APM ANNALS OF PALLIATIVE MEDICINE
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Peer Review File

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Reviewer A

The paper is well written and could be accepted as is. The paper concerns a current and important global problem concerning alternative therapeutic treatment. The topic is important.

Reviewer B

The authors have presented their experience with endobronchial ultrasound-guided aspiration and local isoniazid injection in the treatment of mediastinal lymph node tuberculosis. The study is hospital based and uses a retrospective design. The approach taken is quite innovative and almost certainly has a role to play in very selected cases. There are, however, a number of serious concerns with the paper as it stands. These cast doubt upon the findings/conclusions of the study. They are summarized below.

Major:

Comment 1: The length of anti-tuberculosis drug treatment in both arms of the study (chemotherapy alone vs chemotherapy plus endobronchial ultrasound) is much longer than standard therapy. It is unclear why. What determined the duration of therapy? Was it based upon the recovery criteria? All outcomes, other than deterioration, might be considered acceptable. The authors need describe in greater detail precisely how the patients were treated (independent of their bronchoscopic intervention). It is conceivable that patients in the bronchoscopic intervention arm were followed more closely.

Reply: Thanks for these good comments. According to the descriptions in the *Clinical Tuberculosis* published in 2011, 3HREZ/9~15HRE is recommended for the chemotherapy of mediastinal lymph node tuberculosis. It states that, there is a large amount of tissues with caseous necrosis in the lymph nodes, and thus the intensive therapy may prolonged to 6 months. In the Technical Specifications for Tuberculosis

Prevention and Control in China published in 2020, intrathoracic lymphatic tuberculosis can be treated for 1 year. We revised the course of treatment in the present study: patients who were treated for 1 year were observed and compared. In the chemotherapy group, deterioration was observed in 1 patient (presence of new enlarged lymph nodes).

Change in the text: Page 8 line 148-151

Comment 2: Only 30-40% of cases in each arm were culture-positive, permitting first-drug susceptibility testing and therefore tailored chemotherapy. No mention is made of whether treatment was directly-observed or not. This raises questions about the relative adequacy of chemotherapy in each arm.

Reply: Thanks for this comment. (1) not all the patients had concomitant pulmonary tuberculosis; (2) patients without concomitant pulmonary tuberculosis were excluded, and the positive rate of Mycobacterium sputum culture was calculated separately, in the CT group, the positive rate was 41%; in the IT group, the positive rate was 45%. In China, the positive rate of Mycobacterium sputum culture is about 30-60%, which is consistent with our findings. In recent years, with the development of molecular biological technique (such as the introduction of Gene-Xpert technique), the positive rate of Mycobacterium sputum culture is increasing. Relevant statistical analysis was also done in the present study. In addition, all the patients received DOTS. Revisions were made in the text.

Change in the text: Page 8 line 149

Comment 3: Consideration was given to endobronchial ultrasonograpy when the nodes were considered "refractory". Presumably it was based upon 1 month scans versus baseline in some cases and 3 month scans versus baseline in others. The authors need to elaborate on this. What steps were taken to determine the adequacy of therapy?

Reply: Thanks for this comment. There were inclusion criteria in the present study: Patients aged 17-70 years were initially diagnosed with MTLA and had poor response to chemotherapy for more than 1 month. There was mediastinal lymph node liquefaction; chest enhanced CT showed the largest diameter of liquefaction was >1.5 cm, or the largest diameter of liquefaction was <1.5 cm, but there were presence of persistent clinical symptoms such as fever ≥38.5^{o}C or dyspnea. In the IT group, pus aspiration was done at the mediastinal lymph nodes with EBUS-TBNA, and isoniazid was locally injected once every 2-3 weeks. The size of abscess cavity on ultrasonography was used to determine the dose of isoniazid injected.

Change in the text: Page 7, Lines 125-132

Comment 4: Following upon comment 2 above can the authors indicate just how many of the suppurating intrathoracic nodes were causing extrinsic compression on other mediastinal structures. These would seem to be the most important nodes to aspirate. Pain would also be an indication for intervention but interestingly it is not listed as a symptom. In the experience of this reviewer these are very rare.
Reply: Thanks for this comment. In the present study, the lymph nodes 2R, 4R, 7, 10R and 11R are susceptible to enlargement and abscess formation, causing compression on other mediastinal structures, and 4R and 7 lymph nodes are the most common. The persistent enlargement of these lymph nodes may compress surrounding tissues and organs, and even the lesions lymph nodes may rupture and invade the trachea, bronchus or lung, which may result in some symptoms such as cough and dyspnea. In 2015, Goussard et al also reported a child who received treatment for airway obstruction due to Mediastinal lymphadenopathy. In our study, few patients had pain as the main symptom, and these patients were not further analyzed.
Change in the text: Page 3, Line 52; Page 11, Line 223-227

Comment 5: Some of the symptoms the authors report (e.g. shortness of breath) are unusual in pulmonary TB or intrathoracic lymph node TB. Fever, is not a common symptom in cervical lymph node TB in the absence of HIV co-infection, even when the nodes are suppurating. Was the HIV status of the patients known? If most of the symptoms were due to co-existent pulmonary TB, the time to resolution of these symptoms in the chemotherapy alone arm is extraordinarily long.
Reply: Thanks for this comment. In our study, all the patients received examination for HIV infection, and results were negative. In the present study, about 90% of patients had concomitant pulmonary TB and thus fever was a common symptom in these patients. The majority of patients had low grade fever. As mentioned by this reviewer, the time to resolution of these symptoms in the chemotherapy alone arm is relatively long (about 1-2.5m).
Change in the text: Page 14, Lines 293-297; Line 451: Table 1

Comment 6: There is in the opinion of this reviewer there is no evidence that anti-tuberculosis drugs fail to penetrate caseous lymph nodes and therefore no evidence for the use of intra-nodal isoniazid. In those rare instances where differential penetration of anti-tuberculosis drugs into a closed space have been described (e.g. a

tuberculous empyema), isoniazid appears to be one of the drugs that penetrates well.
Reply: Thanks for this comment. The goal of the treatment is to reduce tissues with caseous necrosis in the lymph nodes by aspiration and, at the same time, and increase the local concentration of a drug (then the therapeutic efficacy was assessed). We mean, as compared to systemic chemotherapy, local injection may increase the local concentration of a drug. In the future study, we may observe the therapeutic efficacy of pus aspiration of the lesioned lymph nodes alone.
Change in the text: Page 15, Line 319-322

Comment 7: Some of the statements made by the authors are very questionable (e.g. lines 82-83 in the introduction; lines 102 in the introduction - this is not a systematic review.
Reply: Thanks for this comment. No study has been conducted to investigate the effect of EBUS-TBNA on the MLNT, and EBUS-TBNA is mainly used for the purpose of diagnosis in TB patients. Our hospital is a hospital designated for the treatment of tuberculosis, a lot of patients with TB are treated in our hospital and, in this study, we attempt to use a new technique in the treatment of TB. This study reports our experience in the use of this technique in the treatment of MLNT.
Change in the text: Page 5, Line 94

Minor
Comment 8: The authors should include the amount of radiation exposure in there comments about safety. A large number scans were performed in these patients - a concern especially for women.
Reply: Thanks for this comment. In our study, the dose of radiation was 8-10 mSv in a single chest enhanced CT, which is a safe dose. As mentioned in the text, repeated chest CT is unnecessary, and chest CT is performed at the end of treatment.
Change in the text: Page 9 line 183-185

Comment 9: Can the authors clarify what "course" means in Table 1
Reply: Thanks for this comment. The “course" means the course of disease in Table 1.
Change in the text: Page 22, Line 455

Comment 10: In line 260 the authors appear to be referring to Figure 3, not Table 2.
Reply: Thanks for this comment. We have revised it according to this comment.
Change in the text: Page 12 line 248

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Reviewer C

Thank you very much for the opportunity to review this very interesting manuscript on the use of endobronchial ultrasound to guide aspiration and local isoniazid injection for treatment of mediastinal lymph node tuberculosis. Overall, the results are quite impressive, and if confirmed in future studies could be quite important for an uncommon disease process that is challenging to treat in current practice. However, it is difficult to generalize the results in the current format without first clarifying some aspects of the manuscript. Therefore, I recommend a number of modifications and clarifications to the manuscript before it is suitable for publication.

Major Considerations:

The Introduction and Discussion require stronger framing of the current literature on the subject, justification for why this study is important, and what this study adds to current knowledge. For example:

Comment 1: Please discuss what, if any, previous literature exists describing EBUS-TBNA for treatment of MTLA.

Reply: Thanks for this comment. No study has been conducted to investigate the effect of EBUS-TBNA on the MTLA, and EBUS-TBNA is mainly used for the purpose of diagnosis in TB patients.

Change in the text: Page 5, Line 95-96

Comment 2: Please discuss the data describing how EBUS-TBNA is better than better than TBNA (which you refer to in the Discussion)?

Reply: Thanks for this comment. In the EBUS-TBNA, the additional ultrasound can visualize the location, size and blood flow of lymph nodes more safely and precisely. We have revised these in the text and relevant data were compared.

Change in the text: Page 13 line 272-279

Comment 3: How does the treatment response described here in this manuscript compare to outcomes in those who receive surgery?

Reply: Thanks for this comment. Zuo et al (20) examined video-assisted thoracoscopic surgery (VATS) for the treatment of MLNTA. All of the patients underwent the radical debridement and drainage of abscesses by VATS and were also administered with the intensified anti-tuberculosis treatment. In all the 16 MLNTA

patients, two patients developed complications after surgery, of whom one developed recurrent laryngeal nerve injury, and the others had poor wound healing. In contrast, our study showed our treatment was less traumatic and more safe.

Comment 4: The basic research design needs to be presented more clearly in the Methods section. For example:
- This appears to be a retrospective review, but portions of the Abstract and Methods read more like a prospective clinical trial. This should be clarified further for the reader. See details in Abstract and Methods sections of "Specific Considerations" below.
- Please clarify inclusion and exclusion criteria. Were the inclusion criteria established before patients were treated (in which case it is really more of a prospective rather than a retrospective study) or after they were treated? It is difficult to tell here if patients were given a choice of which treatment they wanted? Flow chart 1 speaks to this in some regard, though it is not clear in the text.
Reply: Thanks for this comment. This was a retrospective study as mentioned by this reviewer, and we just summarized and analyzed the available data. Of note, there were two teams involved in this study: one conducted pus aspiration and local injection plus chemotherapy (informed consent was obtained before surgery), and the other conducted routine chemotherapy. In this study, data were collected from patients who received treatments in the same period and further analyzed, and then the characteristics were compared between two groups.
Change in the text: Page 6, Line 110-112 ; Page 27, Line 480

Comment 5: Please explain and justify the sample size. You mention in Line 118 that thirty cases were considered sufficient, but without further explanation.
Reply: Thanks for this comment. The incidence of MTLA is relatively low and thus the sample size was small in the study period in the present study. More studies with large sample size are needed to confirm our findings, which was one of limitations of this study.
Change in the text: Page 16, Line 341-342

Comment 6: Language clarity:
- There are numerous places where the grammar is incorrect. I would recommend that these be addressed as errors in grammar can distract the reader from the content.
- I would recommend that inclusion/exclusion criteria, equipment, etc, not be formatted as a list with numbers but rather in prose.

Reply: Thanks for these comments. We have revised according to these comments.
Change in the text: Page 6-7, Line 123-139

Specific Considerations

Abstract:
Comment 7: All items should be written out in the text first before being abbreviated (EBUS-TBNA and IT, for example, are not written out first).
Reply: Thanks for this comment. We have revised it.
Change in the text: Page 3 line 35-36, line 43

Comment 8: Line 42-47: You state this was a retrospective study, which would imply patients were treated without a predefined study protocol and you are now going back and analyzing their outcomes. However, the language here suggests that the patients were "recruited" to the study, and that they were then assigned or "divided into" a specific treatment arm, which would make this a prospective trial. Please clarify.
Reply: Thanks for this comment. These descriptions were misleading, and we have revised them.
Change in the text: Page 3 line 43; Page 6, Line 111-112

Comment 9: Line 54: Units of time need to be added.
Reply: Thanks for this comment. We have revised this sentence.
Change in the text: Page 3 Line 52.

Comment 10: Line 60-62: Units of time to recovery need to be added.
Reply: Thanks for this comment. The time to recovery was added to the Abstract.
Change in the text: Page 3-4 Line 58-60.

Comment 11: Line 66-70: "This treatment can be promoted in clinical practice". As this is a retrospective single center study with relatively small sample size, I would recommend that in the abstract your conclusion be more tailored to your present findings. Future studies will be required to attempt and replicate these findings and standardize protocols before widespread adoption is appropriate. Furthermore, at the conclusion of the Discussion you say further studies are needed to replicate these findings and standardize treatment protocols that could "pave way for clinical promotion of this treatment", which seems somewhat different from what is stated in the abstract.

Reply: Thanks for this comment. As mentioned by this reviewer, findings from retrospective studies should be confirmed in more prospective studies, which will be helpful for the promotion of this technique in clinical practice. We have revised these descriptions in the text.
Change in the text: Page 4 line 65-68 and Page 16 line 342-344

Introduction:
Comment 11: Line 80: Is this meant to say injection or infection?
Reply: Thanks for this comment. It means infection. We have revised it.
Change in the text: Page 5 line 77

Comment 12: Line 98-101: It would be helpful to describe here for the benefit of the reader some of the results you have referenced regarding diagnostic accuracy of EBUS-TBNA for MLNT.
Reply: Thanks for this comment. Relevant information was added to the text.
Change in the text: Page 5 line 95-96.

As discussed above,
Comment 13: Line 102: This incorrectly defines the study as a systematic review.
Reply: Thanks for this comment. We have revised this sentence in the Introduction section.

Methods:
Comment 14: Lines 109-117: As in the Abstract, you state this was a retrospective study, which would imply patients were treated without a predefined study protocol and you are now going back and analyzing their outcomes. However, the language here suggests that the patients were "recruited" to the study and that they were then assigned or "divided into" a specific treatment arm, which would make this a prospective trial. Please see comments regarding clarification of study design listed under "Major Considerations" above.
Reply: Thanks for this comment. This was a retrospective study, and this description was misleading. We have revised it in the Methods section.
Change in the text: Page 6, Line 107-119.

Comment 15: Line 118: Please explain why 30 patients was defined as the minimum sample size for this study.
Reply: Thanks for this comment. The incidence of MTLA was relatively low, and

thus the sample size was small in the study period in our study. This description was misleading and we have revised it.

Comment 16: Lines 122-125: This sentence is more appropriate for the Discussion than the Methods section.
Reply: Thanks for this comment. We have revised this sentence.
Change in the text: Page 7 line 127-130.

Comment 17: Lines 130-143: Please clarify inclusion and exclusion criteria. Current wording is not clear. For example, you could consider rephrasing 2nd inclusion criteria as "Poor treatment response after one month of chemotherapy as defined by: mediastinal lymph node liquefaction; largest diameter of liquefaction on enhanced chest CT >1.5cm, or maximum diameter <1.5cm but presence of persistent clinical symptoms such as fever >38.5 degree C or dyspnea".
Reply: Thanks for this comment. We have revised the inclusion and exclusion criteria according to this comment.
Change in the text: Page 7 line 124-132

Comment 18: Line 221: This seems to imply that patients were assigned to a treatment arm prior to enrollment, which is not consistent with a retrospective review. Please clarify.
Reply: Thanks for this comment. This was a retrospective study, and we revised this misleading sentence in the text.
Change in the text: Page 6 Line 107-112 and Page 27 Line 480

Discussion:
Comment 19: Lines 306-307: Units of time to recovery need to be added.
Reply: Thanks for this comment. We have added the units of time to recovery.
Change in the text: Page 14 line 294-297

Comment 20: Please discuss if EBUS-TBNA has been previously described in the literature for treatment (i.e. aspiration and local injection) of MLNT.
Reply: Thanks for this comment. To our knowledge, no study has been conducted to investigate the treatment of MTLA with EBUS-TBNA. Currently, EBUS-TBNA is mainly used in the diagnosis of TB.
Change in the text: Page 5, Line 93-94

Comment 21: Please discuss the data describing why EBUS-TBNA is better than better than TBNA.

Reply: Thanks for this comment. In the EBUS-TBNA, the additional ultrasound can visualize the location, size and blood flow of lymph nodes more safely and precisely. Traditional TBNA can be employed to observe the location of lymph nodes by CT, but it fails to precisely localize the lymph nodes.

Change in the text: Page 15, Line 272-279

Comment 22: How does the treatment response described here compare to outcomes in those who receive surgery?

Reply: Thanks for this comment. Zuo et al (20) examined video-assisted thoracoscopic surgery (VATS) for the treatment of MLNTA. All of the patients underwent the radical debridement and drainage of abscesses by VATS and were also administered with the intensified anti-tuberculosis treatment. In all the 16 MLNTA patients, two patients developed complications after surgery, of whom one developed recurrent laryngeal nerve injury, and the others had poor wound healing. In contrast, our study showed our treatment was less traumatic and more safe with few adverse effects.