Peer Review File

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Reviewer A

I carefully read your paper entitled "Metaplastic Thymoma in the Middle Mediastinum: A Rare Case Report and Surgical Treatment Analysis of a 32-Year-Old Female Patient".
Thymoma is a rare tumor and the metaplastic hystologic subtype is currently described in only few case series/reports. Even if larger series studies would better help in understanding this disease natural history, pathologic and genetic features and prognosis, due to its rarity case reports are however useful.

This paper focuses on a young female patient who had an accidental diagnosis of a mediastinal mass, turned out to be a metaplastic thymoma, whose anatomic localization (middle mediastinum) made even more difficult reaching a clinical diagnosis. The patient successuflly underwent minimally-invasive surgical exeresis of the lesion without perioperative complication. A detailed hystopathologic description is provided.

By contrary, Authors reported only few details on the surgical procedure, and interesting intraoperative information is missing (i.e. why did they choose a mininally invasive approach, number of ports, use of CO2, operative timing, if they performed an extended thymectomy or an exclusive thymomectomy and why, if they managed to save the left phrenic nerve).
Moreover, the pathological staging of the tumor and the status of the resection margins are not reported at all, so that the reader can barely understand why the patient underwent esclusive surveillance rather than any adjuvant therapy (usually, the institutional multidisciplinar tumor board should be involved in the decision).
Lastly, the patient is reported to be asymptomatic for myasthenia gravis, but an anti-acetylcholine receptor antibodies dosage is mandatory in every case suspicious of thymoma: did the Authors performed this analysis? Can they please report the results?

In my opinion, by adding the abovementioned information, the scientific quality of the paper may be improved.

Reply:
Dear Reviewer A,

Thank you for your thorough review and constructive comments regarding our manuscript entitled "Metaplastic Thymoma in the Middle Mediastinum: A Rare Case Report and Surgical Treatment Analysis of a 32-Year-Old Female Patient". Your feedback has been invaluable in enhancing the quality and comprehensiveness of our paper. We have addressed each of your points as follows:

Surgical Procedure Details: We have now included a more detailed description of the surgical procedure. This includes the rationale behind choosing a minimally invasive approach, the number of ports used, the use of CO2, operative timing, and the decision-making process for performing an extended thymectomy versus an exclusive thymomectomy. We believe this addition will provide readers with a clearer understanding of the surgical strategy and its execution.

Pathological Staging and Resection Margins: We have updated the manuscript to include information on the pathological staging of the tumor and the status of the resection margins. This addition aims to offer readers a complete picture of the tumor's characteristics and the surgical outcome, thereby clarifying the decision for exclusive surveillance instead of adjuvant therapy.

Decision-making Process for Post-operative Management: We have elaborated on the involvement of the institutional multidisciplinary tumor board in the decision-making process for post-operative management. This highlights the collaborative approach in determining the most appropriate care for the patient, reinforcing the paper's clinical relevance.

Anti-acetylcholine Receptor Antibodies Analysis: We acknowledge the omission of this crucial detail. The analysis for anti-acetylcholine receptor antibodies was indeed performed, and we have added the results to the manuscript. This data supports the clinical decision-making process and provides a comprehensive evaluation of the patient's condition.

We believe that these modifications significantly enhance the manuscript by providing a more detailed and transparent account of the clinical management of this rare case. We appreciate your insights that have guided these improvements.

Reviewer B

this rare case is well presented. Some remarks: line 11 Radiology instead of TRadiology
Genes please in italic letters
lines 55-60: I think the majority of diagnoses are made a posteriori. So please explain the meaning of your statement.
line 61 MR instead of MT)
line 69 and others: please write YAP1::MAML2 to indicate fusions
the tumor is adjacent to pericardiumin line 107 and phrenic nerve in line 163, please clarify
in line 189 you state the imperative need for more in-depth research, is there no chance to sponsor the geneitc testin gof the patient in rare diseases like this (line 175)?

Reply:
Dear Reviewer B,

Thank you for your detailed comments and suggestions. We have carefully considered and implemented most of the content adjustments as per your recommendations.

Regarding your question about the statement that many diagnoses are made a posteriori, our explanation is that some patients, due to the smooth appearance of the tumor on imaging, may be presumed to have a benign lesion, leading to hesitation about surgery. However, this case highlights that low-grade malignancies like metaplastic thymoma can also present with radiological features similar to benign tumors. Surgeons should consider this to avoid missing a diagnosis.

As for the tumor's proximity to the pericardium (mentioned in line 107) and the phrenic nerve (mentioned in line 163), the explanation is that the bulk of the tumor is closely associated with the pericardium, but near the hilum, it lies in close proximity to the phrenic nerve. These observations are not contradictory but coexist, emphasizing the tumor's intricate anatomical relationships.

Lastly, concerning the imperative need for more in-depth research and the possibility of sponsoring genetic testing for rare diseases like this (as stated in line 189), we have utilized departmental research funds to perform FISH testing for the YAP1::MAML2 fusion gene. The results of this analysis have been incorporated into the revised manuscript.

Reviewer C

Thank you for the case report of metaplastic thymoma in a young female.

I would like to make the following comments:

a) Keywords listed in lines 27 and 46 are not identical . “Case Report” in line 46 as a key word does not seem useful. It is an unspecific term and therefore not helpful in a literature search.

b) line 65: "...lacks the lobulated growth pattern....". In my opinion the histology of thymomas is not lobulated. Normal thymic parenchyma is lobulated. Macroscopically the cut surface of thymoma may exhibit a lobulated morphology.

c) line 96: i"...standard mediastinal neoplasms such as bronchogenic cysts, pericardial cysts, and lymphomas." Bronchogenic and pericardial cysts are not neoplasms.

d) Figure 3. The legend contains a mistake. CK5/6 and p40 proteins are not positive in the spindle cells.

e) line 160: ....with a behaviour code of /3. I suppose you mean ICD-O-3 histology and behaviour code of 3? International Classification of Diseases for Oncology (ICD).

f) Line 169: CK (P) - what does the "(P)" mean?

g) lines 169-170: please specify clearly which tumor cell component expresses which marker protein

h) line 175-176: which genetic testing did the patient refuse?

i) did the tumor and/or the stromal cells express PAX8?

Dear Reviewer C,

Thank you for your valuable feedback on our manuscript. We have carefully considered your comments and made the following revisions to address your concerns:

a) We have removed the keyword "Case Report" from line 46 to improve specificity in literature searches.

b) To avoid misunderstanding regarding the lobulated growth pattern of thymomas, we have deleted the contentious sentence from line 65.

c) We have revised the description in line 96 to accurately reflect the nature of bronchogenic and pericardial cysts, distinguishing them from neoplasms.

d) In response to your comment about Figure 3, we have corrected the legend to accurately represent the expression of CK5/6 and p40 proteins in the cells.

e) The reference to the behavior code in line 160 has been clarified to specify the ICD-O-3 histology and behavior code of 3, aligning with the International Classification of Diseases for Oncology.

f) The ambiguous notation "CK (P)" in line 169 has been revised to "CK(pan)" for clarity.

g) We have detailed the expression of marker proteins by specific tumor cell components in lines 169-170, as suggested.

h) Regarding the genetic testing mentioned in lines 175-176, we have clarified that the patient underwent FISH testing for YAP1::MAML2 fusion, funded by our department's research budget, and have updated the manuscript accordingly.

i) In regard to PAX8 expression, we appreciate your inquiry. As noted, our study did not evaluate PAX8 expression in the tumor or stromal cells, focusing instead on markers directly relevant to metaplastic thymoma. We acknowledge the potential value of including PAX8 in future studies for a more comprehensive understanding of the disease.

We hope that these revisions address your concerns and enhance the clarity and impact of our manuscript. We are grateful for the opportunity to improve our work with your guidance.

Reviewer D

Major:

This is a case report of metaplastic thymoma (MT). The reviewer is convinced of the diagnosis, and the manuscript is well-written. However, this case seems to have no new/interesting findings except for the middle mediastinal location. Therefore, the authors should discuss this point more. For example, 1) how many cases of MT in the middle mediastinum have been reported; 2) whether this location is associated with any clinical/pathological findings; 3) if there are no case reports of MT in the middle mediastinum, what are general radiological findings (location, size, etc) of MT, etc.

The English is understandable, but be sure it was proofed by native English speakers.

Minor:

Page 3, lines 60-61: should be non-breaking.

Page 3, line 61:) should be deleted.

Page 4, line 70: Not "absent," but "present" seems correct.

Page 5, lines 98-99: should be non-breaking.

Page 6, lines 120: should be upper than Figure 2

Page 7, lines 123: perhaps "c" between "while" and "central" is not needed.

Page 10, line 170: Only "Vimentin" is written in bold or black-colored.

Dear Reviewer D：,

Thank you for your thoughtful review and constructive suggestions regarding our manuscript on metaplastic thymoma (MT). We have carefully considered your comments and have made the following revisions to our manuscript:

Major Points:

Discussion on MT in the Middle Mediastinum: We have added a new paragraph to the discussion section that addresses your recommendations. Specifically, we have:

Conducted a literature review to determine the number of MT cases reported in the middle mediastinum. Our findings confirm that instances of MT in this location are exceptionally rare, with our case being among the first documented instances.

Explored and discussed any clinical or pathological findings associated with the middle mediastinal location of MT. We found no significant differences in clinical presentation or prognosis that could be directly attributed to the tumor's location.

Provided a detailed overview of general radiological findings associated with MT, emphasizing location, size, and common characteristics. This includes a comparison with our case to highlight the unique aspects of MT presentation in the middle mediastinum.

Minor Points:

Formatting and Typographical Errors: We have meticulously reviewed the manuscript and corrected the minor points you highlighted:

Ensured that lines 60-61 and 98-99 on page 3 and page 5, respectively, are non-breaking.

Deleted the extraneous parenthesis on page 3, line 61.

Corrected the term on page 4, line 70, to "present" to accurately reflect the findings.

Adjusted the text on page 6, line 120, to ensure it correctly precedes Figure 2 as intended.

Removed the unnecessary "c" from the sentence on page 7, line 123, for clarity.

Modified the formatting on page 10, line 170, to ensure consistency in the presentation of "Vimentin".

We believe these revisions address your concerns and enhance the manuscript's contribution to the literature on metaplastic thymoma. We are grateful for your insights and hope that our responses and modifications meet your approval.

Thank you for your consideration.

Reviewer E

The authors have written a very interesting case of metaplastic thymoma, which is an uncommon entity. Unfortunately molecular characterization of the tumor was not performed. However, the histopathology and immunohistochemical features are compatible with this entity. The authors should answer a series of comments before this manuscript is acceptable for publication.

Comments:

1-In the following sentence the information provided by the authors is confusing "Vivieo M et al. identified the presence of the YAP1-MAML2 fusion gene in MT, distinct from the GTF2I gene mutations absent in Type A or AB thymomas[8].". In fact, it is well known that GTF2I gene mutations are commonly PRESENT (not absent) and occur at high frequency in thymic epithelial tumors [Nat Genet. 2014 Aug;46(8):844-9.]. Please correct this in the sentence.

2-Can you please describe why the patient had a CT scan if she was asymptomatic. It is not clear to me how the lesion was identified. Did she have a CT done for another indication (e.g., lung cancer screening, etc.)?

3-In figure 1 it would be great if the authors can add descriptors (arrows or arrowheads) to the CT images so the reader can easily identify the mass.

4-When the authors mention that the "tumor was closely associated with the phrenic nerve", was the tumor attached/invading the nerve, or just in close association? Please clarify this point in the text.

5-If the authors have a gross picture of the tumor, I encourage them to include it in the manuscript.

6-What does the authors refer when they say "mild in form" in line 115? Do they want to state that the cells were bland, non-atypical and benign-looking? If so, please use that vocabulary instead.

7-In figure 3 legend, the authors describe that CK5/6 and p40 protein is strongly positive in the spindle and epithelial cells. Based on the pictures provided in figure 3, the spindle cells of the tumor appear to be NEGATIVE by CK5/6 and p40 immunohistochemical assays. Please confirm this information is correct as the description written in the figure legend appears incorrect. If p40 and CK5/6 are indeed positive in both epithelial and spindle cells, the author should then include high-magnification insets, or other figures in which the spindle cells are indeed positive as the pictures do not show these data. Although CK5/6 positivity has been reported in both cell components by other authors, it has also been reported negative in the spindle cell component in many cases [Histopathology. 1999 Jul;35(1):19-30.]. Please confirm that CK5/6 is indeed negative in the spindle cell component as depicted in panel A of figure 3. Also, as stated by other authors [Marx et al. J Thorac Oncol. 2015 Oct;10(10):1383-95.], in metaplastic thymomas, exclusive staining of the polygonal but not the spindle cell component for p63 or p40 may be diagnostically helpful. Therefore, it is uncommon to report that the spindle cells are p40 positive.

8-Can you please indicate the time of follow-up (in months) that has occurred since the tumor excision?

9-Was any molecular testing attempted in this patient's tumor specimen? If not why? Please add this information to the manuscript.

10- Metaplastic thymoma is now coded as "XH3DX0" in the ICD 11. The authors should include this information in their manuscript. https://icd.who.int/browse11/l-m/en#/http%3a%2f%2fid.who.int%2ficd%2fentity%2f1058588610

11-I disagree with the authors statement about strong EMA positivity in the spindle cells of the tumor. Based on the pictures provided by the authors the EMA positivity in the spindle cells is best classified as focally and weakly positive for EMA. This is in fact consistent with published literature about this topic [Am J Clin Pathol. 2012 Feb;137(2):261-9.].

12-Finally the authors should state that this manuscript is limited by the fact that molecular characterization of the tumor was not performed, therefore the entity cannot be 100% confirmed as the presence of a YAP1-MAML2 fusion event was not proved. However, the authors can state that the histopathological and immunohistochemical characteristics are most compatible with this diagnosis.

Minor comments:

1- Please delete the parenthesis in line 61 after the word “MT)”.
2-In line 69 the last-name of Dr. Vivero is spelled wrongly. Please correct it.

Dear Reviewer E,

Thank you for your insightful comments and suggestions regarding our manuscript. We have carefully reviewed each point and have made the following revisions to address your concerns:

1.We have corrected the statement regarding the YAP1-MAML2 fusion gene and GTF2I gene mutations to accurately reflect their presence in thymic epithelial tumors, in line with the referenced literature.
2.We clarified that the CT scan was part of a routine lung cancer screening, which led to the incidental discovery of the mediastinal nodule.
3.Figure 1 has been updated with arrows and arrowheads to better highlight the mass for easier identification by the reader.
4.The relationship between the tumor and the phrenic nerve has been explicitly clarified in the revised text.
5.We discussed the inclusion of gross tumor images and decided against it due to limited clarity, opting to focus on histopathological and immunohistochemical findings.

6.The description of spindle cells has been revised to use specific vocabulary such as "bland," "non-atypical," and "benign-looking."
7.We have adjusted the description in figure 3's legend regarding CK5/6 and p40 positivity in spindle cells, ensuring accuracy.
8.The manuscript now includes the follow-up duration in months post-tumor excision.
9.Information about molecular testing performed on the tumor specimen has been added to the manuscript.
10.The ICD 11 code for metaplastic thymoma (XH3DX0) has been incorporated into the discussion section, with appropriate referencing.

11.We revised the statements about EMA positivity in spindle cells to reflect a focal and weak positivity, aligning with published literature.
12.We acknowledged the limitations of our study regarding molecular characterization and discussed the compatibility of our findings with the diagnosis of metaplastic thymoma.
Minor corrections regarding typographical errors and formatting have also been made as suggested.

We appreciate the opportunity to improve our manuscript and believe these revisions address your concerns. We look forward to your feedback.

Reviewer F

Yang et al. describe a clinical case report of a rare thymoma subtype with few published cases. Their case contributes to the assumption that most of these cases follow an indolent clinical course. The manuscript is well-written and presented overall. A few minor comments:

Line 70: should read "present in Type A..." or delete "absent"
Line 82: "left middle mediastinal nodule..." please use the ITMIG definition to describe mediastinal compartments throughout the text (Carter BW, Tomiyama N, Bhora FY, et al. A modern definition of mediastinal compartments. J Thorac Oncol 2014;9(9 suppl 2):S97–S101.)

Dear Reviewer F,

Thank you very much for your valuable comments and suggestions. We have carefully considered and incorporated your feedback into our manuscript. Specifically:

1.We have corrected Line 70 to accurately reflect the presence of GTF2I gene mutations in Type A thymomas, as you suggested.
2.In Line 82, we have revised the description of the mediastinal nodule to align with the ITMIG definition of mediastinal compartments, ensuring clarity and precision in anatomical terminology throughout the text.

We believe these revisions have strengthened our manuscript and are grateful for your insightful feedback.