Peer Review File

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Reviewer A

The authors investigated the significance of CK in surgically resected thymic epithelial tumors and the relationship between CK and clinicopathological factors. They retrospectively evaluated the relationship between preoperative CK levels and prognosis in 120 patients with thymic epithelial tumors who underwent surgical resection at two centers. Authors claim that preoperative serum CK might reflect the host nutritional status in patients with resected thymic epithelial tumors; therefore, CK could be a biomarker of postoperative prognosis.

Comment 1: I liked the general outline of the study and I agree with authors "the findings demonstrated that low CK levels were associated with poor prognosis in patients with surgically resected thymic epithelial tumors. CK might be a biomarker of the nutritional status and prognosis in thymic epithelial tumors". However, this needs to be described in a tentative way as sarcopenia and malnutrition is also a poor prognostic factor in all cancers. DFS are affected by some other factors but OS is affected with CK only according to the Tables 3 and 4.

Reply 1: We appreciate these helpful comments. As you noted, the prognosis of resected thymic epithelial tumors can be affected by various factors, including sarcopenia and the nutritional status. Therefore, we revised the manuscript as follows:

<Conclusions; page 13, line 2-4>

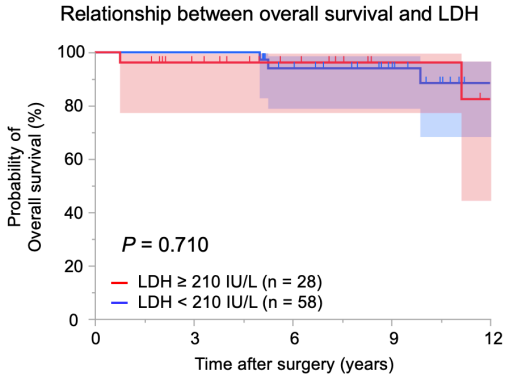
<u>CK might be a prognostic biomarker of thymic epithelial tumors. However, given that sarcopenia and malnutrition are also poor prognostic factors in all cancers, further detailed analysis including the relationship between CK levels and the nutritional status is necessary.</u>

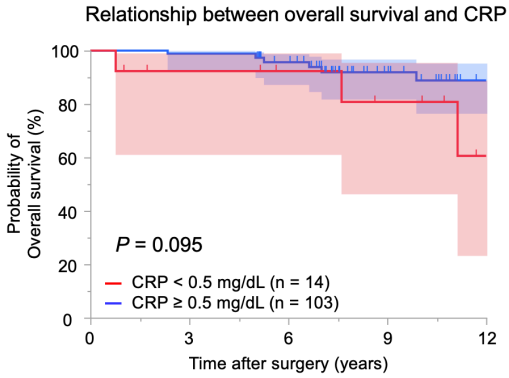
Comment 2: Limitations were discussed clearly; however, I believe an expert statistician review could be helpful.

Reply 2: We thank you for this helpful comment. This article had already been reviewed by an expert statistician, Mototsugu Shimokawa, PhD (professor).

Comment 3: Did the authors study other factors such as LDH and CRP as prognostic significance. Please indicate if you did.

Reply 3: We appreciate your query. Per your remarks, we investigated the prognostic significance of LDH and CRP in patients with thymic epithelial tumors whose data were available (n = 117 and n = 86, respectively). The cutoffs of LDH and CRP were 210 IU/L and 0.5 mg/dL, respectively, according to previous reports.[1-2] Overall survival did not differ between the high LDH (n = 28) and low LDH groups (n = 58, P = 0.710) or between the high CRP (n = 14) and low CRP groups (n = 103, P = 0.095). These results indicated that other factors such as LDH and CRP did not significantly affect prognosis.





References:

1. Janik S, Raunegger T, Hacker P, et al. Prognostic and diagnostic impact of fibrinogen, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio on thymic epithelial tumors outcome. Oncotarget. 2018;9(31):21861-75.

2. Liu Q, Fu X, Su X, et al. Elevated pretreatment serum lactate dehydrogenase level predicts inferior overall survival and disease-free survival after resection of thymic carcinoma. J Thorac Dis. 2017;9(11):4550-60.

Comment 4: The article is well written. I suggest to decrease the graphs and remove those without clinical significance.

Reply 4: We thank you for this constructive comment. As you noted, the associations of CK levels with BMI and CRP were less important than other associations (**Figure 2**). Thus, we removed the following sentences from the revised manuscript:

<Results; page 9>

・BMI was slightly lower in the low CK group than in the normal CK group, but this difference was not statistically significant (P = 0.175, **Figure 2A**).

・Serum CRP levels were significantly higher in the low CK than in the normal CK group ($P < 0.001$, **Figure 2C**).

Comment 5: What do they suggest based on their finding? Do they recommend neoadjuvant treatment?

Reply 5: We greatly appreciate these queries. Thymomas can recur up to several decades after the initial surgery. In addition, the Kaplan–Meier curves of OS revealed a prominent difference between the normal and low CK groups at 8 years after surgery in our study. Therefore, we propose that longer and intensive follow-up should be considered for patients with low CK levels who underwent surgical resection of thymic epithelial tumors. CK levels could be useful in determining appropriate follow-up durations and intervals in patients with thymic epithelial tumors. Thus, we revised the text as follows:

<Discussion; page 11, line 9-10>

Furthermore, CK levels might be useful in determining appropriate follow-up durations and intervals in patients with thymic epithelial tumors.

Reviewer B

This paper examines the relationship between blood CK levels and prognosis in cases of resected thymic epithelial tumors. The results of this paper are very interesting as they suggest that a decrease in CK levels, which can be easily measured in clinical settings, may be a factor related to recurrence and prognosis after surgery for thymic epithelial tumors.

The problems are listed below.

Comment 1: The results may have been influenced by the fact that the study included thymic carcinoma and thymoma. Thymoma, as the authors note, is a relatively low-grade tumor. It is not uncommon for stage IV thymoma types A and B to survive five years. Therefore, if only completely resected thymomas were considered, there would be no difference in prognosis between the groups over a 6-year observation period. If there was a difference in prognosis between each group, wouldn't there be more cases of advanced thymoma Type C in the low CPK group?

Reply 1: We thank you for these constructive comments. As you noted, Fisher's exact test indicated that low CK levels were associated with thymic carcinoma (P = 0.017). This result was consistent with the finding that tumor malignancy reflects CK levels. Thus, we investigated the relationship between CK levels and prognosis in patients with thymomas, and we separated the WHO histological type into three categories (A/AB/B1, B2/B3, TC/NETT) in multivariate analysis. We revised **Tables 3–4** and added **Supplemental Figure 1**, and the text was revised as follows:

<Results; page 10, line 7-11>

To distinguish thymomas from TC and NETT, survival in patients with thymoma was also investigated. In patients with thymoma, RFS did not differ between the low and normal CK groups (P = 0.355, **Supplemental Figure 1**). However, low CK levels were linked to significantly shorter OS (10-year OS: 68.4% vs. 92.7%, P = 0.020, **Supplemental Figure 1B**).

Comment 2: Did low CK affect death from thymic tumors? Please describe the cause of death for each group, especially whether it was due to other diseases or the underlying disease.

Reply 2: We thank you for these constructive comments. Per your remarks, we added a new paragraph to the Results and summarized the causes of death in ***Supplemental Table 1***.

<Results; page 9, line 27-29 and page 10, line 1-2>

The numbers of recurrences and deaths were 17 and 12, respectively, and the recurrence and mortality rates were 14.2% and 10.0%, respectively. We described the clinical and pathological features and the prognosis of patients who experienced recurrence of thymic epithelial tumors in **Supplemental Table 1**. In addition, the main causes of death were thymic epithelial tumors (n = 4, 33.3%), other cancers (n = 3, 25.0%) and pneumonia (n = 2, 16.7%).

Comment 3: Are there any reports that there is a relationship between CK levels and the presence of thymoma? If not, don't people with low CK levels have a poor prognosis regardless of whether they undergo surgery or have thymoma?

Reply 3: We appreciate these queries. As you noted, it is possible that low CK levels portend a poor prognosis, perhaps because low CK levels reflect a poor nutrition status. We suspected that one possible biological mechanism was immunosuppression after thymectomy in patients with low CK levels. Thus, we added the following paragraphs:

<Discussion; page 11, line 22-29 and page 12, line 1-13>

CK activity appeared to decrease because of tumor progression in some research. Decreased CK levels are more commonly detected in tumor cells than in normal tissues in various human cancers (33, 34). Previous reports revealed that low CK levels were related to tumor progression in gastric and esophageal cancers (13, 14). However, our result indicated that CK levels were not significantly associated with factors of tumor malignancy, such as the WHO histological type and Masaoka–Koga stage. In addition, low CK levels were not associated with poor DFS in patients with thymoma (**Supplemental Figure 1A**), although low CK levels were associated with poor DFS in patients with thymic epithelial tumors including TC and NETT (**Figure 3A**). The reason for the discrepancy between CK and tumor malignancy could be the slow progressive nature of thymomas. CK levels could reflect tumor malignancy in patients with TC and NETT but not in those with thymoma. Further studies investigating the relationship between CK level and malignancy are needed.

Conversely, low CK levels were associated with poor OS in both thymic epithelial tumors and thymomas (**Figure 3B** and **Supplemental Figure 1B**). To elucidate the relationship between low CK levels and poor OS in thymic epithelial tumors, we focused on thymic immunological functions. The thymus is the primary organ responsible for generating immunocompetent T cells, and it decreases in size with aging (28). However, thymic immunological function persists even late in life, and age-related thymic involution contributes to the reduction

of thymopoiesis, which precedes T-cell–related immunocompetence (29). Thymectomy leads to decreases in naive T helper and memory lymphocyte counts, which result in immunodeficiency (30). Furthermore, sarcopenia and malnutrition increase the risk of various infections or cancer-related death (12, 31). Therefore, the potential immunocompetence caused by thymectomy could affect patients with low CK levels, which could explain the poor OS in such patients.

Comment 4: Why do some people have low CK levels? If there is no difference in BMI, I don't think there is much of a relationship with undernutrition. Cholesterol levels also affect the presence or absence of hyperlipidemia and the medications taken. Is malnutrition the cause of low albumin levels? Have you ever seen a higher number of patients with decreased liver function in the low CK group? Comorbidities for each group must be described.

Reply 4: We thank you for these helpful comments. **Figure 1** illustrates that BMI was slightly lower in patients with low CK levels than in those with normal CK levels, but this difference was not significant. Therefore, this figure did not appear be clinically significant, and it has been removed.

Furthermore, as you stated, we investigated the past history of hyperlipidemia and liver diseases. We identified 14 patients with hyperlipidemia and 3 patients with liver diseases, and we excluded these patients to explore the relationships of CK levels with cholesterol and albumin levels. Thus, we reanalyzed the data in **Figure 2** and revised the text as follows:

Changes in the text:

<Results; page 9, line 20-24>

Furthermore, we focused on serum albumin and total cholesterol levels to elucidate the relationship between CK levels and the nutrition status. We identified 14 patients with hyperlipidemia and 3 patients with liver diseases, and these patients were excluded from the analysis. Serum albumin and total cholesterol levels were significantly lower in the low CK group than in the normal CK group, as observed in the prior analysis (both $P < 0.001$, **Figure 2A and 2B**).

Comment 5: Discussion is important. Even if it is understood that undernourished people have a worse prognosis than well-nourished people, it is difficult to understand how this relates to thymoma recurrence and

prognosis. It is understandable that cachexia caused by cancer leads to malnutrition and affects prognosis in rapidly progressing cancers, but why is malnutrition associated with thymoma, a low-grade malignant tumor? This Discussion cites research results that show that CK is associated with tumor progression. If this is the case, like a tumor marker, CK would have to decrease if the tumor recurs, but I have no experience of that.

Reply 5: We thank you for your remarks. As you indicated, we described the possible biological mechanisms by which low CK levels were associated with poor prognosis in the Discussion as explained in the response to

Reviewer C

This article focused on prognostic impact of preoperative CK in resected thymic epithelial tumors.

I have several serious comments.

Major points

Comment 1: This article distinguished from type A, AB, B1 from B2, B3 and carcinoma in Table 2, 3 and 4. I think that thymic carcinoma should be discussed and assessed independently form thymoma. Authors should know the complete different nature of thymoma and thymic carcinoma.

Reply 1: We thank you for these constructive comments. As you noted, we must distinguish thymomas from thymic carcinoma and thymic neuroendocrine tumors. Thus, we revised **Table 1–4** and added **Supplemental Figure 1**, and the manuscript was revised as follows:

<Results; page 9, line 5-8>

Myasthenia gravis was present in 10 patients (8.3%). Histological analysis revealed that 15 (12.5%), 28 (23.4%), 25 (20.8%), 32 (26.7%), and 10 patients (8.3%) had WHO type A, AB, B1, B2, and B3 thymoma, respectively, and eight (6.7%) and two patients (1.6%) had thymic carcinoma (TC) and neuroendocrine thymic tumors (NETT), respectively.

<Results; page 10, line 7-11>

To distinguish thymomas from TC and NETT, survival in patients with thymoma was also investigated. In patients with thymoma, RFS did not differ between the low and normal CK groups (P = 0.355, **Supplemental Figure 1**). However, low CK levels were linked to significantly shorter OS (10-year OS: 68.4% vs. 92.7%, P = 0.020, **Supplemental Figure 1B**).

Comment 2: If authors want to include thymic carcinoma in the current analysis, how about thymic neuroendocrine tumor (NET)? I wondered why the did not include cases of thymic NET.

Reply 2: We thank you for this query. As you stated, we should consider cases of NETT. We incorrectly classified NETT as thymic carcinoma in our previously submitted manuscript. We identified two patients with NETT and revised **Tables 1–4** accordingly.

Comment 3: why did authors include curability and adjuvant therapy as a potential prognostic factor?

Reply 3: We thank you for this question. We did not need to focus on adjuvant therapy to investigate the prognostic significance of CK. Regarding curability, we revised the text to categorize incomplete resection as subtotal resection. Thus, we removed this information from **Table 1**.

Reviewer D

The result that preoperative serum creatine kinase is associated with post-operative survival in thymic epithelial tumor seems something new in this category of neoplasm, but I have some questions.

Comment 1: Although the operative procedures are classified as extended thymectomy, tumor resection and complete thymectomy, there is no description on completeness of resection. Is there any patients undergoing subtotal resection?

Patients undergoing incomplete resection, these patients should be excluded in analysis of tumor-free survival.

10 patients had the Stage IV disease. Were they all treated by complete resection?

Reply 1: We thank you for these remarks. As you explained, we should describe subtotal resection. We removed curability (R0/R1) from **Table 1**, and we added subtotal resection (n = 8) as a surgical procedure. In addition, patients who underwent incomplete resection were excluded from the analysis of DFS. Thus, we revised **Tables 1–4** and the text as follows:

<Results; page 9, line 11-13>

Tumor resection (n = 56, 46.7%), complete thymectomy (n = 37, 30.8%), extended thymectomy (n = 19, 15.8%), and subtotal resection (n = 8, 6.7%) were also performed.

Comment 2: Cause of death is important in evaluating overall survival of thymoma, because a considerable portion of patients die from non-tumor related cause. I ask the authors to reveal the cause of death in dead patients. In addition, I request the authors to show the details of recurrence, and the treatment for recurrent lesions. Surgery, radiotherapy or chemotherapy?

Reply 2: We thank you for these constructive comments. Per your remarks, we summarized the causes of death and provided details of recurrence and the treatment for recurrent lesions in ***Supplemental Table 1***.

<Results; page 9, line 27-29 and page 10, line 1-2>

We described the clinical and pathological features and the prognosis of patients who experienced recurrence of thymic epithelial tumors (**Supplemental Table 1**). In addition, the main causes of death were thymic epithelial tumors (n = 4, 33.3%), other cancers (n = 3, 25.0%) and pneumonia (n = 2, 16.7%).