Peer Review File

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Reviewer A

The authors present a case with lipofiroadenoma (LFA) of thymus. They surgically resected the tumor and diagnosed histopathologically. They also performed whole exome sequencing and RNA sequencing, which are novel attempts for LFA. The manuscript demonstrates clinicopathological features of the case appropriately.

Minor comments:

Comment 1: The novelty of this report is genetic analyses of LFA although no abnormality was detected. I would like to know which NGS systems and kits were used

Reply 1: The technical details of the molecular analyses were added to the manuscript

Changes in the text: page 4-6 from line 22 on page 4 inserted:

For WES total DNA was isolated using the AllPrep DNA/RNA/Protein Mini Kit (QIAGEN) according to standard protocol on the QiaCube (Qiagen). DNA-seq libraries were generated with 150 ng DNA using the KAPA HyperPrep Kit in combination with the HyperExome capture kit (Roche) and subsequently sequenced on an NovaSeq 6000 system (2x150 bp) (Illumina). The DNA sequencing data of the tumor and the normal (DNA extracted from blood) were processed as per the GATK 4.0 best practices workflow for variant calling, using a wdl and cromwell based workflow (https://gatk.broadinstitute.org/hc/en-us/sections/360007226651-Best-Practices-Workflows). This included performing quality control with Fastqc (version 0.11.5) to calculate the number of sequencing reads and the insert size Picard (version 2.20.1) for DNA metrics output and MarkDuplicates. (3) mRNA sequencing was performed as previously described.(4) In brief, total RNA was isolated using the AllPrep DNA/RNA/Protein Mini Kit (QIAGEN) according to standard protocol on the QiaCube (Qiagen). RNA-seq libraries were generated with 300 ng RNA using the KAPA RNA HyperPrep Kit with RiboErase (Roche) and subsequently sequenced on an NovaSeq 6000 system (2x150 bp) (Illumina). The RNA-seq data were processed as per the GATK 4.0 best practices workflow for variant calling, using a wdl and cromwell based workflow (https://gatk.broadinstitute.org/hc/en-us/sections/360007226651-Best-Practices-Workflows). This included performing quality control with Fastqc (version 0.11.5) to calculate the number of sequencing reads and the insert size Picard (version 2.20.1) for RNA metrics output and MarkDuplicates.(3) The raw sequencing reads were aligned using STAR (version 2.7.0f) to GRCh38 and gencode version 29.(4) Finally, expression counts were determined at gene level using Subread Counts. (5) Fusion gene detection was performed using STARfusion.

Comment 2: In addition, the results of the genetic analyses should be demonstrated briefly in Abstract.
Reply 2: a brief one-line sentence was added to the abstract indicating the absence of genetic events
Changes in the text: added to the Abstract (lines 10-12): *The histology of this lipofibroadenoma was similar to previously described cases. No gene mutations or rearrangements were identified.*

Comment 3: Whether each component of LFA (i.e. epithelial, lipomatous and fibrous component) is neoplastic or not is still unknown. Therefore, it should be clearly adressed which part of the LFA was submitted for genetic analyses (Was the specimen macro/micro-dissected? or were whole-tissue-slides containing all components submitted?).
Reply 3: No dissection of tumour tissue was performed. It is the policy of the institute to check sampled tumour tissue by frozen section, all components were present in the procured tissue. Moreover, given the highly intermingled components of this particular tumour type it may be assumed that (even without visual control) all components are present in the samples. Finally, given the absence of molecular alterations in the samples and the observations above, it can reliably be assumed that (micro)dissection would have been of no added value, if a genetic alteration had been present, this almost certainly would have been detected with or without microdissection.
Changes made to the manuscript: page 4, line 8, the sentence was altered to reflect the presence of the tissue components. Fresh tissue, *checked by frozen section for the presence of representative constituents, was procured for molecular studies*

Comment 4: Discussion (page 3, line 18): Please provide a reference which proposed the entity of "thymoliposarcoma".
Reply 4: Several cases of thymoliposarcoma have been reported. A summary of these can for instance be found in the review article currently added to the manuscript
Changes made to the manuscript: a reference for thymolposarcoma was added tot the manuscript on page 6, line 14: den Bakker MA, Marx A, Mukai K, et al. Mesenchymal tumours of the mediastinum--part I. Virchows Arch. 2015;467(5):487-500.

Comment 5. Table: Previously reported cases of LFA are well summarized. The case 8 (ref. 11) and case 11 (ref. 10) seems to be the same since clinical characteristics and macroscopic images are almost identical. It is recommended to review these references and make some comments.
Reply 5: Reviewer A is to be commended for noting this fact. Upon diligently checking these publications it is evident that these are indeed duplicate publications. The age and sex of the patient are identical; the tumor size (in three

dimensions to the millimeter) are also identical and it is obvious that the gross image in the 2021 Kurebashi et al. Pathology International publication is a digitally modified ('photoshopped') version of the article by Hamada (Jpn J Lung Cancer) et al. Only three authors are common to both publications. Upon identifying these facts the corresponding author of this case report has contacted the editorial boards of both journals and the corresponding authors, outlining the observations. The editorial board of Pathology International responded that the coincidence of these publications had been noted by readers of Pathology International and a corrigendum was added to the article which now also included a reference to Hamada et al.
As both publications concern the same case, it is now counted as a single case of LFA and both references are given. Because an additional case of LFA has come to our intention, total number of LFA cases remains the same (note Comment 1 by Reviewer B)
Changes to the manuscript:
Table 1 was modified

1) To reflect the duplicate publication (case 8)
2) To include the additional LFA case described by Matyjek, A. (see Comment 1 by Reviewer B below) (case 11)

Comment 6: Figure 1A: Please add arrow(s) indicating tumor on the image.
Reply 6: arrows have been added to Figure 1A and 1B
Changes to the manuscript: The figure legends of Figure 1A and 1B were modified to indicate the presence of the arrows.

Comment 7: Novelty of this paper lies in genetical studies but the detail is not provided. The manuscript would be acceptable when it is demonstrated.
Reply 7: See reply to Reviewer A, comment 1.

Reviewer B
The manuscript is written very clearly, concisely and in good English. It presents a case of rare mediastinal tumor, lipofibroadenoma and adds some new insights about this disease in a good and interesting discussion at the end.
I would have some minor suggestions to the authors:

Comment 1. Page 2, lines 7-8. Please, note there is a recently published case of lipofibroadenoma analysed by NGS (https://pubmed.ncbi.nlm.nih.gov/34366048/ PMID: 34366048).
Reply 1: We thank the reviewer for indicating the existence of this report. However, only limited genetic analysis (Archer fusionplex analysis for gene fusion transcripts) was performed and therefore is not comprehensive. Nevertheless, this report has been incorporated in our manuscript
Changes to the manuscript:

1. The reported case by Matyjek was incorporated in Table 1 (case 11)
2. In the word 'full' was added to the abstract on page 2, line 9, to indicate that although in a previous publication molecular analysis was performed, this was not comprehensive, the current paper remains the first with full genetic analysis, the sentence now reads: *The resected tumour was extensively investigated, including the first instance of full molecular analysis of this rare entity*
3. In the discussion, the last paragraph (page 8, lines 24 – page line 2) was modified to read: *In a single recently described LFA case no fusion transcripts were detected (23), taken together with our case, there are currently no features which support a neoplastic process*.

Comment 2. Page 3, line 28: There is also another paper reporting thymoangiolipoma coexisting with MG (https://pubmed.ncbi.nlm.nih.gov/33489056/ and PMID: 33489056).
Reply 2: Thank you for making us aware of this reference, this has been added to the manuscript
Changes to the manuscript: reference added (Anbardar et al. 2020; page 6 line 24)

Comment 3. Page 3, line 18: Could you, please, add a reference directing to thymoliposarcoma?
Reply 3: please refer to Reviewer A - Comment 4, and our reply (reference added)

Comment 4. Page 3, line 26 and page 4 line 26 „HMGA-2": please, keep the recommended style of gene names writing (in italics).
Reply 4 and changes to the manuscript: this has been corrected in both instances

Comment 5. Fig. 1 and 3. I would suggest adding some markers on CT and microscopic images pointing the most important elements. Please, note, Mediastinum is a multidisciplinary journal and such markers would be helpful for specialists who may not be fluent in radiological or histopathological images.
Reply 5: Arrows have been added to figure 1 (note Comment 6 by reviewer A). Sample indicators were added to image 3A to indicate adipose and fibrous tissue. While we acknowledge the fact that the image may be difficult to interpret for non-pathologist, adding numerous indicators is unlikely to clarify the histology sufficiently to readers who have no experience in interpretating histology slides.
Changes to the manuscript:
- An '*' was added to image 3A to indicate fatty tissue; an '#' was added to image 3A to indicate fibrous tissue
- The legend to figure 3A was modified to reflect these changes and now reads (also see Reviewer B comment 6 below):

Figure 3. Histology. HE stained sections (A & B low power view; C & D medium power view)

A. *Fibro-fatty tissue with ramifying cords of epithelial cells. Mature fatty tissue, present in several areas in this slide (a single area indicated by *). Pink fibrous collagenous tissue is indicated (#). Darker branching strands of epithelial cells ramify through both fibrous and fatty tissue. HE stain*
B. *Scattered small calcifications were present, again note the slender strands of epithelial cells*
C. *Discrete collections of spindled epithelial cells, comparable to those of a spindle cell thymoma*
D. *Lymphoid component with a Hassall's corpuscle*

Comment 6. Fig. 3 and 4. Please add the information about magnification of microscopic images.
Reply 6: The histology images were generated by taking representative images from whole slide imaging (virtual microscopy), which were then further modified to provide optimal histological images to illustrate the specific features of lipofibroadeoma. Providing 'exact' magnifications is not useful because a) virtual imaging allows any magnification, not just discrete magnification such as produced by a conventional microscope, b) after producing these images by editing such as cropping and image resizing, the original magnification, even if this is known, is no longer valid, c) individuals who have experience with histology can easily judge the approximate magnification, for individuals who do not have experience with viewing histology images, providing an exact magnification is not helpful.
To provide a rough guide indications of the (original) magnification as "Overview", Low Power (LP), Medium Power (MP) and High Power (HP) were added were relevant.
Changes to the manuscript: The figure legends of Figures 3 and 4 were modified, for the legend of Figure 3, please refer to Reviewer B – comment 6 and changes to manuscript above.
The heading of legend of Figure 4 now reads*: Figure 4. Immunohistochemistry. (medium power views)*

Reviewer C
Comment 1: The publication of new cases on these rare entities, especially rare thymic tumors, contributes to expanding our knowledge about them. As a thoracic surgeon, I think it is important to emphasize the fact that the majority of these specimens have been removed by the thoracoscopic approach.
Reply 1: While we agree that the current preferred procedure is thoracoscopic resection, this cannot be concluded from the published cases, of the 13 cases 5 tumours were removed by thoracotomy, 4 were removed by VATS / thoracoscopy / robot-assisted removal and for the remaining 4 cases no details are provided.

We therefore find there is insufficient support to add a statement to this extent in the manuscript.
Changes to the manuscript: none

Comment 2: Another noteworthy point is the follow-up recorded in Table 1. Do you think that the follow-up time of these patients should be standardized taking into account the biology of the tumour? Would it be more justified if it is associated with a B1 thymoma?
Reply 2: To all intents and purposes and based on the 13 cases described to date, lipofibroadenoma should be considered a benign thymic tumour and if not admixed with another subtype such as B1 thymoma (as in three published cases) limited follow-up seems warranted. In the single cases with a less favorable outcome described by Matyjek with progressive renal failure the outcome cannot definitely be ascribed to the resected lipofibroadeoma. In our opinion this is an example of unfortunate co-incidence. Nevertheless, lipofibroadenoma numbers are small and care must be taken not to sign this rare tumour off to easily. To emphasize the benign behavior of lipofibroadenoma and acknowledge the association with B1 thymoma a minor modification was made to the manuscript
Changes to the manuscript: Added (at page 8, line 11): *While the patient described in the report by Matyjek and co-workers made an initial good recovery from the operative procedure to remove a large LFA, she did suffer progressive ANCA-vasculitis associated renal failure.(24) Although it was suggested that the vasculitis may have been associated with the LFA given the co-occurrence of two rare diseases, this may be contested as the ANCA-associated vasculitis progressed after removal of the LFA. Current evidence suggests that LFA behaves in a benign fashion and only limited follow-up after removal is indicated. In those cases where as associated sub-type is present, as in the three reported cases associated with B1 thymoma it would be prudent to base the follow-up on the associated thymoma.*

Comment 3: It should be said that the anatomical-pathological findings and its differential diagnosis with thymolipoma are clearly exposed. Again, congratulations on your work.
Reply 3: Many thanks for your supportive comment

Additional changes made to the manuscript
The font size was changed to 12 points throughout, this affects the original line numbers, the changes to the manuscript are given in regard to the new line numbers.