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Reviewer A

Comment 1: To date it is questionable if the authors' conclusion is supported by the data provided. Between the low PMI and the normal PMI group there was a significant difference in mean age of 10 years (59 vs. 69). Here the p-value (0.002) is more than tenfold lower than for the association of low PMI with worse 5yr overall survival (0.026). It should be proven that this difference alone does not explain the difference in survival. Age dependent PMI sarcopenia reference values are needed to clear this question, as provided by Hamaguchi et al (Nutrition 2016). Here the PMI declined significantly after 50 years of age, with a faster decrease noted after the age of 60. Furthermore, a higher age implies a higher mortality rate on its own.

Reply 1: We thank the reviewer for their important comment. As the Reviewer stated, we could not conclusively demonstrate that age and PMI were independent prognostic factors in this study due to the small cohort size, which did not allow for multivariate analysis. However, age was not a prognostic factor in univariable analysis (Table 3). Additionally, we previously demonstrated that both age and PMI were independent prognostic factors in a study involving 135 patients with lung squamous cell carcinoma (Ozeki N, et al. Int J Clin Oncol. 2020;25:876-84). We predict that studies with larger cohort sizes will reveal PMI as a prognostic factor of TSQCC. In accordance with the Reviewer's comment, we have revised our conclusion to temper the interpretation of our findings as follows.

Change in the text:

Abstract (page 3, line 49): "Our findings suggest that PMI might be considered as a potential prognostic factor in TSQCC…"

Discussion (page 13, line 221): "In the present study, our findings suggest…"

Conclusions (page 17, line 293): "This study suggested that…"

Comment 2: In thymic tumors (although less common in thymic carcinoma) myasthenia is a well known complication, which probably would result in a decrease of PMI and which might be of prognostic relevance. Please add information on that issue.

Reply 2: As saliently stated by the Reviewer, myasthenia gravis is an extremely rare presentation in patients with thymic carcinoma. In fact, none of the patients in the current cohort had autoimmune diseases, such as myasthenia gravis. Accordingly, we have added the following statement in the Results section.

Change in the text:
Page 11, line 180: “No patient in the present study had autoimmune disorders, such as myasthenia gravis.”

Comment 3: What is the rationale for choosing a value of 58.2 as a limit for low PMI?
Reply 3: We thank the Reviewer for their inquiry. The median PMI of the cohort was selected as the limit for low PMI. As described in the Limitations section, this retrospective study was performed in a single center and the cohort included patients with similar ethnic backgrounds in one country. Therefore, the general applicability of our findings should be considered with care.
Change in the text: No change.

Comment 4: In section “methods” you state that there have been 215 patients with thymic tumors. I feel including these patients in the analysis would improve the message of the paper as one could compare these different entities.
Reply 4: We thank the Reviewer for their inquiry regarding this important detail. In this study, we aimed to assess the relationship between TSQCC and PMI as well as FXR1 expression. We thought that including the analysis of other thymic epithelial tumors could complicate the interpretation of the results due to the heterogenous characteristics of thymomas. Therefore, only patients with TSQCC were included in the present study. In future studies, we will expand our analyses to other thymic epithelial tumors.
Change in the text: No change.

Comment 5: You state a 5 year overall survival rate of 53 % according to reference (2). The 5 yr OSR in your sample was much higher (93%), is there any explanation for the difference?
Reply 5: We thank the Reviewer for their question. One possible explanation is differences in population. The previous study included not only squamous cell carcinomas but also undifferentiated and small cell carcinomas, among others. In contrast, in the present study, we only focused on squamous cell carcinoma. In addition, 29% of the patients in the previous study had inoperable cancer. We consider that these differences in cohorts might explain the differences in the outcomes of the studies. As explained in Response 1 to Reviewer D, we have revised the reference and modified the corresponding sentence.
Change in the text:

Page 4, line 60: “TCs have a 5-year survival rate of 51%, which is significantly lower than that of thymoma, which is 94% (2).”

Comment 6: In “PMI measurement” a correction for sex is described. Can you explain the precise background of this equation (line 118f)?

Reply 6: We thank the Reviewer for their inquiry. As described in the Methods section, we used the mean PMI values (males, 8.85; females, 5.77) to adjust the total psoas area for individual height. The mean PMI values were based on a study by Hamaguchi et al. who found that the PMI was significantly higher in males than in females during their analysis of the PMI sex distribution in 541 patients who were undergoing living-donor liver transplantation (Nutrition 2016;32:1200–5). Therefore, we adjusted the PMI values by correcting for sex. We have added the following sentence to the Methods section to clarify our approach.

Change in the text:

Page 7, line 120: “Previously, Hamaguchi et al. examined the sex distribution of PMI in 541 patients who underwent living-donor liver transplantation and found that the mean PMI was significantly higher in males than in females (8.85 vs. 5.77) (18). These mean PMI values were used to adjust the total psoas area for individual height using the following equation…”

Comment 7: In table 1, first line under low PMI it should read (31.0 - <58.2). In the same line, the mention of p< 0.001 is of doubtful significance.

Reply 7: We thank the Reviewer for pointing this out and apologize for the oversight. We have corrected the range of low PMI to “31.0–58.1” in Table 1. We confirm that the P-value in the original Table 1 is correct.

Change in the text: (31.0-58.1) in Table 1

Comment 8:

1. Line 77 have instead of has, and Line 87 are instead of is

Reply 8: We thank the Reviewer for their careful observation. The indicated errors have been corrected in page 5, line 79 and page 6, line 92.

Change in the text:

Introduction (page 5, line 79): “no study has assessed the relationship between TSQCC and PMI.”

Introduction (page 6, line 92): “are”

Reviewer B

Comment 1: The authors showed the clinical significance of PMI and one of the myogenesis- associated gene（FXR1) in thymic cancer. I could understand each result of DFS in PMI, the expression of FXR1, and PD-L1. However, I feel it difficult to understand what relationships the PMI had with those prognostic marker (FXR1 and PD-L1). The authors should explain the scientific background focusing the relationships between PMI, FXR1, and PD-L1, and your motive of the present study in "Introduction" in more details.

Reply 1: We thank the Reviewer for their comment, which was addressed by other reviewers as well. As mentioned in the Introduction, FXR1, which has been found to play an essential role in normal myogenesis, is highly expressed in myocytes. Mutations or altered expressions of RNA-binding proteins can cause dysfunction in multiple biological and pathological processes, causing diseases such as cancer (Deng M, et al. Cell Death Dis. 2022;13:170). FXR1 overexpression in cancer is a candidate biomarker for poor survival in various types of cancers, including lung cancer (Qian J, et al. Proc Natl Acad Sci U S A. 2015;112:3469-74). Therefore, we hypothesized that FXR1 expression was a prognostic factor in patients with TSQCC as well. As indicated in our response to Reviewer A, only univariate analyses could be performed due to the small cohort size, and hence definitely determining whether PMI, FXR1, and PD-L1 were independent prognostic factors was not possible. Therefore, PMI and FXR1 expression were investigated in an exploratory approach. Further, PD-L1 was evaluated in TSQCC based on its controversial role as a prognostic factor. The following revisions in the Introduction section correspond to the above details.

Change in the text:

Page 5, line 82: "…and FXR1 is highly expressed in myocytes. Mutations or altered expressions of RNA-binding proteins can cause dysfunction in multiple biological and pathological processes, leading to diseases such as cancer (12)."

Page 5, line 88: "Therefore, we hypothesized that FXR1 expression was a prognostic factor in patients with TSQCC."

Comment 2: I fell that there are the significant relationships with PMI and the expression of FXR1 by intuition. However, your data did not show any significant relationships among them. You had better mention your speculation of those unexpected results and previous reports on other malignancies regarding PMI and FXR1 in "Discussion".

Reply 2: We thank the reviewer for this important comment. The prognostic potential of FXR1 has been demonstrated in lung as well as other cancers (Qian J, et al. Proc Natl

Acad Sci U S A. 2015;112:3469-74). However, no study to date has investigated PMI or other musculoskeletal mass indices and FXR1 expression as potential prognostic markers for lung cancer. To the best of our knowledge, this is the first study to investigate the relationship between skeletal muscle mass and FXR1 expression in patients with TSQCC. The present study is only exploratory, and the results show that FXR1 is widely and strongly expressed in TSQCC, regardless of PMI status. FXR1 expression may not be a prognostic factor. However, our findings suggest that FXR1 as a predictor of treatment if targeted therapies for FXR1 are developed in the future. In response to the Reviewer's suggestion, we have added the following statement in the Discussion section.

Change in the text:

Page 15, line 257: "Although not directly indicating FXR1 as a prognostic factor in patients with TSQCC, this finding highlights the possibility of a relationship between the skeletal muscle mass and the microenvironment of TSQCC."

Reviewer C

Comment 1: Authors examined PMI, and FXR1and PD-L1 expression with IHC. Why did they focus on these factor or molecules? Is there any relationship between PMI and FXR1/ PD-L1? Please describe this explanation in the background of abstract and text.

Reply 1: We thank the Reviewer for their important comment, which has been mentioned by other reviewers as well. As stated in the Introduction, FXR1 plays an essential role in normal myogenesis and is highly expressed in myocytes. Mutations or altered expressions of RNA-binding proteins can cause dysfunction in multiple biological and pathological processes, causing diseases, such as cancer (Deng M, et al. Cell Death Dis. 2022;13:170). FXR1 overexpression is a candidate biomarker for poor survival in various types of cancers, including lung cancer (Qian J, et al. Proc Natl Acad Sci U S A. 2015;112:3469-74). Therefore, we hypothesized that FXR1 expression was a prognostic factor in patients with TSQCC. We investigated the potential role of PMI and FXR1 as prognostic factors in an exploratory study setting. We also evaluated PD-L1 in TSQCC based on its controversial role as a prognostic factor. To clearly state our aim, we have revised the Introduction to include additional sentences and a new reference as follows**.**

Change in the text:

Page 5, line 82: "…and FXR1 is highly expressed in myocytes. Mutations or altered expressions of RNA-binding proteins can cause dysfunction in multiple biological and pathological processes, leading to diseases such as cancer (12)."

Page 5, line 88: “Therefore, we hypothesized that FXR1 expression was a prognostic factor in patients with TSQCC.”

Comment 2: The evaluation methods for FXR1 and PD-L1 should be also briefly included in Abstract.

Reply 2: We thank the Reviewer for their recommendation. Accordingly, we have added the following statement in the Abstract.

Change in the text:

Page 3, line 40: “Immunohistochemical analysis was performed to determine the FXR1 and PD-L1 expression levels.”

Comment 3: A proportion of patients would receive radiation or chemotherapy in addition to surgical treatment. These modalities and the ratio should be mentioned in the table of patients’ characteristics. Did they influence to the outcome?

Reply 3: We thank the Reviewer for their important comment. In our cohort, postoperative radiotherapy, which was administered in 11 patients, did not impact the prognosis. The 5-year OS rates did not show significant differences between the patients with and without postoperative radiotherapy (90.9% and 95%, respectively; $P = 0.788$). Similarly, we did not observe a significant difference in the 5-year DFS rate between the patients with and without postoperative radiotherapy (30.7% and 67.7%, respectively; $P = 0.105$). Accordingly, we have revised Tables 1 and 3. We have also added the following sentence in the Results section.

Change in the text:

Page 11, line 179: “Postoperative radiotherapy was administered to 11 patients (32%).”

Comment 4: There is a description of the median follow up as 103 months in Results. Is this true? The range is 1-133 months and the study period is 2006-2018. Lots of censored cases are found until 60 months in the survival curve in Fig.4. Reconfirmation is necessary.

Reply 4: We thank you the Reviewer for their salient comment. This was a simple but important mistake. We apologize for the oversight. Following the review of the data, we confirm that the median follow-up was 60.2 months. We have corrected the number to “60” in the Results section (page 12, line 199).

Change in the text: 60

Comment 5: Because of the small sample size, the subset survival analyses by the stage does not make sense. Fig.5 could be deleted.

Reply 5: We agree with the Reviewer. Accordingly, we have removed Figure 5 from the revised manuscript.

Change in the text: Figure. 5 was deleted.

Comment 6: What is the outcome, if excluding R1,2 resection cases?

Reply 6: We thank the Reviewer for their inquiry. Accordingly, we have performed additional analysis after excluding the five patients who underwent R1 resection. The 5-year OS rates were 81.8% and 100% in the low and normal PMI groups, respectively (P = 0.055). The 5-year DFS rates were 49.5% and 77.5% in the low and normal PMI groups, respectively (P = 0.04). These results were consistent with the findings of the entire cohort. We have added the following sentence to the Results section to provide additional details.

Change in the text:

Page 10, line 178: "R0 resection was performed in 29 patients (75%), whereas R1 resection was performed in 5 patients (15%)."

Comment 7: L.86; "Skeletal muscle mass plays in TSQCC progression.", L.234; "Skeletal muscle mass could reflect the tumor biological nature." I cannot understand or speculate the biological effect or influence of Skeletal muscle to the tumor biology. Please explain in detail for this description, scientifically.

Reply 7: We thank the Reviewer for their important comment, which has been mentioned by other reviewers as well. As stated in the Introduction, FXR1 plays an essential role in normal myogenesis and is highly expressed in myocytes. Mutations or altered expressions of RNA-binding proteins can cause dysfunction in multiple biological and pathological processes, leading to diseases such as cancer (Deng M, et al. Cell Death Dis. 2022;13:170). FXR1 overexpression is a candidate biomarker for poor survival in various types of cancers, including lung cancer (Qian J, et al. Proc Natl Acad Sci U S A. 2015;112:3469-74). Therefore, we hypothesized that FXR1 expression was a prognostic factor in patients with TSQCC. We investigated the potential role of PMI and FXR1 as prognostic factors in an exploratory study setting. We also evaluated PD-L1 in TSQCC based on its controversial role as a prognostic factor. To clearly state our aim, we have revised the Introduction to include additional sentences and a new reference as follows.

Change in the text:

Page 5, line 82: "…and FXR1 is highly expressed in myocytes. Mutations or altered expressions of RNA-binding proteins can cause dysfunction in multiple biological and pathological processes, leading to diseases such as cancer (12)."

Page 5, line 88: "Therefore, we hypothesized that FXR1 expression was a prognostic factor in patients with TSQCC."

Reviewer D

Comment 1: In Introduction section, some descriptions are not related to references.
Reply 1: We thank the Reviewer for their suggestion. We have revised the reference and modified the following sentence. Second, we modified the following sentence.
Change in the text:
Page 4, line 60: "TCs have a 5-year survival rate of 51%, which is significantly lower than that of thymoma, which is 94% (2)."
Page 4, line 67: "…with a clinical response to programmed death-1 (PD-1) antibodies, as suggested in a phase II study (6)."

Comment 2: In Method section, reference 17 did not discuss about PMI evaluation.
Reply 2: We thank the Reviewer for their careful observation and apologize for the oversight. We have revised the manuscript to cite reference 10 (page 7, line 118).
Change in the text: 10

Comment 3: In Result section, should the analysis could further subgroup stage III-IV to stage III-IVA and stage IVB? Because stage IVB thymic cancer has worse prognosis than stage III-IVA disease.
Reply 3: We thank the Reviewer for their suggestion. According to the Masaoka–Koga classification, there was one patient with stage IVa disease, while eight patients had stage IVb disease. All the patients had stage IVa disease according to the TNM classification. In other words, stage IV disease occurred due to lymph node metastasis in most cases and intrapulmonary or distant metastasis was not observed. To address the Reviewer 's concern, we have deleted Figure 5 (Comment 5 by Reviewer C).
Change in the text: Figure. 5 was deleted.

Comment 4: In table 3, univariate analysis showed high or low PD-L1 expression is associated with survival difference. Is this difference still noted by Kaplan-Meier survival curve? In addition, the abbreviations of table 3 did not showed CYFRA and its full name.
Reply 4: We thank the Reviewer for their salient suggestions. Indeed, the 5-year OS was significantly better in patients with high PD-L1 expression than in those with low PD-L1 expression according to the Kaplan–Meier method using the log-rank test (100.0% vs 85.1%, P = 0.018). We have added the full definition of CYFRA (cytokeratin fragment) to Table 3.

Change in the text: CYFRA, cytokeratin fragment

Comment 5: In conclusion section, FXR1 may not be a candidate target for cell-specific therapy, because its expression is widely observed regardless of the PMI status, and not related to prognosis.

Reply 5: We partially agree with the Reviewer's point of view. We also suggest that solid conclusions may be difficult to derive based on the findings of our study, since we were not able to perform multivariate analysis due to the small sample size. Nonetheless, albeit based on an exploratory study, our findings indicating the wide and strong expression of FXR1 in TSQCC independent of the PMI status raises further questions regarding the relationship between skeletal muscle mass and the microenvironment in TSQCC. In response to the Reviewer's comment, we have revised our Conclusion to temper the interpretation of our findings as follows. In addition, we added the following sentence in Discussion section.

Change in the text:

Abstract (page 3, line 49): "Our findings suggest that PMI might be considered as a potential prognostic factor in TSQCC…"

Discussion (page 13, line 221): "In the present study, our findings suggest…"

Conclusions (page 17, line 293): "This study suggested that…"

Page 15, line 257: "Although not directly indicating FXR1 as a prognostic factor in patients with TSQCC, this finding highlights the possibility of a relationship between the skeletal muscle mass and the microenvironment of TSQCC."

Reviewer E

Comment 1: The group analysed was small, which consequently prevented multivariate analysis. Therefore, we cannot be sure whether the results are reliable. Could the authors address this and suggest what other variables correlate with sarcopenia? Does age matter or gender matter? Other laboratory findings?

Reply 1: We agree with the reviewer's point of view. We also suggest that solid conclusions may be difficult to derive based on the findings of our study, since we were not able to perform multivariate analysis due to the small sample size. However, per out response to Comment 1 of Reviewer A, we previously demonstrated that both age and PMI were independent prognostic factors in a study involving 135 patients with lung squamous cell carcinoma (Ozeki N, et al. Int J Clin Oncol. 2020;25:876-84). Therefore,

we predict that studies with larger cohort sizes will reveal PMI as a prognostic factor. We have revised our Conclusion to temper the interpretation of our findings as follows.

Change in the text:

Abstract (page 3, line 49): "Our findings suggest that PMI might be considered as a potential prognostic factor in TSQCC…"

Discussion (page 13, line 221): "In the present study, our findings suggest…"

Conclusions (page 17, line 293): "This study suggested that…"

Comment 2: More detailed information on postoperative treatment needs to be added. This will also be relevant in future studies planned by the authors, as a significant variable in the multivariate analysis relating to OS.

Reply 2: We thank the Reviewer for this important suggestion. As per our response to Comment 3 of Reviewer C, postoperative radiotherapy, which was administered to 11 patients, did not impact the prognosis. The 5-year OS rates did not show significant differences between the patients with and without postoperative radiotherapy (90.9% and 95%, respectively; $P = 0.788$). Similarly, we did not observe a significant difference in the 5-year DFS rate between the patients with and without postoperative radiotherapy (30.7% and 67.7%, respectively; $P = 0.105$). Accordingly, we have revised Tables 1 and 3. We have also added the following sentence in the Results section.

Change in the text:

Page 11, line 179: "Postoperative radiotherapy was administered to 11 patients (32%)."

Comment 3: It would be valuable to broaden the discussion relating to potential methods of preventing sarcopenia and optimising the condition of patients before surgery.

Reply 3: We thank the Reviewer for their valuable recommendation. As described in the Discussion section, exercise is a non-pharmacological intervention that improves the psychological status of patients with lung cancer. Furthermore, nutritional supplements, including branched-chain amino acids, vitamin D, whey protein, and hydroxymethylbutyrate-enriched milk, play an important role in preventing and improving sarcopenia (page 16, lines 270–272). Whether improvement in sarcopenia following these interventions can enhance the postoperative prognosis remains unclear due to the luck of the randomized control trial. Therefore, further studies are required to explore this possibility. In response to the Reviewer's comment, we have added the following statement in the Discussion section.

Change in the text:

Page 16, line 274: “Whether improvement in sarcopenia following these interventions can enhance the postoperative prognosis remains unclear due to the luck of the randomized control trial. Therefore, further studies are required to explore this possibility.”

Reviewer F

Comment 1: Consider the confound factors: It will be depending on the staging. In the current results (from the Figure), the research question is not resolved. Divided into treatment intent and analyze them.

Reply 1: We thank the Reviewer for their careful observation. We agree that confounding factors should be considered. Our findings suggest that PMI is a potential prognostic factor in TSQCC based on the results of univariate analysis (Table 3) and not multivariate analysis. We agree that there might be a confounding relationship between PMI and stages. However, there was no difference in p-Stage between the low and normal PMI groups. In addition, our subgroup analysis based on p-Stage stratification indicated that the PMI status was a prognostic factor in advanced TSQCC. We were not able to perform multivariate analysis due to the small cohort size, and hence solid conclusions may be difficult to derive from the current study results. As per our responses to the previous comments of the other reviewers, we have revised our Conclusion to temper the interpretation of our findings as follows.

Change in the text:

Abstract (page 3, line 49): “Our findings suggest that PMI might be considered as a potential prognostic factor in TSQCC…”

Discussion (page 13, line 221): “In the present study, our findings suggest…”

Conclusions (page 17, line 293): “This study suggested that…”

Comment 2: I think a test is not required for the Patient's characteristics (Table 1.)

Reply 2: While we are not certain regarding the Reviewer’s comment, we wished to clarify that the patient characteristics were not significantly different between the two patient groups. Therefore, we have retained the corresponding *P*-values in Table 1.

Change in the text: No change.

Comment 3: Multiple analyses will be needed.

Reply 3: We thank the Reviewer for this important suggestion. While we wholeheartedly agree with the significance of multivariate analyses, we were not able to conduct the

analysis due to the small number of patients and events. We have revised the following sentence in the Discussion section to acknowledge this limitation.

Change in the text:

Page 16, line 284: "…perform multivariate analyses due to the small number of patients and events."

Comment 4: What brought you to analyze the FXR1 for thymic carcinoma? Clarify the background.

Reply 4: We thank the Reviewer for their comment, which was addressed by other reviewers as well. As mentioned in the Introduction, FXR1, which has been found to play an essential role in normal myogenesis, is highly expressed in myocytes. Mutations or altered expressions of RNA-binding proteins can cause dysfunction in multiple biological and pathological processes, leading to diseases such as cancer (Deng M, et al. Cell Death Dis. 2022;13:170). FXR1 overexpression in cancer is a candidate biomarker for poor survival in various types of cancers, including lung cancer (Qian J, et al. Proc Natl Acad Sci U S A. 2015;112:3469-74). Therefore, we hypothesized that FXR1 expression was a prognostic factor in patients with TSQCC as well. As indicated in our response to Reviewer A, only univariate analyses could be performed due to the small cohort size, and hence definitely determining whether PMI, FXR1, and PD-L1 were independent prognostic factors was not possible. Therefore, PMI and FXR1 expression were investigated in an exploratory approach. Further, PD-L1 was evaluated in TSQCC based on its controversial role as a prognostic factor. The following revisions in the Introduction section correspond to the above details.

Change in the text:

Page 5, line 82: "…and FXR1 is highly expressed in myocytes. Mutations or altered expressions of RNA-binding proteins can cause dysfunction in multiple biological and pathological processes, leading to diseases such as cancer (12)."

Page 5, line 88: "Therefore, we hypothesized that FXR1 expression was a prognostic factor in patients with TSQCC."

Comment 5: Clarify whether all patients were completely resected or not.

Reply 5: In this study, R0 resection was performed in 29 patients (75%), while R1 resection was performed in 5 patients (15%) (Table 1). We have revised the Results section to provide this important information.

Change in the text:

Page 10, line 178: "R0 resection was performed in 29 patients (75%), whereas R1 resection was performed in 5 patients (15%)."

Comment 6: Clarify the future perspective from the results of the research.

Reply 6: We thank the Reviewer for their salient suggestion. As described in the Discussion section, we believe that the results of our study have several implications for the design of future clinical studies involving TSQCC. We suggest that simple enhancements in exercise and nutritional support targeting skeletal muscle mass may improve the prognosis of patients with TSQCC. In addition, in this first study to evaluate the expression of FXR1 in thymic carcinoma, the extensive FXR1 expression in TSQCC raised the possibility of FXR1 as a new biomarker for molecular targeted therapies in the future.

Change in the text: No change.

Comment 7: Page 4. line 61: specify the number of subtypes. Figure 5. I think "IV" will be deleted in figure (B) and (D).

Reply 7: As recommended by the Reviewer, we have specified the number of subtypes (14 subtypes) (page 4, line 62). We have also deleted Figure 5 in response to Comment 5 by Reviewer C.

Change in the text: 14 (page 4, line 62)