

Peer Review File

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Review Comments (Round 1)

Reviewer A

Overall comments:

This review article described general and clinical perspectives of mediastinal thymic cysts. Separate sections include pathophysiology, histology, diagnosis, symptoms, and treatment. I appreciate the author's efforts to review the rare disease comprehensively. However, I felt that each section is detail-oriented and may not provide clinically useful information. Each section mentioned various descriptions of thymic cysts, including rare conditions, and did not mention the most critical or clinically valuable features. In addition, I was unclear if the authors intended to discuss solely thymic cysts or wanted to include cystic changes in the neoplastic process. No figures or tables were provided.

1. The exact prevalence of thymic cysts is unclear even in the literature. However, I do not know how the authors estimated it as 0.01%. The number of 0.01% reported in the literature by Henschke (Radiology. 2006 May;239(2):586-90) is the rate of new mediastinal mass on annual screening studies. I recommend that authors look at ACR white paper (J Am Coll Radiol. 2018 Aug;15(8):1087-1096).

Reply: We agree that the true incidence of thymic cysts is difficult to estimate. We have revised our manuscript to reflect this and added the suggested reference (ACR white paper - (J Am Coll Radiol. 2018 Aug;15(8):1087-1096)

This has been revised on page 3.

Mediastinal thymic cysts are exceedingly rare and the true incidence is difficult to estimate. In patients undergoing imaging for lung cancer screening, the prevalence of any mediastinal mass was 0.77% and mediastinal thymic cyst was 0.01%. Other studies have reported that mediastinal thymic cysts represent only 1 to 4 percent of all mediastinal masses diagnosed.

2. Pathophysiology: The most commonly used classification of thymic cysts are congenital/acquired or unilocular/multilocular. I thought dividing those as congenital vs. inflammatory was confusing.

Reply: We agree that there are multiple ways to categorize mediastinal thymic cysts. We choose congenital/inflammatory based on based on the extensive review of

literature performed by Choi et al, the authors suggest a simpler classification system of congenital/inflammatory as suggested by one of the largest histological imaging comparisons to date (AJR Am J Roentgenol. 2001 Oct;177(4):881–5).

No changes made

3. **Histology:** I believe the diagnostic feature of a thymic cyst is the presence of thymic tissue within or adjacent to the cyst wall. This paragraph could be more informative if discussing how thymic cysts were pathologically differentiated from bronchogenic or pericardial cysts (even though many cases are not straightforward).

Reply: Thymic cysts are notable as degenerated Hassall's corpuscles, we added a reference to emphasize this as well as a description of these structures. (Am J Surg Pathol. 1990 Mar; 14(3) 284-303).

This has been revised on page 5.

Histologically, thymic cysts are most readily identified by the inclusion of Hassall's corpuscles in the cyst wall (22,36–40). These swirl shaped keratinized structures surrounded by epithelial cells are unique to the thymus (40). Cyst walls are also prominent for fibrous stratified squamous epithelium (8,28).

4. **Diagnosis:** The first paragraph lacks a general description of the thymic cyst (usually a well-demarcated hypoattenuating mass, which may fluctuate in size in different positions or over time). More description is necessary about the usefulness of CE-MRI in the cases of thymic cysts complicated with hemorrhage or inflammatory proteinaceous content. I would recommend CTA rather than echocardiography in case of vascular diseases considered.

Reply: Thank you for this suggestion, we have made all of the suggested changes about general description (Added J Thorac Dis. 2017 Dec; 9(12) 5203-5211), utility of MRI (Added (J Comput Assist Tomogr. 1995 Apr; 19(2) 188-191), and CTA - (Added Int J Cardiovasc Imaging. 2021 Jun; 37(6) 1961-1966).

This has been revised on page 6

Computed tomography or CT imaging, preferably with contrast, is frequently obtained in the evaluation of mediastinal masses. Based on the largest series analyzing the CT characteristics of thymic cysts, these masses appear to be most commonly well defined, round / oval, and laterally positioned in the anterior mediastinum (35).....Large series demonstrate that the diagnostic sensitivity of CT in making an accurate diagnosis was less than 55% (35). Incorrect diagnosis was

especially common with masses less than 3cm and Hounsfield units greater than 20 on imaging (35).....If a question of diagnosis exists, additional imaging should be obtained with echocardiography which can provide more dynamic information with doppler flow to help exclude vascular or cardiac chamber involvement (7,51,52).

5. **Treatment:** This is a good review of the different approaches for the treatment of the mediastinal cysts. However, I have some remarks. Personally, I do not agree with some statements, for example: Surgical resection is usually required because these lesions can continue to increase in size and cause compressive symptoms. [Burjonrappa SC, Taddeucci R, Arcidi J. Mediastinoscopy in the treatment of 256 mediastinal cysts. JSLs. 2005 Apr-Jun;9(2):142-8.] – in my experience in many cases, especially in elderly patients, small anterior mediastinal cysts do not require surgical treatment. Nevertheless, the authors referred to the publication and might have a different opinion. The other notion, I do not agree is: Cysts in the subcarinal area are difficult to see from thoracoscopy and contralateral pneumothorax can be easily achieved during the dissection. In my experience, VATS is a convenient approach in such cases.

Reply: Thank you for comment. We have added an additional article in the support of mediastinoscopy in select patients (JSLs. 2005 Jun; 9(2) 142-148). We also added pursuit of adjunctive imaging as an option to guide treatment.

It is unclear what the reviewer is referring to regarding subcarinal dissection via VATS. No discussion of this was included in the text. We agree that VATS can adequately obtain sufficient exposure to subcarinal masses.

The manuscript was revised on page 10.

Reports of mediastinoscopy for resection have also been described (37,66) however should only be undertaken if complete resection is possible. Incomplete resection from this approach has resulted in deaths from sepsis (42).

6. **Some other remarks:** The authors completely neglected EBUS and EUS in diagnosis of the mediastinal cysts, especially those located in the middle and posterior mediastinum. The authors omitted to mention the use of transcervical approach and subxiphoid approach and I suggest it should be complemented

Reply: Thank you for identifying this omission, we added a discussion of EBUS/EUS in the discussion of diagnosis with emphasis on the risks of aspiration and low diagnostic yield. We also added discussion of transcervical and subxiphoid approaches for resection.

The manuscript was revised on page 7 and 10.

Endoscopic ultrasound is a newer technology that can potentially aid in diagnosis of thymic cysts (58–60). As previously discussed, caution should be taken with endoscopic aspiration of cyst contents when diagnosis of thymic or mediastinal cyst is suspected, as analysis of fine needle aspiration is usually non-diagnostic 73 percent of the time, with risk of infection, sepsis, and death (41,42,50,60).

Subxiphoid and transcervical approaches seem to be effective in selected patients with anterior midline location cysts with sufficient operator familiarity (9,23,31,68)

Reviewer B

General Comments:

I think one point should be outlined in the manuscript is that in recent years an increasing number of small anterior mediastinal lesions, including thymic cysts, have been found incidentally during the lung cancer screening. Therefore, differentiating these small thymic cysts from other tumors is vitally important in clinical practice.

Reply: We added information about adjunctive imaging modalities including MRI, PET, radiomics, that can aid in accurate diagnosis.

Changes in text:

Diagnosis, paragraph 5, 6

Despite the known limitations in imaging, recent advances in technology such as “radiomics” have proven to be valuable to assisting in accurate diagnosis of cysts by using computer analysis of acquired images (53). Magnetic resonance imaging has often been described as an additional adjunctive imaging modality with cysts to aid in diagnosis (19,43). T2 sequences appearing very bright, is a specific, but not sensitive quality that can help aid in identifying thymic cysts from other pathologies (54). T1 MRI sequences also appear to be of value, with signal intensity ratios of 1 to 1.5 being useful in identifying thymic cysts.

Positron emission tomography is another adjunctive imaging modality to differentiate cysts, with low maximum standardized uptake value, suggesting benign features (55). Nuclear medicine imaging with radiotracer uptake of iodine-131 (56) and technetium-99 sestamibi (57) have been detailed in cases although these features do not appear to be common.

1. Page 2 - line 25: Please insert the sentence like “ However, CT screening for lung cancer has led to the increase of incidental detection of anterior mediastinal lesions, and the differentiation of small thymic cysts is becoming vitally important.”

Reply: We added the suggested sentence highlighting importance of topic in context of expansion of lung cancer screening.

The manuscript was revised on page 3.

Despite the low prevalence of this pathology, an increasing number of mediastinal cysts are likely to be incidentally discovered with expansion of lung cancer screening (5). Accordingly, the diagnosis and management of mediastinal thymic cysts will become increasingly important.

2. Page 4 - line 70: I believe thymic cysts associated inflammatory processes are basically multilocular cysts. Consider rephrasing “these cysts” to “multilocular cysts”.

Reply: Although the majority of inflammatory cysts are multilocular, exceptions have been reported as noted in the 2nd paragraph of pathophysiology (page 4).

Please see page 4 for this discussion.

3. Page 4 - line 76-79: As a matter of fact, most of incidentally found, untreated thymic cysts are benign [Yoon SH, Choi SH, Kang CH, Goo JM. Incidental Anterior Mediastinal Nodular Lesions on Chest CT in Asymptomatic Subjects. J Thorac Oncol. 2018 Mar;13(3):359-366. doi: 10.1016/j.jtho.2017.11.124. Epub 2017 Dec 9. PMID: 29233791.]. Therefore, I feel this paragraph is a little misleading and needs revision.

Reply: There is a discrepancy between large series suggesting low risk of malignancy and the plethora of case reports suggesting higher potential risk for malignancy. We have added additional references to support this (J Thorac Dis. 2017 Dec; 9(12) 5203-5211 and J Thorac Oncol. 2018 Mar; 13(3) 359-366). We also added a discussion that the case reports may have inherent selection bias, which could overestimate the malignant risk.

The manuscript was revised on page 9.

Determining a method to stratify asymptomatic cysts based on risk of neoplastic potential poses further challenges. While nearly all neoplastic cysts are multilocular, only approximately half have this appearance on CT imaging (13,15,35). Solid components seem to be common features of neoplasia (12,15), however these do not always appear to be apparent on cross-sectional imaging, and authors have shown that solid components are most likely benign features (13).

4. Page 5 - line 108: Although thymic cysts often contain high proteinous fluid, and shows >20 HU, some thymic cysts would contain serous fluid and their CT value would be close to zero (same as water). I have the impression that the CT value would be somewhere between 0 and 30 HU.

Reply: Changes made based on data from (J Thorac Dis. 2017 Dec; 9(12) 5203-5211) with range of hounsfield units of 0-62.

The manuscript was revised on page 6.

Gross appearance is also variable, being smooth or lobular and unilocular or multilocular based on type. Internal components vary and frequently contain solid components (13). Cyst fluid when simple varies from 0 and 62 Hounsfield units (9,13,17,35). Although routinely non-calcified (13) focal (47) and grossly calcified (44,48) mediastinal thymic cysts have been reported.

5. Page 6 - line 106: I feel in this manuscript, authors include thymic cyst associated with neoplasm. If the cyst has relatively large solid component (like thymoma with cystic component), I think needle biopsy for solid component can be an option. Please clarify this point.

Reply: We agree that needle biopsy of solid component is a diagnostic option.

The manuscript was revised on page 7 and 8.

However, for thymic cysts with significant solid components, which are commonly seen in thymoma or thymic carcinoma, percutaneous needle biopsy is an option for diagnosis with appropriate patient counseling on risks (15)

6. Page 6 - line 125: Please rephrase “cysts appearing bright on T2 sequences” to “cysts with very high signal intensity on T2 weighted images.”

Reply: We have updated the manuscript as suggested.

The manuscript was revised on page 7.

T2 sequences appearing very bright, is a specific, but not sensitive quality that can help aid in identifying thymic cysts from other pathologies (54)

7. Page 6 - line 127: 18F-FDG PET can be also useful to differentiate pure cystic lesions from neoplastic lesion. Please add explanation about this point with

following citation. Sugawara H, Ito K, Watanabe H, Morita T, Yatabe Y, Watanabe SI, Kusumoto M. Clinical usefulness of PET/MRI in differentiating anterior mediastinal masses. Nucl Med Commun. 2022 Jan 1;43(1):92-99. doi: 10.1097/MNM.0000000000001483. PMID: 34887372.

Reply: We have updated the manuscript as suggested.

The manuscript was revised on page 7.

Positron emission tomography is another adjunctive imaging modality to differentiate cysts, with low maximum standardized uptake value, suggesting benign features (55). Nuclear medicine imaging with radiotracer uptake of iodine-131 (56) and technetium-99 sestamibi (57) have been detailed in cases although these features do not appear to be common.

8. Page 9 - line 179: Please insert sentence like “Therefore, it is extremely important to identify small accompanying solid components with contrast-enhanced CT, MRI, or 18F-FDG PET.” with some citations.

Reply: We have updated the manuscript as suggested to highlight the importance of identifying solid components and discuss the limitations of radiographic accuracy.

The manuscript was revised on page 6 and 7.

Definitive diagnosis based on imaging is challenging and ultimately diagnosis is based upon histological evaluation of surgical specimens. Large series demonstrate that the diagnostic sensitivity of CT in making an accurate diagnosis was less than 55% (35). Incorrect diagnosis was especially common with masses less than 3cm and Hounsfield units greater than 20 on imaging (35). Tissue sampling before surgical excision should not be undertaken, as biopsy of cysts results are often unrevealing (39,49) and risk bacterial seeding, subsequent sepsis and death (41,42,50). Additionally, given preoperative diagnostic uncertainty, and overall rarity of this pathology, workup requires a broad differential.

Despite the known limitations in imaging, recent advances in technology such as “radiomics” have proven to be valuable to assisting in accurate diagnosis of cysts by using computer analysis of acquired images (53). Magnetic resonance imaging has often been described as an additional adjunctive imaging modality with cysts to aid in diagnosis (19,43). T2 sequences appearing very bright, is a specific, but not sensitive quality that can help aid in identifying thymic cysts from other pathologies (54). T1 MRI sequences also appear to be of value, with signal intensity ratios of 1 to 1.5 being useful in identifying thymic cysts.

Positron emission tomography is another adjunctive imaging modality to differentiate cysts, with low maximum standardized uptake value, suggesting benign

features (55). Nuclear medicine imaging with radiotracer uptake of iodine-131 (56) and technetium-99 sestamibi (57) have been detailed in cases although these features do not appear to be common...

However, for thymic cysts with significant solid components, which are commonly seen in thymoma or thymic carcinoma, percutaneous needle biopsy is an option for diagnosis with appropriate patient counseling on risks (15)

9. Page 9 - line 180: The statement “however these do not always appear to be apparent on cross-sectional imaging, and authors have shown that solid components are most likely benign features (11).” is misleading. First, this literature is a little old and current modalities can have better diagnostic abilities. In my opinion, this statement is only true for “multilocular cysts associated with idiopathic diseases” and the statement is somewhat overgeneralization. Please rewrite the sentence.

Reply: Agree with the reviewer that diagnosis is challenging via imaging. No changes made.

10. Page 9 - line 182: About the sentence “Because no reliable risk stratification can be made based on the presence or absence of imaging features”. I do not think so. If the cyst is simple, unilocular without definite solid component, a huge number of literatures suggest the cyst would be benign. However, I admit some multilocular cysts are challenging to do risk stratification. Please rephrase the sentence.

Reply: Literature comparing imaging findings to histological results, suggests that CT is ineffective for risk stratification. Added (J Thorac Dis. 2017 Dec; 9(12) 5203-5211), the largest study on the topic underscoring this, where in 108 patients, CT imaging made the correct diagnosis <55% of the time. This is consistent with smaller studies where 8 of the 18 thymic cysts described in (Diagn Pathol. 2018 Jun 26;13(1):41) appeared to be unilocular on imaging, and were in fact multilocular histologically. Results were similar in (AJR Am J Roentgenol. 2001 Oct;177(4):881–5) where 4 of 8 cysts appeared unilocular on imaging and were in fact multilocular.

The manuscript was revised on Diagnosis, paragraph 3

Definitive diagnosis based on imaging is challenging and ultimately diagnosis is based upon histological evaluation of surgical specimens. Large series demonstrate that the diagnostic sensitivity of CT in making an accurate diagnosis was less than 55% (35). Incorrect diagnosis was especially common with masses less than 3cm and hounsfield units greater than 20 on imaging (35). Tissue sampling before surgical excision should not be undertaken, as biopsy of cysts results are often unrevealing

(39,49) and risk bacterial seeding, subsequent sepsis and death (41,42,50). Additionally, given preoperative diagnostic uncertainty, and overall rarity of this pathology, workup requires a broad differential.

11. Page 10 - line 211: The word “whenever” is far too strong. Please tone down.

Reply: We have changed the manuscript too include “shared decision making approach”

The manuscript was revised on page 10.

Because no reliable risk stratification can be made based on the presence or absence of imaging features, a shared decision making approach with patients is appropriate regarding management. Risk of malignancy is likely less than 1 percent (65) and the rate operative of complications is 1 to 5 percent (10,35).

Reviewer C

1. Proper nouns should be capitalized. For example: Hassall’s corpuscles, Hodgkin’s lymphoma, Reed-Sternberg cells.

Reply: Thank you for identifying this problem. We have made appropriate changes throughout the manuscript.

2. More than conventional CT features, one study recommended a CT-based radiomics nomogram as an effective and convenient tool for differentiating thymic cysts from thymic epithelial tumors. Radiomics and AI have developed rapidly in recent years, and played an important role in imaging diagnosis.

At present, studies of MRI have found other sequences that were more accurate than using only T2 sequence. In fact, most solid tumors also appeared bright on T2 sequence. The most important factor to identify malignancy is whether there was a solid component in the cyst.

Reply: Thank you for the references, we have added a discussion on radiomics and MRI sequencing. (Front Oncol. 2021 Dec 10; 11 744021). (Korean J Radiol. 2019 May; 20(5) 854-861)

The manuscript was revised on page 7.

Despite the known limitations in imaging, recent advances in technology such as “radiomics” have proven to be valuable to assisting in accurate diagnosis of cysts by using computer analysis of acquired images (53). Magnetic resonance imaging has often been described as an additional adjunctive imaging modality with cysts to aid in diagnosis (19,43). T2 sequences appearing very bright, is a specific, but not sensitive

quality that can help aid in identifying thymic cysts from other pathologies (54). T1 MRI sequences also appear to be of value, with signal intensity ratios of 1 to 1.5 being useful in identifying thymic cysts.

Review Comments (Round 2)

Reviewer A

> Re-Review: Thank you for the author's effort in revising the manuscript. However, the manuscript could be better and more comprehensive if it focuses on more general informative discussion as a streamline and describe the uncommon features as an addition.

Reply: Thank you for the suggestion, further efforts have been made per below to streamline as requested.

> Re-Review: Again, please read carefully the papers I suggested. Be clear the prevalence of anterior mediastinal mass (AMM) vs thymic cyst. In the report by Henschke, 0.01% is an annual incidence of incidentally found AMM. We never know the exact incidence of thymic cyst based on CT appearance. Please put a reference if there is any report mentioning "0.01%

Reply: Thank you for the comment.

Per the paper by Henschke (citation #1), 40 of 9263 individuals had anterior mediastinal masses (Table 1) or 0.4%. Also, on page 589 of the same paper by Henschke, under the heading "thymic masses" on lines 10 and 11, it was noted only 1 of 9263 was a thymic cyst or 0.01%.

Additionally, a more recent paper by Yoon (citation #2), on page 4,14 of 56,358 individuals had a thymic cyst for 0.03%. No changes made based on detailed review of available evidence. If the reviewer requires further clarification regarding the provided studies, do not hesitate to have them reach out to the authors.

> Re-Review: Again, the most commonly used classification of thymic cysts are congenital/acquired or unilocular/multilocular. In the paper by Choi, they are using those terminologies, focusing on the significance of multilocular cysts with neoplastic process. Please modify

Reply: Again, thank you for the comment.

Although the congenital / acquired, unilocular / multilocular is the most common classification system, it is not the most accurate. Evidence which is presented throughout the paper suggests that there is a blurred line between the unilocular and multilocular classification. For example in the paper by Choi where the majority of patients were non-neoplastic, all 8 were histologically multilocular, however half appeared unilocular on imaging.

The authors acknowledge the reviewers perspective, and have changed to the congenital / acquired, unilocular / multilocular classification.

> Re-Review: I still feel the use of MRI for thymic cyst is underscored in this paragraph. Because MRI is the key modality to diagnose thymic cysts with superior diagnostic accuracy to CT (Eur J Radiol. 2009 Feb;69(2):280-8)

Reply: Despite its superior accuracy, MRI is uncommonly used in clinical practice as an initial modality, especially in the era of incidental findings from lung cancer CT screening. The inaccuracy of CT is stated throughout the diagnosis section with specific criteria that reduce accuracy, as well as MRI features and criteria that increase diagnostic accuracy. No changes made.

> Re-Review: Regarding the tables, major cohort studies should be shown in the tables, not only case reports or small case series. I would suggest not to stress on the very uncommon findings such as I131 or Tc99 uptake.

Reply: Case reports with uptake of I131 and Tc99 have been removed. An additional table containing the larger studies has been added.

Reviewer B

>The sentence "and authors have shown that solid components are most likely benign features (13)" seems to be incorrect. I read the reference (13) and recommend the following sentence instead. "and previous research have shown that solid components among multilocular thymic cyst can represent various non neoplastic tissue such as thymic hyperplasia, normal thymic remnants, hemorrhagic cysts, or numerous small cysts septated by thick inflammatory thymic tissue (13)."

Reply: Changes made as requested.