Peer Review File
Article information: https://dx.doi.org/10.21037/jtd-23-847

Reviewer A

Comment 1: While the study is of value and importance of assessing intrapleural tPA and its systemic effects, the study suffers as the authors propose from some drawbacks, and many of which are severe such that any meaningful conclusion cannot be drawn from reviewing the study, which primarily include the fact that this is a very small study, single center and not blinded, and thus could have several biases.

Reply 1: Thank you for your review and comments. We understand that this is a smaller single-center study, so it may be hard to definitively conclude that intrapleural tPA and DNase do not cause measurable changes in systemic coagulation. This adds to current literature and understanding. Further, we hope this study can continue to promote further extensive multicenter trials, which will be necessary to recruit a large enough sample size to make a more definitive conclusion. While blinded randomized control trials help limit bias, tPA and DNase have been shown superior to intrapleural saline in reducing the surgical need for those with intrapleural lytics and, for this reason, may be challenging to randomize even in further studies.

Changes In Text: NA

Comment 2: I would suggest that this study be presented as a letter to editor as a pilot study while highlighting the several issues, or once a larger number of patients is obtained, then re-consider submission then.

Reply 2: We appreciate the feedback on the study. We believe this is a unique study for a widely used therapy and helps to form further hypotheses on the systemic risks of the therapy. We have outlined the limitations in the study (lines) and hope that this study may be used as preliminary data for a more extensive multicenter study needed to collect a large enough sample size.

Changes In Text: NA

Reviewer B

Comment 1: The inclusion criteria are imprecisely defined – it would be preferable to define the exact criteria used for diagnosis of complicated parapneumonic effusion. Further to this, the results suggest that the aetiology of some included participants' pleural effusion was not in fact complicated pleural effusion –complex malignant effusion and hydropneumothorax are also listed. This is a major discrepancy and needs to be clarified. Should these patents not have been included?

Reply 2: Thank you for your review and comments. Initially, we had not included full definitions based on previously published papers but have since added definitions for complicated parapneumonic effusions, empyema, and multiloculated effusions. We have also clarified the one patient previously listed as hydropneumothorax and added the patient to the complicated

parapneumonic effusion/empyema list as the patient grew out pseudomonas. While our patient with the multiloculated malignant effusion had pH <7.1 and glucose of 12, there were no cultures positive and no obvious pneumonia treated, so it was not counted in the complicated parapneumonic group. We feel that both patients meet the criteria for intrapleural lytics and, therefore, should be included in the study.

Changes In Text:
We added "multiloculated malignant effusions" to the introduction lines 123-124 with citation 14 to document this indication for intrapleural lytics.

We have added a more precise definition to complicated parapneumonic effusions, empyemas, and multiloculated effusions on lines 200-203 to include "defined as pH <7.2, glucose <60 or LDH >1000 or multiloculated effusions defined as septations seen on ultrasound imaging or computer tomography (CT) scans. Empyema was defined as frank purulent drainage or culture-positive pleural fluid."

We have updated lines 292 to 293 for reader clarification on the patient with the hydropneumothorax, now classified with the complicated parapneumonic/empyema group.

We have updated Table 1 for clarification on the hydropneumothorax now classified with the complicated parapneumonic effusion/empyema.

Comment 2:
It is unclear how many samples were obtained from each participant. Was coagulation assessed before and after doses of intrapleural tPA/DNase on multiple occasions per participant or only once? This would affect which statistical test would be most appropriate i.e., Wilcoxon signed rank test vs repeated measures ANOVA or Friedman test, depending on normality.

Reply 2:
We have added clarification that each participant only had one set of samples obtained.

Changes In Text:
We have added the following text to lines 231-233 "Data for only one tPA and DNase therapy was used for each patient. Each patient had assessment of their coagulation profile before and after intrapleural therapy."

Comment 3:
The methods suggest they may have included participants who had already received intrapleural tPA and DNase in this episode of care. Given the systemic absorption of these medicines from the pleural space is unknown, in such instances the authors could not be certain the prior doses did not affect the subsequently assessed baseline coagulation profile, and this has not been acknowledged. How many participants had already received intrapleural tPA/DNase at the time of the baseline coagulation studies?

Reply 3:

We have added clarification information to the text on why those with previous intrapleural therapy were included. We clarified that there was a minimum of one hour after the chest tube had been released to wash out previous doses. This is based on data showing that the half-life of systemic tPA is five minutes and lasts up to one hour. Since labs are also a direct comparison for the patient before and after therapy and the findings are based on differences, even if there were ongoing systemic effects, which we do not believe there to be, we should still see an increase in the change with the following therapy.

Changes In Text:
We have added the following text to describe better the washout period of one hour and why this was done on lines 221-226 ". If patients had received any intrapleural tPA and DNase, a one-hour washout period after draining intrapleural tPA and DNase was used to minimize any previous dose effects on the baseline coagulation profile. One hour was chosen as an adequate washout period as the half-life of systemic alteplase is five minutes, and systemic effects last up to one hour[23]."

We have also added the following text on lines 303-307 to describe further why previous doses should not affect the outcome as we should still see and change in the pre and post-labs even if the pre-labs have some residual effects: "While some patients were enrolled after their first intrapleural tPA and DNase treatment, the combination of a one-hour washout period and the direct comparison of pre and post coagulation profiles should not affect the detection of absorption as we would expect that there would still be a measured change if tPA or DNAse were absorbed."

Comment 4:
Given the absorption of the tPA/DNase form the pleural space is unknown, how did the authors choose their timeframe for repeat assessment of coagulation profile?

Reply 4:
We chose 50-120 minutes to give adequate dwell time to see if there is absorption but not more than 1 hour after the chest tube had been unclamped. This is due to fibrinolytic systemic effects lasting up to one hour.

Changes In Text:
We had added the clarifying statement on lines 228-231: "Post-infusion phlebotomy was completed at this interval to give adequate dwell time to allow for possible absorption but not longer than one hour after the chest tube had been unclamped, as fibrinolytic effects can be for up to one hour."

Comment 5:
The authors have not described how they have defined pleural bleeding in this study, which has been a limitation of many of the studies of intrapleural tPA/DNase. Without this definition, it is easy for bleeding to be over or under-reported because this treatment is known to cause hemorrhagic discoloration of pleural fluid. Please describe how pleural bleeding was defined here.

Reply 5:
We acknowledge that our study was limited in the definition of intrapleural hemorrhage. As you have stated, this has been a key issue with multiple studies. Our patient with intrapleural hemorrhage was identified by more hemorrhagic fluid transformation. Due to the scope of our study being about systemic absorption and not specifically looking for intrapleural hemorrhage, we did not have consent to repeat lab testing on samples of the patient's pleural fluid, and the treatment decisions and pleural fluid analysis were left to the treatment teams.

Changes In Text:
We have added the additional text to define intrapleural hemorrhage in our patient on lines 311-313: "This patient had hemorrhagic discoloration of fluid without the need for blood transfusions. This patient had no significant changes in comparing their pre and post coagulation profiles."

We have added the additional text to express the limitations of the definition on lines 384-387: "We acknowledge that our study's definition of intrapleural hemorrhage is limited and would have benefited from further testing, including pleural fluid hematocrit. Further investigation on the fluid was not performed as it was left to the treating team's discretion."

Comment 6:
Data was summarized using medians and interquartile ranges. Why were medians and IQRs chosen blanketly? Were all variable not normally distributed.

Reply 6:
All of the variables in Table 2 were roughly normally distributed, as best can be ascertained with a small sample size (n = 17). Given the size of this sample, data was analyzed using non-parametric statistics, which are traditionally accompanied by medians and interquartile ranges for continuous variables.

Changes In Text:
NA

Comment 7:
Suggest caution with the statement in Discussion "Our data suggests that this therapy may be potentially safe in this higher risk patient population and may further expand it's use." This has not been tested in this study. Would suggest limiting your comments to suggesting further study in this population is needed.

Reply 7:
We agree that due to the sample size, the study's conclusions can be limited. We have changed the text to clarify better.

Changes In Text:
We have removed the previous statement and added additional text on lines 351-353: "Our data suggests that there is no measurable change in systemic coagulation with intrapleural tPA and

DNase; however, larger studies are needed to better examine how this affects the potential systemic bleeding risks."

Comment 8:
Throughout the text "lab draws", "labs", "blood work" could be better described. Please consider alternatives including but not limited to "phlebotomy", "serological analysis", "biochemical analysis", "assessment of coagulation profile."

Reply 8:
See updated text changes.

Changes In Text:
The wording has been updated to phlebotomy, biochemical analysis, and more appropriate terms.

Comment 9:
Several acronyms, including tPA, DNAse, IRB and MA are used without preceding definition.

Reply 9:
Changes have been added as necessary to update definitions.

Changes In Text:
Definitions added to acronyms as needed.

Comment 10:
Fibrinolytic is preferable to lytic because it is more specific.

Reply 10:
Lytics have been updated throughout the text to fibrinolytic for more specificity.

Changes In Text:
"Lytic" has been replace with "fibrinolytic" throughout the text for clarity.

Comment 11:
I would suggest consistency in choosing between "instillation" and "infusion" regarding intrapleural medicine administration.

Reply 11:
The wording has been updated to infusion for consistency.

Changes In Text:
Installation has been changed to infusion throughout the text.

Comment 12:
"D-Dimers" was used in an instance where it seems to not have referred to a plural in line 130.

Reply 12:
We have updated the text to remove the plural.
Changes In Text:
Line 157 D-Dimers changed to D-Dimer

Comment 13:
There are some instances where “lytic therapy” has been used seemingly to describe results of studies of intrapleural tPA-DNase. Mores specific language is suggested because other intrapleural fibrinolytic agents have been trialed, with differing results.

Reply 13:
We have updated appropriate areas for more specific language of tPA and DNase.

Changes In Text:
Several areas have been updated, from fibrinolytic or lytic therapy to tPA and DNase, to be more specific based on the study or specific medications used.

Comment 14:
“Floor” and “ceiling” of assays can be more accurately described. It would seem you are describing the minimum and maximum reported quantified values of the test. Perhaps something like “the assay in our institution reports a quantified value for glucose between the ranges of X and Y. Values above Y are reported as “>Y” and for the purposes of this study, where the value was reported as >Y, this was recorded as the value of Y.

Reply 14:
The text has been updated to help with clarity and more specifics.

In Text Changes:
The text has been updated to clarify lines 254-261: “Assays at our institution are reported as quantified values with normal ranges, and values above or below the ranges are reported as greater than or less than the minimum or maximum range. For the purposes of this study, either the maximum or minimum value was used as the reported value. Pleural fluid pH less than 6.6 was recorded as the value of 6.6. Pleural fluid glucose less than 10 mg/dL was reported at 10mg/dL. Pleural fluid LDH less than 25 (U/L) was reported with the value of 25 (U/L), and greater than 25,000 (U/L) was recorded as 25,000 (U/L). Systemic fibrinogen assays greater than 1,000 (mg/dL) were reported as 1,000 (mg/dL).”

Comment 15:
No units were reported for some variables that in fact have units eg glucose, LDH.

Reply 15:
Units have been added.

Comment 15:
Units have been added and updated as appropriate.

Comment 16:
In the introduction in line 103, suggested removal of "especially for poor surgical candidates." This component of your statement has not been tested, to my knowledge.

Reply 16:
We agree that there is no randomized trial to study this as poor surgical candidates would not likely have the option for VATS, and therefore, treatment options would probably be limited to tPA and DNase. We have removed the statement for clarification.

In Text Changes:
Line 118 especially for poor surgical candidates has been removed for clarification.

Comment 17:
For a more comprehensive description of the post-MIST2 tPA/DNase literature, suggest consideration of inclusion of the following references:
o Majid A, Kheir F, Folch A, Fernandez-Bussy S, Chatterji S, Maskey A, et al. Concurrent Intrapleural Instillation of Tissue Plasminogen Activator and DNase for Pleural Infection. A Single-Center Experience. Ann Am Thorac Soc. 2016;13(9):1512-8.
o Smith D, Shaw H, Ryder T. Intrapleural 3ssue plasminogen ac3vator and deoxyribonuclease administered concurrently and once daily for complex parapneumonic pleural effusion and empyema. Intern Med J. 2023 Apr 8. doi: 10.1111/imj.16084. Epub ahead of print. PMID: 37029951.
o Bishwakarma R, Shash S, Frank L, Zhang W, Sharma G, Nishis SP. Mixing It Up: Coadministra3on of tPA/DNase in Complicated Parapneumonic Pleural Effusions and Empyema. . J Bronchology Interv Pulmonol. 2017;24:40-7.
o Popowicz N, Ip H, Lau EPM, Piccolo F, Dootson K, Yeoh C, et al. Alteplase Dose Assessment for Pleural infec3on Therapy (ADAPT) Study-2: Use of 2.5 mg alteplase as a star3ng intrapleural dose. Respirology. 2022;27(7):510-6.
o Jiang C, Xie M, Cervellione K, Thurm C. Clinical efficacy and bleeding outcomes of 3ssue plasminogen ac3vator and dornase alfa in pleural space infec3on with once daily concurrent administra3on: a retrospec3ve cohort study. BMC Res Notes. 2020;13(1):368.

Reply 17:
These are important studies and were examined as part of the background review for this paper. Due to our paper's scope and not being a comprehensive review of the available literature, they were not included in the study.

In Text Changes:
NA

Comment 18:
Given it's very large sample size that sets it apart from the rest of the post MIST-2

literature, the findings of Akulian et al should be highlighted more. For example, the statement “more recent data suggests that full-dose anticoagulation may not cause increased risk of intrapleural hemorrhage” is contradicted by Akulian et al’s findings and this is not mentioned in this paper.

Reply 18:
Akulian et al. is a well-done large study exploring intrapleural bleeding risk. It was previously mentioned in the paper. We have since moved this to follow lines 134-139 to support the prior statement that full-dose anticoagulation has been associated with an increased risk of intrapleural hemorrhage. However, we do mention the study by Gilbert et al. as this study was done on indwelling intrapleural catheters; systemic anticoagulation may not be associated with intrapleural hemorrhage. While significant data exists that systemic anticoagulation is associated with increased intrapleural bleeding risk, we suggest that further studies are needed to understand some discrepancies and better understand the mechanism of how systemic anticoagulation may affect intrapleural hemorrhage risk.

In Text Changes:
We have moved lines 150-155 to 134-139 to help support previous statements. We added lines 144-147 “Further studies are needed to understand how different patient populations may be at risk for intrapleural hemorrhage and what is the mechanism in which systemic anticoagulation and the pleural space interact to affect intrapleural bleeding.”

Comment 19:
The reference for the sentence “While there are a few case studies that suggest possible correlation between intrapleural lytics and systemic bleeding, multiple larger trials exploring intrapleural lytic effects have not reported significant systemic bleeding.” is of a single case report. Suggest additional reference to the mentioned trials.

Reply 19:
We have previously referenced the study by Akulian et al. and that this large trial did not have systemic bleeding events. See lines 167-169. We have added reference 15 (Akulian et al.) to the above statement to support this statement further.

In Text Changes:
Addition of reference to line 132. Further discussion of Akulian study added on lines 167-169 updated to “However, in the more recent larger trial by Akulian et al., there were no reported episodes of major systemic bleeding associated with intrapleural tPA and DNase.”

Comment 20:
I think the statement “If systemic anticoagulation does increase intrapleural hemorrhage risk it may suggest that these medications can affect the pleural and systemic vasculature interface” needs more justification if it is to be included.

Reply 20:
This statement was meant to be more hypothesis-driven. We have updated the text to clarify.

In Text Changes:
Lines 363-370 have been added and reworded to describe better the future role of studies based on the data we presented. “However, other studies have not consistently found a correlation between systemic anticoagulation and intrapleural hemorrhage.[14] Further studies are needed to better understand the mechanism by which systemic anticoagulation increases the risk for intrapleural hemorrhage. While we acknowledge further studies are needed to continue to evaluate if there is a measurable change in systemic coagulation associated with intrapleural tPA and DNase, we hope this study can also drive further hypotheses on the mechanism in which pleural space and systemic circulation may communicate.”

Comment 21:
What was the severity of the bleeding reported in the patient that suffered intrapleural bleeding? How much did their Hb decrement, did they require blood transfusion and of how many units of blood?

Reply 21:
Unfortunately, the patient with reported intrapleural bleeding did not have pleural hematocrit or a significant need for transfusion to support this definition further. As our study approval by the IRB was for observational purposes of lab draws, the treating team determined decisions on pleural fluid hematocrit. We report it as intrapleural hemorrhage as this is the official reason for stopping intrapleural tPA and DNase treatments. We acknowledged that this is a study limitation, described the patient further, and discussed that no transfusions were needed.

In Text Changes:
Additional text was added for clarification:
Lines 384-387: “We acknowledge that our study’s definition of intrapleural hemorrhage is limited and would have benefited from further testing, including pleural fluid hematocrit. Further investigation on the fluid was not performed as it was left to the treating team’s discretion.”

Lines 311-313: “This patient had hemorrhagic discoloration of fluid without the need for blood transfusions. This patient had no significant changes in comparing their pre and post coagulation profiles.”

Comment 22:
Rather than saying that patients “required” surgical intervention, it would be preferred to say the patients were referred for and underwent surgical intervention despite intrapleural therapy because you have not presented evidence that it was required.

Reply 22:
The text has been updated from “required” to underwent surgical intervention for clarification.

In Text Changes:
The text has been updated from “required” to underwent surgical intervention for clarification.

Comment 23:
Suggesting modifying Table 1 for better clarity. The format of the right column's values would be better described after each variable in the left column e.g. Male (n, %). Currently, there is a mixture of description in the heading of the right column and after variables in the left column, which is confusing to the reader.

Reply 23:
Thank you for pointing this out. We have modified Table 1 by putting all of the continuous variables at the bottom under a separate bolded heading.

In Text Changes 23:
Continuous variables have been moved to the bottom of Table 1.

Reviewer C

Comment 1:
Can the authors elaborate on the criteria used to define complicated parapneumonic effusion rather than just referring readers to the references?

Reply 1:
Thank you for your review and comments. Initially, we had not included full definitions based on previously published papers but have since added definitions for complicated parapneumonic effusions, empyema, and multiloculated effusions. We have also clarified the one patient previously listed as hydropneumothorax and added the patient to the complicated parapneumonic effusion/empyema list as the patient grew out pseudomonas. While our patient with the multiloculated malignant effusion had pH <7.1 and glucose of 12, there were no cultures positive and no obvious pneumonia treated, so it was not counted in the complicated parapneumonic group. We feel that both of these patients meet the criteria for intrapleural lytics and, therefore, should be included in the study.

Changes In Text:
We added "multiloculated malignant effusions" to the introduction lines 123-124 with citation 14 to document this indication for intrapleural lytics.

We have added a more precise definition to complicated parapneumonic effusions, empyemas, and multiloculated effusions on lines 200-203 to include "defined as pH <7.2, glucose <60 or LDH >1000 or multiloculated effusions defined as septations seen on ultrasound imaging or computer tomography (CT) scans. Empyema was defined as frank purulent drainage or culture-positive pleural fluid."

We have updated lines 292 to 293 for reader clarification on the patient with the hydropneumothorax, now classified with the complicated parapneumonic/empyema group.

We have updated Table 1 for clarification on the hydropneumothorax now classified with the complicated parapneumonic effusion/empyema.

Comment 2:
Can the authors specify which tPA was used in the study? I assume it is alteplase however this is not clear.

Reply 2:
We have specified that alteplase is used.

In Text Changes:
Alteplase is specified in line 235.

Comment 3:
The authors mention that patients could be on VTE prophylaxis but not full dose anticoagulation. What about antiplatelet agents?

Reply 3:
Patients were allowed to be on antiplatelet medications. While this can affect the MA on TEG scans it would still be expected to see a difference in the pre and post coagulation profiles if intrapleural tPA and DNase had an effect on the MA.

In Text Changes:
We have added line a line on 206 to clarify that those on antiplatelet medications can be included.

Comment 4:
Did the authors also collect hemoglobin and platelet count data?

Reply 4:
Hemoglobin and hematocrit were obtained at baseline but not tested after. While looking for differences in hemoglobin and hematocrit could help us identify intrapleural or systemic hemorrhages, the study aimed to look for measurable change in systemic coagulation.

In Text Changes:
None

Comment 5:
Given the half-life of intravenous alteplase is ~5min (half-life in pleural fluid is unclear), can the authors comment on why they chose 50-120min post lytic infusion as the time frame for post-infusion labs to be collected?

Reply 5:
We chose to look at the post-infusion time of 50 min to 120 min as it is currently unknown when or if there is systemic absorption as the half-life of tPA is 5 min, but fibrinolytic effects may be seen for up to 1 hour. We felt that this time range would give us the best chance to find an effect. This is up to 1 hour after absorption and not more than 1 hour after the chest tube was unclamped.

In Text Changes:
We have added lines 224-226: “One hour was chosen as an adequate washout period as the half-life of systemic alteplase is five minutes, and systemic effects last up to one hour.”

Comment 6:
It would be useful to provide a basic description of what TEG is, and what its various parameters measure. Also consider including a figure of a normal and an abnormal/fibrinolysis TEG pattern.

Reply 6:
Unfortunately, due to word count and the scope of the paper, a review of TEG scans is not possible in the article, but further literature is easily accessible online.

In Text Changes:
NA

Comment 7:
Units are lacking for pleural fluid glucose and LDH

Reply 7:
We have updated the units for fluid glucose and LDH.

In Text Changes:
We have added units to pleural glucose on line 258 and LDH on line 259.

Comment 8:
Can the normal reference ranges for the variables in table 2 be included?

Reply 8:
Yes, we have updated Table 2 to have normal ranges.

In Text Changes:
Updated table 2 with reference ranges.

Comment 9:
Can the authors specify the threshold for statistical significance? I assume it is $p<0.05$

Reply 9:
Yes, we will add that to the methods.

In Text Changes:
“P-values less than 0.05 are considered statistically significant.” was added on line 278.

Comment 10:
Were there changes in haemoglobin or platelet counts pre and post lytic infusion?

Reply 10:
We did not measure hemoglobin or platelets after tPA and DNase infusion. While measuring hemoglobin may have helped identify intrapleural or systemic bleeding, the paper aimed to evaluate measurable changes in coagulation profiles. While we did not measure post platelets, post-MA times should give a functional assessment of platelets and if there was a significant change after intrapleural tPA and DNase.

In Text Changes:
None

Comment 11:
Can more details be provided on the patient who had an intrapleural bleed? Was there hemodynamic instability? Did haemoglobin drop? By how much? Was blood transfusion needed? Also, what was this individual's TEG, PT/INR, aPPT, fibrin, D-dimer, haemoglobin and platelets before and after lytic infusion?

Reply 11:
We did add further description to the patient with intrapleural bleeding. Unfortunately, the definition was based on bloody discoloration and temporary hypotension, but no pleural hematocrit studies were sent. This was limited by the study's IRB approval that treatment teams would decide how to proceed on tPA and DNase complications. We have also listed this as a limitation. We described that this patient had no changes in their coagulation profiles.

In Text Changes:
Lines 311-313: "This patient had hemorrhagic discoloration of fluid without the need for blood transfusions. This patient had no significant changes in comparing their pre and post coagulation profiles."

Lines 384-387 added: "We acknowledge that our study's definition of intrapleural hemorrhage is limited and would have benefited from further testing, including pleural fluid hematocrit. Further investigation on the fluid was not performed as it was left to the treating team's discretion."

Comment 12:
Line 267 "...may not be clinically significant absorption of tPA from the pleural space."
This statement is misleading as post infusion bloods were draw on average 50-120min after lytic infusion and hence we do not know what is happening prior to 50min.

Reply 12:
We acknowledge that there may be absorption outside of this time period and have mentioned this in the paper. Based on the systemic half-life and fibrinolytic effects of tPA, we believe that if there were clinically significant absorption, we would see it during this time. We acknowledge further studies are needed to examine the timing to see if this is an effect at different time points. We would also suggest that even if there is brief absorption that cannot be measured after a very short period of time, it may not have clinical importance. We have updated the statement to say suggests for further clarification.

In Text Changes:
Added “suggests” to line 330.

Reviewer D

Comment 1:
What was the dwell time of the intra-pleural lytics?

Reply 1:
Dwell time was for 1 hour. This is now specified.

In Text Changes:
Added comments about dwell timeline 238-239: “Intrapleural tPA and DNAse were allowed to dwell for one hour before chest tubes were unclamped.”

Comment 2:
What is the half-life of tPA?

Reply 2:
The half-life of tPA is approximately 5 minutes, but fibrinolytic effects can last up to an hour.

In Text Changes:
Added lines 224-226: “One hour was chosen as an adequate washout period as the half-life of systemic alteplase is five minutes, and systemic effects last up to one hour.”

Comment 3:
Can tPA be tested directly in the blood or only the side effects?

Reply 3:
To our knowledge, there is no direct test for tPA (alteplase) levels in the blood.

In Text Changes:
NA

Reviewer E

Comment 1:
Recommend thoroughly reviewing the introduction for repetition. There is no need to restate every major trial supporting the use of lytic therapy. Rather focus on the limitations of use and what gap in the clinical landscape this pilot is aiming to fill.

Reply 1:
Thank you for this excellent comment. We are working to balance the introduction base on your and other reviewer’s comments asking for additional review. We aim to minimize redundancy while providing the foundation for clinical practice and this trial.

In Text Changes 1:
NA

Comment 2:
Recommend explicitly stating the results of the one patient that had intrapleural bleeding. If their systemic coagulation factors were normal, then this points to other risk factors for intrapleural bleeding (and strengthens your point).

Reply 2:
We did add further description to the patient with intrapleural bleeding. Unfortunately, like many intrapleural tPA and DNase studies, the definition was based on bloody discoloration, but no pleural hematocrit studies were sent. This was limited by the study's IRB approval that treatment teams would decide how to proceed on tPA and DNase complications. We have also listed this as a limitation. We described that this patient had no changes in their coagulation profiles.

In Text Changes:
Lines 311-313: "This patient had hemorrhagic discoloration of fluid without the need for blood transfusions. This patient had no significant changes in comparing their pre and post coagulation profiles."

Lines 384-387 added: "We acknowledge that our study's definition of intrapleural hemorrhage is limited and would have benefited from further testing, including pleural fluid hematocrit. Further investigation on the fluid was not performed as it was left to the treating team's discretion."

Comment 3:
You note that baseline coagulation factors were drawn for some patients after they had lytic instillation. Could you provide pharmacokinetic justification for this? As well as the number of patients who's baseline coagulation factors were drawn >1hour after lytic drainage.

Reply 3:
Not every patient was enrolled in the study before their first treatment of tPA and DNase. We allowed for an adequate was out period >1 hour after the patient's chest tube had been opened and allowed to drain. This is due to the terminal half-life of tPA being 1 hour. Further, since this study had a direct comparison of pre and post-labs to look for a difference, we would expect to see a difference in lab values still. Nine patients had labs drawn before the first treatment, four on their second treatment, three on their third, and one on their fourth (See Table 1).

In Text Changes:
None

Comment 4:
You note that the potential for missing transient elevations, and state that "further evaluation" is needed, how do you propose addressing this potential missed change?

Reply 4:

We would expect that different time points could be explored with a larger trial and more patients.

In Text Changes:
None