in the article that "many cases with a short observation period in all groups". This should be clarified.

Reply: Thank you for your suggestive comments. We have added minimum and maximum follow-up time in the text.

Changes in the text: The average observation period was 42.9 (0.1-133.0) months.(P11, L1)

Comment 4: I am not sure that figure 3 belongs in the article, as it covers a period of 10 years whereas the average observation time is 38 months.

Reply: The horizontal scale of Figure 3 has been changed to 8 years. Because We have a case of recurrence over 6 years after the operation.

Changes in the text: Figure 4 (previous Figure 3)

Comment 5: To summarise, the main limitations of the article are: too few patients, short follow-up time compared to the natural history of the disease and an empirical classification of calcifications even if its origin has been partially explained in the discussion.

Reply: I agree with your opinion. Limitations were added in discussion.

Changes in the text: While the results of this study are encouraging, any conclusions should be tempered by the limitations. The single-institution setting, small number of cases, short observation period, and inclusion of only operated cases may limit the validity of the present results. (P14, L6-8)

Reviewer E

I think this is an interesting paper to discuss the clinicopathological analysis based on the findings of calcification on CT findings for thymic epithelial tumors.
However, I have several questions.
First, in Figure 2, I think you should add a survival curve not only for each group of calcifications, but also for the presence or absence of calcifications alone. The number of cases in each group is too small in the group-specific survival curves, and it would be difficult to perform statistical analysis.

Reply: I really appreciate for your review of our manuscript. We have added survival analysis with or without calcifications.

Changes in the text: Figure 4A

As for the survival curves, I think the authors should analyze thymoma and thymic carcinoma separately. Or, it would be better to analyze only thymoma excluding thymic carcinoma.

Reply: I really appreciate for your review of our manuscript. We have excluded thymic carcinoma cases.

In Tables 1 and 2, the ± values in Factors of tumor size and recurrence free periods, are SD (standard deviations) or SE (standard errors)? Please clarify.
In the Results, please specify what the" ±" means in line 13 on page 8 SE (standard error) or SD (standard deviation)?

Reply: In materials and methods, we indicated the values were presented as the mean ± standard deviation. In text and tables, the statement was added.

Changes in the text: Tables 1 and 2. Values were presented as the mean ± standard deviation (SD). (P7, L11-12, tables 1-2)