Peer Review File

<mark>Reviewer A</mark>

Comment 1: This is a good retrospective review! Multilocular effusion requires surgical intervention. What is special about locus in anterior mediastinum?

Reply 1: Thank you for reviewing our manuscript. We appreciate your pertinent comment. Previous studies concluded that the existence of a locule with uncontrolled infection was a valid predictor of resistance to medical treatment. Because chest tube drainage is quite difficult and thrombolytic therapy may not be effective on the mediastinum side, the presence of locules on the anterior mediastinal side, which we defined the condition as LAM, may be a strong indicator for resistance to the medical treatment. We have added the following text for clarification (see Page 13, line 254-257).

Changes in the text: In our study, LAM, which has not been discussed previously, was a strong indicator for surgical management, possibly because chest tube drainage is quite difficult and thrombolytic therapy is not effective on the anterior mediastinal side.

<mark>Reviewer B</mark>

Comment 1: The authors retrospectively review the outcomes of 167 patient with pleural infection treated at a single institution in a 12-year period. They examine the factors that were associated with failure of medical treatment. The manuscript is well written and the aims are clear. I think this is a useful contribution to our growing knowledge on the optimum treatment for pleural infection. I have the following comments/suggestions:
- In statistics, the author mention that 'univariate' analysis was carried out using logistic regression. The authors probably meant 'multivariate'.

Reply 1: Thank you for reviewing our manuscript. We appreciate your insightful suggestion. We agree with you and other reviewes and have added multivariate analyses. We have revised the text from univariate to univariate and multivariate: (see Page 9, line 166-169).

Comment 2: In the methods, it is stated that chest tubes were inserted under fluoroscopic or CT guidance. Is ultrasound used at all to guide tube insertion? In centres in Europe, CT-guidance is usually reserved for causes where collections are posteriorly loculated or where ultrasound views are not clear.

Reply 2: We agree with your advice. We always use the ultrasound for tube drainage and besides, we insert the tube under CT guidance or fluoroscopy for safer insertion. We have added the following text for clarification (see Page 8, line 140).

Changes in the text: in addition to the ultrasound

Comment 3: The study spans 12 years of practice. Did the authors notice any change in patterns of management or in outcomes between the first half and second half of

this period?

Reply 3: As your comment, though there is no significant difference, the number of patients who underwent surgery with VATs at an early stage increased, and the mortality was decreased in the second half of this period. Based on the guidelines and this research, we would like to proceed with prospective research for early surgery for patients with findings indicators for surgical management in patients with pleural infections.

Comment 4: Can the authors explain what they mean by irrigation that was carried out for non-surgically treated patients? It would be useful to briefly explain to the readers what volumes of saline (or other agents?) used, and the frequency.

Reply 4: Although there is no clear evidence, irrigation using saline was often performed for non-surgical therapy for empyema in Japan, because thrombolytic therapy was not allowed of covered by insurance in Japan. The purpose of irrigation was to wash away the fibrin for preventing constituting the septum. We agree with you and have added the following text (see Page 8, line 133-134).

Changes in the text: Irrigation was performed every day after tube drain placement by using 500 or 1000 mL of saline depending on the size of the cavity.

Comment 5: What was the size of chest tubes used in medical patients? If different sizes used, any differences in outcome? This factor would be a useful addition to the multivariate analysis (in case different tube sizes were used).

Reply 5: Thank you for your thoughtful comments. Because we usually use the drain 24Fr. (8mm) tube in both surgery and non-surgery cases, we could not compare the outcome by the tube size.

Comment 6: In the results, I suggest moving table 1 in the supplement to the main paper. If the maximum number of tables/figures is exceeded, figure 2 can be moved to the supplement. Also in supplementary table 1: what is P/F ratio? Can authors clarify if this the PaO2/FiO2? Also it is more customary to present CRP results in mg/L.

Reply 6: We agree with you and have moved supplement table 1 to the main paper. We also agree with your comments and have modified our table as advised (see Tables 1, 2, and 3).

Comment 7: The average hospitalisation periods in your cohort is between 26-42 days according to type of management. This is much longer than what is typically reported in the literature (please see results from Cargill et al, reference 20). Do the authors have an explanation?

Reply 7: Because our hospital previously performed non-surgical treatment sufficiently and surgery was performed for the cases resistance to non-surgical treatment, thus, we think that the average hospitalization period is longer than other reports. Recently, it has been possible to shorten the length of hospital stay by performing surgery at an early stage.

Comment 8: Finally, I suggest the authors, add to the limitations paragraph that the study spanned a long period of time which must have involved changes in practices and thresholds to refer patients to surgery.

Reply 8: We agree with you and have added the following text to the limitations paragraph (see Page 15, line 302-304).

Changes in the text: Moreover, this study spanned over a long period during which changes in practices and thresholds to refer patients to surgery may have evolved.

Comment 1: Sakai et al present a paper involving a single centre retrospective review of 167 consecutive patients treated for pleural infection from 2008-2020. They then subdivided the cohort into those successfully treated medically with urokinase and those who required a VATS for source control. A univariate logistic regression analysis was performed on various patient factors, laboratory values and imaging characteristics. They also did another analysis looking at patients who underwent surgery within 7 days of symptoms and after 7 days of symptoms. The final analysis demonstrated that multifocal loculations and anterior mediastinal loculations were radiographic predictors of need for surgery. Furthermore, performing surgery <7 days from symptoms resulted in a shorter hospital stay and a faster recovery.

Overall, the authors should be commended for their work on a very challenging topic. Specifically, how to select for patients with empyema who will benefit from surgery. The study is well constructed but can benefit from a few modifications.

1) The authors need to better define the inclusion criteria for the study. Currently, any patient treated for pleural infection are included. This is a bit vague. Further details into how a diagnosis of pleural infection is confirmed would improve the validity of this study. If information regarding pH, gram stain, purulent drainage or culture are available this should be added. Based on table 1, this information appears available. I would recommend creating a table with columns for surgically managed patients and medically managed patients and the variables analyzed. This way readers can compare the groups and see if they were similar or not. It also would be more intuitive for readers to see differences in variables.

Reply 1: Thank you for reviewing our manuscript. We appreciate your insightful suggestions. We agree with you and have revised the text (see Page 7, line 107-109). We also agree with you and have made Supplementary Table 2.

Changes in the text: All cases were diagnosed to acute empyema via blood test, chest X-ray, ultrasound, computed tomography (CT) scan, and thoracentesis, and classified based on the ACCP category and Light's classification.

Comment 2: The use of 7 days as early vs delayed surgery is a bit arbitrary. Would the authors be able to expand on how they arrived at this cut-off? Typically, early stage or exudative stage encompassed this first 2 weeks of symptoms. Thought I acknowledge there is overlap at the 1–2-week mark. The 2–6-week period after symptoms represents the fibrinopurulent stage where controversy lies as to whether surgical or medical management should be pursued. However, in this case the

presence of loculations already indicates you are in the fibrinopurulent stage. As such, I'm not sure how much value the <7 vs >7 day adds. I would argue outcomes differences will become even more distinct the shorter the interval between presentation and surgery.

Reply 2: Thank you for your insightful suggestion. As described in the introduction, clinical stages cannot be defined exactly because of the difficulty in diagnosing intrathoracic phases based only on clinical findings and the fact that the disease duration depends on the self-reported appearance of symptoms. Thus, we classified the patients underwent surgery to two groups; Surgery≤7 days and Surgery>7 days based on the median days from hospitalization to surgery. We agree with you and have added the following text to the limitations paragraph (Page 11, line 223).

Changes in the text: using median days from admission to surgery

Comment 3: The authors should perform a multivariable analysis after the univariate analysis to ensure there was no confounding. This is because many of the variables analyzed in the univariate analysis are co-dependent.

Reply 3: Thank you for your insightful suggestion. We agree with you and have added a multivariate analysis (Table 2), and have added the following test (Page 7, line 144-146, Page 8, line 188-189).

Changes in the text: Univariate and Multivariate evaluations were performed using logistic regression analysis. Multivariate analysis used the significantly different variables detected by the univariate analysis and variables used for classification in the ACCP category and guidelines (Page 9, line 166-169).

Multivariate analysis detected the existence of multiple locules and the presence of LAM was a significant indicator for resistance to non-surgical therapy (Page 11, line 215-217.).

Comment 4: Supplementary table of patient characteristics should again be divided into two columns, medical vs surgically managed.

Reply 4: We agree with you and have made Supplementary Table 2.

Comment 1: I read with interest your work entitled 'Multifocal locules including the Anterior Mediastinum Side as a Surgical Indicator in Pleural Infection'. Indications and timing of surgery in pleural infections is an important topic. This paper presents a retrospective single-center study of 167 consecutive patients and tries to assess the risk factors associated with surgical referral. Authors found that multifocal locules, especially locule(s) on the anterior mediastinum were at risk of failure of medical treatment.

Though the idea of the study is relevant, this paper suffers from major bias and limitations.

Specific points:

Methods:

- Loculations have only been evaluated by CT scan, but thoracic ultrasonography

would have been helpful and more precise.

Reply 1: Thank you for reviewing our manuscript. We appreciate your insightful suggestion. We agree with you and we have been always used ultrasound for the diagnosis of pleural infection and tube drainage. The efficiency of ultrasound for the diagnosis of pleural infection has already been well-known and thus, we researched the additional predictors.

We have added the following text for clarification (see Page 8, line 140).

Changes in the text: in addition to the ultrasound

Comment 2: It is a shame that the investigators could not use DNase in addition to a fibrinolytic agent. Data in literature are clear about the benefits of using this combination (Chadda et al., Use of fibrinolytics and deoxyribonuclease in adult patients with pleural empyema: a consensus statement, Lancet Respir Med, 2021). Of note, contrary to what the authors wrote, DNase is not classified as a fibrinolytic agent.

Reply 2: We agree with you and we expect to be able to use DNase in addition to a fibrinolytic agent in Japan. We agree with your comment and have revised the manuscript (see Page 8, line 138-139).

Changes in the text: because combination therapy using tissue plasminogen activator and DNase was not allowed in Japan.

Comment 3: One major limitation is the following indication bias: 'a surgical approach was considered after a respiratory-center discussion if locules persisted', so loculations were an indication to surgical referral, so it is not surprising that authors found that loculations are associated with the need for surgery…

Reply 3: We agree with your comment and there may be some selection bias in this study. However, not only the image findings including locules but also many factors such as the inflammatory data, comorbidities, the data of pleural effusion were considered in determining surgical indications, based on the ACCP guidelines, Light's classifications, and past studies. Moreover, the isolated locules on the anterior mediastinal side (LAM) were not considered until this analysis.

Comment 4: 'antibiotics were continued until inflammatory parameters improved': This is not exactly what current guidelines recommend (i.e > 3 weeks course and clinical, biochemical and radiological improvement).

Reply 4: We agree with you and have added the following text to the paragraph (see Page 9, line 156-158). We also evaluate the clinical condition and radiological findings to continue the antibiotics based on the guidelines.

Changes in the text: Antibiotics were continued until clinical condition, radiological findings, and inflammatory parameters including the white blood cell count and C-reactive protein level, improved.

Comment 5: 'Disease related death' criteria were too selective, especially 'pneumonia on the side with the pleural infection' or 'respiratory failure due to the

pleural infection' could underestimate mortality rates. Authors should prefer a more robust criterion, such as day-28 or 3-month mortality rate.

Reply 5: Thank you for your inshightful suggestion. Patients who did not undergo surgery were characterized by residual pulmonary dilatation and subsequent respiratory failure and pneumonia after discharge. However, the criteria were too selectibe as you commented. Therefore, we have limited the definition of treatment-related death to those associated with empyema, and changed the outcome to 3-month and 6-month mortality (Page 12, 225-226, Supplementary Table 3).

Changes in the text: recurrence of the pleural infection and Disease related death was observed only in the non-surgical group. Mortality was not observed among the patients who underwent surgery within 7 days of admission.

Comment 6: The definition of LAM is unclear and should be more precise: how was 'anterior' and 'mediastinum locules' defined anatomically?

Reply 6: Thank you for your inshightful suggestion. We have defined "LAM" more anatomically as follows (Page 7, line 122-123);

Changes in the text: If isolated locules on the anterior mediastinal side (LAM), locules in the space surrounded by the lung, anterior mediastinum, and chest wall were observed, we defined the condition as LAM.

Comment 7: Statistical analysis:
- Why did the authors use a Wilcoxon test? Patients were not paired. Mann-Whitney test would have been more appropriate.

Reply 7: As you commented, we used the Mann-Whitney test, thus we corrected it. (Page 9, line 166).

Comment 8: There was no multivariate analysis, which is another major limitation. Authors should have performed multivariate logistic regression with surgical referral as event (y) and try to identify 5 or 6 factors (10 events per factor rule) associated with thoracic surgery.

Comment 9: Due to the high number of univariate analyses, correction of type I error should be performed, such as Benjamini-Hochberg test.

Reply 8 and 9: Thank you for your inshightful suggestion. According to the reviewers' advise, we included the multivariable analysis to adjust for confounding. We included the established risk factors, which was shown in the guideline, in the statistical model. (Table 2).

Comment 10: Results:
- There is an inconsistency between the abstract, where it is reported that the study period was 2006-2020, and the Methods section where it is '2008-2020'.

Reply 10: We agree with you and have revised the the study period (2008-2020) (see Page 9, line 176).

Comment 11: 59% of documented pleural infections is an impressive number. Did

the investigators perform blood-culture bottle of retrieved pleural fluid?

Reply 11: We agree with your comment because the positive rate is higher than the previous reports. However, we did not use the blood-culture bottle. The reason may be that many advanced cases, which were treated at other facilities and did not improve, are tranfered and treated at our facility.

Comment 12: Prognosis cannot be rightly assessed without performing Cox regression (because it takes into account the time in the analysis)

Reply 12: We agree with your advice. The prognosis could not be compared because the number of events was very small especially in patients underwent surgery. Therefore, we did not make explicit mentions of prognosis. We will analyze the prognosis by future prospective study.

Comment 13: Discussion:
- To me, this work cannot carry the findings mentioned in the discussion. Due to the statistical issues mentioned above and the study design, authors cannot affirm that LAM was 'a strong indicator' or that they 'clarified that early surgery could decrease the duration of hospitalization and the rate of disease-related death'

Reply 13: Thank you fo your suggestion. We agee with you and have revised the discussion as follows (see Page 13, line 254-259, 263-264);

Changes in the text: In our study, LAM, which has not been discussed previously, was a strong indicator for surgical management, because chest tube drainage is quite difficult and thrombolytic therapy may not be effective on the anterior mediastinal side. There is no report about the anatomical positional relationship of the locules and resistance of the non-surgical therapy, the results of this study may be useful for establishing criteria for surgical indication.   (Page 13, line 254-259).
We clarified that early surgery within 7 days of admission could decrease the duration of hospitalization and improved C-reactive protein level faster, which suggests that early consultation with a thoracic surgeon and surgery in patients with these imaging results may improve the prognosis (see Page 13, line 263-264)

Comment 14: Authors should discuss the impact of the absence of using intrapleural fibrinolytic agent plus DNase. So far, it is the only treatment that has proven a reduction in surgical referral in pleural infections in a large RCT (Rahman et al., Intrapleural use of tissue plasminogen activator and DNase in pleural infection. N Engl J Med, 2011).

Reply 14: We agree with your suggestion. Because combination therapy using tissue plasminogen activator and DNase is not allowed to use in Japan, we could not perform this therapy in our facility, thus, some patients who underwent surgery may treat using combination therapy. We have added the following text as you adviced; (Page 15, line 300-302);

Changes in the text: Combination therapy using tissue plasminogen activator and DNase could not undergo and only monotherapy using urokinase was performed in our facility, some patients who underwent surgery may treat using combination

therapy.

Comment 15: The limitations are not addressed rigorously (confounding factors, limited sample size, absence of use of DNase, etc.)
Reply 15: We agree with your comment and have added the limitations as follows; (Page 15, line 298-300);
Changes in the text: Since the present study was a retrospective and single-center analysis, the number of patients was small and there were biases related to treatment methods and patient groups. Combination therapy using tissue plasminogen activator and DNase could not undergo and only monotherapy using urokinase was performed in our facility, some patients who underwent surgery may treat using combination therapy. Also, this study spanned a long period which must have involved changes in practices and thresholds to refer patients to surgery.

Reviewer E

Comment 1. In page 3 line 39: these enrolled patients were classified into two groups: non-surgical therapy (n=94) and resistant to non-surgical therapy (n=73). I think the word of "initial" should be removed to avoid the misleading information, i.e., these patients treated "initially" by non-surgical therapy underwent decortication "later".
Reply 1: Thank you for reviewing our manuscript. We appreciate your insightful suggestion. We agree with you and removed "initial" (see Page 3, line 45).

Comment 2. In page 5 line 93: 2 patients who had pleural infection after "bronchoscopy" were excluded. Could the authors describe in more detail?
Reply 2: Thank you for your insightful suggestion. There are two cases who affected with empyema after the bronchoscopic biopsy. Lung abscess was occurred after the biopsy and then developed to the empyema. We agree with your comment and have added the following text (see Page 7, line 112).
Changes in the text: lung abscess after bronchoscopic biopsy

Comment 3. I am confused about yield rate of pleural effusion culture. The yield rate is 59.3% (n = 99), also showed in the supplementary table 1. But the positive bacteriological result, showed in the supplementary table 2, is 35.9% (n=60). Could the authors explain the difference?
Reply 3: We agree with your comment. The ACCP guideline classified the bacteriological result into 4 types; unknown, negative, positive, and pus. The yield rate is 59.3% (n = 99) showed in supplementary table 1. The cases with a positive result but not pus were 35.9% (n=60), and 39 cases were positive results and pus (7 cases were negative results and pus). It was complicated and thus, we revised the text in Supplementary Table 1.
Changes in the text: Positive (not pus)

Comment 4. I am interested in the timing about thrombolytic therapy. According to

the authors' treatment strategy, the thrombolytic therapy is usually applied before operation. I wonder the efficacy of preoperative thrombolytic therapy. In my experience, the results are somewhat poor when thrombolytic therapy is used initially, especially for multiple locules. Could the authors share the experience of thrombolytic therapy? Will they use it postoperatively?

Reply 4: Thank you for your insightful suggestion. Some patients with multiple locules can be treated by thrombolytic therapy, however, the duration of hospitalization was longer than the patients who underwent surgery. Also, if the case in which multiple locules are close together may be treated by the thrombolytic therapy, but a case in which locules separated including the anterior mediastinum side (LAM) were difficult to treat by thrombolytic therapy. There is no report about the effect of thrombolytic therapy due to the anatomy of the locules, we will analyze the effect of thrombolytic therapy.

Comment 5. In page 7 line 165-166: I can't understand the sentence: LAM was observed in the pleural space anatomy, a single locule, and multiple locules in 38 (22.8%), and 100 (59.9%), 63 (37."7"%)cases, respectively. It is not consistent to the data showed in supplementary table 2.

Reply 5: We agree with you and have revised the text; 37.1 to 37.7 (see Page 10, line 194).

Comment 6. In Page 9 line 202: ACCP is presented for the first time, so the sentence should be revised as …American College of Chest Physicians (ACCP).

Reply 6: We appreciate your comment. We revised the manuscript and have used "ACCP" first in page 7, line 109.

Comment 7. In page 17 table 1: I suggest PaO2/FiO2 ratio, rather than P/F ratio and pleural space anatomy > 1/2 hemithorax, rather than pleural space anatomy > 1/2.

Reply 7: We agree with your comments and have modified our table as advised (see Table 1 and Table 2).

<mark>Reviewer F</mark>

Comment 1: I would like to congratulate the authors for their interesting study and elegant manuscript.

Reply 1: Thank you for reviewing our manuscript. We appreciate your comment.