Dose de-escalation of intrapleural tissue plasminogen activator therapy for pleural infection: *the ADAPT project*

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***Running Head*:** Reduced dose tPA for pleural infection

Abstract

RATIONALE: Intrapleural therapy with tPA 10mg/DNase 5mg twice daily has been shown to successfully manage over 90% of patients with pleural infection without surgery in randomized and open-label series. The doses chosen are empirical and not established via dose-escalation studies. Potential bleeding risks associated with intrapleural tPA and the drug costs remain important concerns for many clinicians. The ADAPT (Alteplase Dose Assessment for Pleural infection Therapy) project aims to investigate the efficacy and safety of dose de-escalation for intrapleural tPA.

OBJECTIVES: To evaluate the efficacy and safety of a reduced starting dose regimen of 5mg tPA (with 5mg DNase) administered intrapleurally for pleural infection.

METHODS: Consecutive patients with pleural infection treated with open-label tPA 5mg/DNase 5mg were included from four participating centers in Australia, UK and New Zealand. Dose-escalation was permitted at the discretion of the attending physician. Data relating to treatment success, radiological and inflammatory changes (C-reactive protein), volume of fluid drained, length of hospital stay and treatment complications were extracted retrospectively.

MEASUREMENTS AND MAIN RESULTS: 61 patients (41 male, age 57±16 years) with pleural infection were prescribed treatment with an intrapleural regimen of tPA 5mg (with DNase 5mg). Most (n=58, 93.4%) were successfully treated without requiring surgery. Treatment success was corroborated by significant clearance of pleural opacities on chest radiography (from 42% [IQR 22-58] to 16% [8-31] of hemithorax, $p<0.001$), increase in pleural fluid drainage (from 175mL in the 24 hours preceding treatment to 2025mL [IQR 1247-2984] over 72 hours of therapy, $p<0.05$) and a significant reduction in CRP ($p<0.05$). Seven patients (11.5%) had dose-escalation of tPA to 10mg; three patients underwent surgery. Three patients (4.9%) received blood transfusions for gradual pleural blood loss; none were hemodynamically compromised. Pain needing escalation of analgesia affected 36% of patients; none required cessation of therapy.

CONCLUSIONS: These pilot data show that the use of a starting dose of 5mg tPA (with 5mg DNase) given intrapleurally for treatment of pleural infection is safe and effective. This regimen should be tested in future randomized controlled trials.

INTRODUCTION

Pleural infection hospitalises over 65,000 people in the UK and USA each year and its incidence continues to increase worldwide(1-4). Treatment involves evacuation of infected pleural fluid via chest tube drainage and antibiotics. However, one in four patients fail conventional therapy and require invasive surgery and/or succumb to their illness(5).

Treatment failure is frequently due to inadequate drainage of the multi-loculated effusions that develop as a consequence of fibrin deposition within the infected pleural space. Thus, intrapleural fibrinolytic therapy to lyse pleural loculations has attracted ongoing interest since 1949(6) with small studies(7, 8) demonstrating reductions in surgical intervention rates. Subsequently, the MIST (Multi-Centre Intrapleural Sepsis Trial)-1, the largest randomised controlled trial of intrapleural fibrinolytics (streptokinase) failed to demonstrate any benefits over placebo for pleural infection. Similar results were found in the fibrinolytic only (tissue plasminogen activator (tPA)) arm of the MIST-2 trial(9, 10).

Deoxyribonuclease (DNase) was recently found to decrease the viscosity of infected pleural fluid(11). Intrapleural therapy combining DNase and tPA, facilitated pleural fluid drainage in animal studies(12). In the MIST-2, 96% of patients who received intrapleural tPA 10mg/DNase 5mg therapy were successfully treated without needing surgery(9). Similar success rates have been replicated in three open-label series (n=107, 73 and 55) using the same combination doses(13-15).

However, the potential bleeding risks associated with tPA remains the major concern of many clinicians in choosing between tPA/DNase therapy and surgical drainage. tPA also

accounts for ~75% of the combined cost of tPA (10mg) and DNase (5mg) therapy in the USA, UK and Australia.

tPA activates circulating plasminogen and the fibrinolytic cascade. When used for ischaemic stroke, intravenous administration of tPA (0.9mg/kg, max 90mg) is associated with significant risks of symptomatic intracranial hemorrhage (6.4%, *vs* 0.6% with placebo)(16, 17). Intrapleural administration of tPA (±DNase) at 10mg doses rarely causes systemic hemorrhage but pleural bleeding has been reported at rates between 1.8% and 12%(13-15, 18).

Bleeding risks are dose-dependent when tPA is administered intravenously. A recent small study suggested the same may apply to intrapleural tPA use. The study randomized patients to intrapleural treatment with 20mg tPA or urokinase and was un-blinded following serious pleural bleeds in 5 tPA-treated patients (28%)(18). The pleural bleeding rate decreased to 12% when the study continued with tPA at 10mg.

The dosing regimen of tPA 10mg/DNase 5mg administered intrapleurally twice daily, used in MIST-2, has since been widely adopted(9, 13, 14). Interestingly, these drug doses were chosen empirically rather than determined from conventional phase I dose-escalation studies. Given the high clinical success rate with the tPA 10mg/DNase 5mg regimen, we hypothesized that lower doses of tPA (with DNase) could still provide similar treatment efficacy and may improve safety and reduce drug costs

The ADAPT (**A**lteplase **D**ose **A**ssessment for **P**leural infection **T**herapy) project aims to systematically investigate the efficacy and safety of dose de-escalation for intrapleural tPA instillation. In this first study of the ADAPT project, we report patient outcomes from participating centers which adopted a reduced starting dose regimen of 5mg tPA (with 5mg DNase) for intrapleural therapy for pleural infection.

METHODS

Data were collected from Sir Charles Gairdner Hospital (SCGH), Perth, Australia (September 2014-June 2016), North Bristol NHS Trust, UK (March 2015-April 2016), Queen Elizabeth University Hospital (QEUH), Glasgow, UK (February-April 2016) and Wellington Regional Hospital, New Zealand (January-April 2016). The listed centers all employed tPA at 5mg (with DNase 5mg) during the study period as the standard starting dose as part of their local protocols which allowed dose-escalation of tPA at the discretion of the attending physician. Data were extracted retrospectively from case-notes and hospital databases.

All patients in this study were treated according to usual local practice and assessed daily for clinical improvement. Suitability and timing of tPA/DNase therapy and other management decisions were determined by patients' attending physicians. All patients who were prescribed intrapleural tPA/DNase from each centre during the study period received a starting dose of 5mg tPA with 5mg DNase. Decisions regarding dose escalation of tPA or surgical intervention were made by the treating physicians and were recorded. The study was approved by the institutional review board of Sir Charles Gairdner Group Human Research Committee. The ethics committees of the remaining participating centers (NHS North Bristol Central and NHS Greater Glasgow and Clyde Regional Ethics Committees and New Zealand Health and Disability Ethics Committee) provided exemption for the project as it qualified as a retrospective audit and exempted from formal approval.

Pleural infection was defined as in previous studies(9, 13). All patients had clinical presentations consistent with pleural infection and pleural fluid that fulfilled ≥1 of the

following characteristics: purulent fluid, pH ≤7.20, glucose ≤3mmol/L (≤55mg/dL), presence of micro-organism(s) on gram-stain and/or bacterial culture.

Both tPA (5mg) and DNase (5mg) were diluted with 0.9% sodium chloride to 50mL and administered twice daily. The duration of treatment was determined by the attending physicians based on the clinical response to treatment. In three centers, tPA (Actilyse, Boehringher Ingelheim) was instilled intrapleurally via a chest tube which was then clamped for 40-60 minutes and then unclamped to allow fluid drainage for 40-60 minutes. The process was then repeated with DNase 5mg (Pulmozyme, Roche). In one center (n=5), both medications were instilled directly after each other before the tube was clamped.

Case-notes were interrogated for patient demographics, comorbidities and major life-limiting illness (defined as advanced cancer, end stage renal/liver disease, severe chronic obstructive pulmonary disease and heart failure), clinical observations, treatment and outcomes, surgical interventions, complications, and pleural fluid volume drained. Laboratory results especially inflammatory markers, pleural fluid biochemistry and microbiologic cultures were recorded. Data were collected during hospital admission and from routine outpatient clinic follow-up for 90 days. Chest radiographs (CXRs) were assessed for the area of pleural opacity occupied by the pleural effusion by one pulmonologist or radiologist using a validated method(9). The CXR immediately prior to tPA/DNase treatment was compared to the first CXR available at a minimum of 72 hours following the first dose of tPA/DNase and to the CXR at the completion of antibiotic therapy. Where a CXR was not available at the completion of antibiotic therapy, a CXR within 30 days after completion of the antibiotic course or the last available CXR during treatment was

assessed.

Outcomes:

The outcomes included were

i) survival without surgical intervention for pleural infection at 30 and 90 days from initiation of therapy;

ii) length of hospital stay (LOS) following initial dose of tPA/DNase;

iii) percentage change in pleural opacity on chest radiograph

iv) volume of pleural fluid drained;

v) blood C-reactive protein (CRP) level;

vi) complications including

a. systemic bleeding,

b. significant pleural bleeding defined as a decrease in blood haematocrit causing haemodynamic instability or requiring blood transfusion, and

c. pain.

Treatment success was defined as survival to hospital discharge and avoidance of surgical intervention for the 30 days following the initial dose of tPA/DNase.

Statistical analysis:

Statistical analyses were performed using SigmaPlot 12.5 (Systat Software, San Jose, CA). Results are presented as mean (SD) or median (IQR) based on normality of data. The Wilcoxon Signed-Rank test and paired t-test (for non-parametric and parametric data respectively) were used. Multiple group comparisons were performed using analysis of

variance (ANOVA) on-ranks, followed by Dunn's post-hoc test. Significance was defined as $p<0.05$.

RESULTS

A total of 61 patients (41 males) with a mean age of 57 (SD 16) years were included from SCGH (n=36), Bristol (n=16), QEUH (n=5) and Wellington Hospital (n=4). Forty-two patients (69%) had ≥1 comorbidities of which 28% had a major life-limiting illness, Table 1.

The median (IQR) number of days from hospital admission to chest-tube insertion was 2 (1-5) days. Most (93%, n=57) patients received tPA/DNase therapy more than 24 hours following chest tube insertion; amongst whom 39% (n=24) started therapy ≥ 48 hours after tube insertion. The majority of patients (n=36, 59.0%) were treated with 12F chest-tubes, followed by 18F (n=10, 16.4%) and 32F (n=4, 6.5%) drains. In two cases, the drain size was not recorded. Eight patients (13%) had an indwelling pleural catheter (IPC) in situ prior to diagnosis with pleural infection; seven for malignant pleural effusion and one for refractory pleural effusion from congestive heart failure. Eight (13.1%) patients, including three with IPCs, had additional chest drains inserted for drainage.

All patients met the criteria for pleural infection, Table 2. The mean CRP in the 24hrs preceding treatment was 225mg/L (SD 105) and 51% of patients were febrile during this same period. In 37% of patients, the pleural fluid contained pus and/or yielded positive bacterial culture. Positive microbial culture was reported in 21 (34%) patients, with five (8.2%) reported as polymicrobial, Table 3. All patients received broad-spectrum antibiotics such as piperacillin/tazobactam (n=34, 56%), amoxicillin/clavulanate (n=9) and meropenem (n=8). Vancomycin was used as adjunct antibiotics in five patients. In 11 (18%) patients, the antibiotics were escalated during the treatment course. Patients required a median total of

28 (IQR 20-35) days of antibiotics which included 12 (IQR 8-19) days of intravenous antibiotics in hospital or at home.

OUTCOMES

Most (n=57; 93.4%) patients fulfilled our pre-defined criteria of 'success' which included survival to hospital discharge and avoidance of surgical intervention for 30 days following the initial dose of tPA/DNase. One patient subsequently died from advanced malignant mesothelioma 20 days following hospital discharge. Therefore, 91.8% (n=56) of patients were alive at 30 days and did not have surgery. Follow up at 90 days was available in 89.3% (n=50) of these patients, and confirmed resolution of pleural infection with all patients (100%) alive without requiring surgical intervention. The six (10.7%) remaining patients had a median follow-up of 52 (IQR 35-59) days at which time all were alive and did not require surgery.

Patients received a median of 6 (IQR 4-6) doses of intrapleural tPA/DNase. Clinical resolution resulting in early treatment discontinuation occurred in 36% of patients who required a median of 3 doses (IQR 2-4). Less than 5% (n=3) of patients in the study received more than six doses; of which two received 7 and one received 12. Seven patients (11.5%), including five patients with an IPC had their tPA dose escalated to 10mg during the course, on average after 3.3 doses.

The median LOS was 7 (IQR 5-10) days for the entire cohort and for those successfully treated. The LOS was 8 (IQR 6-11.5) days for those who required dose escalation to 10mg tPA and 12 (11.5-13) days for those who needed surgery.

Treatment failures

Three patients were referred to surgery; two due to persistent infection, one underwent video-assisted thoracoscopic surgery and another a mini-thoracotomy. The third patient had successful control of his infection with tPA/DNase treatment but was referred for open decortication of thick residual visceral pleural rind and resultant trapped lung.

An 87-year-old man died following admission with severe pneumonia and a heavily loculated parapneumonic effusion. He received six doses of tPA 5mg/DNase 5mg with significant improvements on CXR and CRP levels. However he had multi-organ failure and was palliated due to his frailty, comorbidities and finding of a necrotic subcarinal mass suspicious of cancer.

Radiology

Significant radiological improvements were observed with tPA/DNase treatment, Figure 1. The percentage of hemithorax occupied by pleural opacity reduced from 42% (median, IQR 22-58) on baseline CXR to 16% (IQR 8-31) after ≥72hrs, $p<0.001$. The pleural opacity was reduced to 5.0% of the hemithorax (IQR 0.5-14.4) at day 30 (IQR 20-45) post-treatment initiation in the 54 (96%) patients where CXR was available. This value was further reduced to 3.8% (IQR 0.1-11.0%) when patients with an IPC *in situ* at presentation were excluded from the analysis.

Volume of pleural fluid drained

The median (IQR) volume of pleural fluid drained in the 24 hours preceding treatment was 175mL (75-450) which increased to 1157mL (844-1756) in the first 24 hours of tPA/DNase treatment and 2025mL (1247-2984) at 72 hours, $p < 0.05$, Figure 2.

CRP

The median CRP at baseline was 220mg/L (IQR 140-301) and decreased by 11% (IQR 0 to -24%) on day 1 of tPA/DNase treatment, Figure 3. However, in 30% (n=16/53) of patients, the CRP remained greater than baseline at 48 hours. By day 4 of treatment a median reduction in CRP of 45% from baseline was observed (95% CI, 37.9-56.8, $p < 0.05$). By day 28 (IQR 15-40), the CRP had reduced to 9.4 mg/L (IQR 3-25).

Adverse events

No systemic bleeding was reported in any patients. Three (4.9%) patients had blood transfusions; all were hemodynamically stable. Two had steadily decreasing blood hematocrit/hemoglobin levels, presumably from drainage of large quantities of hemorrhagic pleural fluid, which triggered a clinical decision for blood transfusion. The first patient drained 6000mL of heavily blood-stained fluid. Her hemoglobin dropped from 107g/L to 73g/L over four days and was transfused two units of packed red cells. She made an otherwise uneventful recovery. The second patient had a long history of thrombocytopenia and was considered a high-risk candidate for surgery. She developed a hematoma at the chest tube insertion site which required a blood transfusion. She had ongoing infection. It was considered that tPA/DNase therapy offered lower risks than surgery and she received six doses of tPA/DNase which successfully eradicated the pleural infection. The hemoglobin

gradually decreased (92g/L to 72g/L over 7 days) without hemodynamic compromise. She received a total of five units of packed red cells. The third patient drained 2575mL of hemorrhagic fluid after the first three doses of tPA/DNase. Following the fourth dose, 400mL of dark venous-looking blood was drained. The fluid had a hematocrit (0.36) similar to that of corresponding serum. Treatment was ceased and the blood loss stopped quickly after. He received one unit of packed red cells at the time as a preventive measure. He remained well and did not record any drop in serum hemoglobin. He was discharged home five days after the start of tPA/DNase therapy.

Chest pain following tPA/DNase administration was common (36%) and responded to short term escalation analgesia or in frequency of opioid therapy in all patients. No patients ceased treatment as a result.

DISCUSSION

Our data showed that intrapleural instillation at a starting dose of 5mg tPA (and 5mg DNase) for pleural infection appeared to have comparable efficacy and safety profile with published data of using higher tPA doses (Table 4). The majority of patients (93.4%) in the present study were successfully treated and avoided surgery for pleural infection. The clinical success was corroborated by significant improvements in radiographic clearance, pleural fluid drainage and inflammatory markers. Treatment with a reduced tPA dose was well tolerated and the incidence of blood transfusions (<5% patients) was comparable to previous studies using ≥10mg tPA. Pleural blood loss was gradual and no patients developed hemodynamic instability.

Pleural infection has a reported mortality of up to 20%(5). In the two largest randomized trials of pleural infection(9, 10), 25-30% of the patients in the control groups failed conventional treatment (systemic antibiotics and chest tube drainage) and required surgery. This contrasts the results from tPA/DNase therapy which cured over 90% of patients without surgery, and produced significantly better radiographic clearances and shorter LOS than conventional treatment. Although intrapleural tPA/DNase therapy has attracted strong interests worldwide, hesitation in its implementation exists in many centers, particularly of concerns over bleeding risks and drug costs.

Doses of tPA used for intrapleural therapy range from 2mg to 100mg daily in published literature(19). A study in horses (n=25) who received treatment with intrapleural tPA for fibrinous pleuropneumonia demonstrated successful treatment with doses ranging from 0.375mg to 20mg of tPA despite typically weighing in excess of 400kg(20). In the MIST-

2 protocol, tPA was administered at 10mg and DNase at 5mg, twice daily up to six doses. These doses were chosen empirically without support from prior dose-escalation studies. The pharmacokinetics of tPA following intrapleural delivery is unknown; likewise, the optimal amount of tPA required to lyze fibrinous loculations remains unclear.

Using a lower starting dose of intrapleural tPA has a number of potential advantages. The bleeding risks with tPA have been shown to be dose dependent following systemic administration, however only causally linked with intrapleural administration. Although the rate of intrapleural bleeding in our study was similar to studies of 10mg tPA, there remains a theoretical potential to reduce adverse events with lower doses in stronger powered studies. We have demonstrated similar treatment success, as measured by survival at discharge and avoidance of surgery at 30 days to other pragmatic studies using intrapleural 10mg tPA (with 5mg DNase) (13-15). Finally, this reduced dosage regimen should reduce total drug costs.

The ADAPT project aims to conduct stepwise dose de-escalation studies to establish the lowest effective dose for intrapleural tPA use in pleural infection. This first ADAPT study confirmed our hypothesis that a lower (5mg) starting dose of tPA provided treatment efficacy highly comparable with studies using higher tPA doses, Table 4. ADAPT-2 is underway to establish the efficacy of tPA 2.5mg/DNase 5mg as a starting regimen. Others have investigated alternatives to the tPA/DNase protocol, eg daily dosing, instilling both drugs simultaneously, use of other fibrinolytics etc(14, 15) .

Although not a direct comparison study, our patient cohort and their disease severity were very similar to those from prior studies. Most of our patients had significant comorbidity, and had complex pleural collections that failed to improve after initial 24 hours of drainage. Indeed, 93% had multi-loculated effusions on ultrasonography. The patients were recruited consecutively to minimize selection biases. The magnitude of the volume of fluid drained over 72 hours, rate of radiographic clearance and reduction in CRP from our study were highly comparable with open-label series using tPA 10mg/DNase 5mg (13).

In total 89% of patients were successfully treated with tPA (5mg) without requiring dose-escalation – thus justifying using 5mg as a starting dose. Only in seven cases of our study did the clinicians raise the tPA dose to 10mg, these patients did not appear disadvantaged in their LOS and other outcomes.

Bleeding has long been a concern of intrapleural fibrinolytic use. The growing clinical experiences suggest that tPA very seldom induces pleural 'hemorrhage', i.e. massive rapid bleeding causing hemodynamic compromise. Instead, intrapleural fibrinolytics (eg tPA), via a monocyte chemotactic protein (MCP)-1 mediated mechanism(21), stimulate production of large quantities (median 1.5 to 2.6L in published series) of hemorrhagic pleural fluid. Patients, especially those with significant comorbidity, may not be able to mount a hematopoietic response to compensate for the loss of red cells resulting in a gradual decline in haematocrit (21).

The study has several limitations. The retrospective nature of our study prohibits comparison with the conventional 10mg tPA dose or with patients treated for pleural

infection who were not administered tPA/DNase therapy. Comparison studies with 10mg tPA are required once the lowest-effective dose of tPA is established through dose de-escalation studies. Secondly, like most other studies on this subject, there were no standard criteria for defining which patient failed tPA/DNase therapy and required surgery. As a pragmatic study, these decisions were left to the attending physician. It is well known that local practice and protocols differ around the world on the threshold of referring patients to surgery. Nonetheless, the vast majority of our patients did not require surgery and their LOS were very comparable to surgical series. It is therefore unlikely that they have been subjected to unusually high threshold for proceeding to surgery. Thirdly, eight patients had IPC related pleural infection. These patients often have better outcomes compared with non-IPC related pleural infection (22), however were included in the analysis to avoid selection bias. The small number of patients within our cohort is unlikely to alter our conclusion but instead help inform the safety profile of the regimen. Lastly, the centers involved had all developed significant experience in the use of tPA/DNase and in pleural infection in general. It is difficult to determine if centers at the early learning phase can reproduce the same success rates.

The treatment success for patients who received a reduced starting dosage of intrapleural tPA (5mg) and DNase (5mg) for pleural infection was 93%, with only 11% of patients requiring dose escalation to 10mg of tPA. These observational data provide evidence that a starting dose of 5mg tPA (with 5mg DNase) intrapleurally for pleural infection is safe and effective. The findings of this hypothesis generating study will need to be prospectively evaluated in future randomized clinical trials against higher published dose regimens.

Table 1: Patient comorbidities

Comorbidity	**n (%)**
Current cancer:	**13 (21.3%)**
Malignant mesothelioma	6 (9.8%)
Non-small cell lung cancer	2 (3.2%)
Ovarian	2 (3.2%)
Haematological	2 (3.2%)
Colon	1 (1.6%)
Endocrine:	**11 (18.0%)**
Type 2 diabetes mellitus	10 (16.4%)
Type 1 diabetes mellitus	1 (1.6%)
Respiratory:	**11 (18.0%)**
Asthma	9 (14.7%)
Severe chronic obstructive pulmonary disease (FEV_1<50%)	2 (3.3%)
Cardiovascular:	**10 (16.4%)**
Ischemic heart disease	6 (9.8%)
Congestive cardiac failure	3 (4.9%)
Atrial fibrillation	1 (1.6%)
Renal disease	**8 (13.1%)**
Previous cancer:	**7 (11.5%)**
Breast carcinoma	3 (4.9%)
Non-Hodgkin's lymphoma	2 (3.3%)
Small Cell Lung cancer	1 (1.6%)
Pancreatic carcinoma	1 (1.6%)
Liver Disease:	**5 (8.2%)**
Cirrhosis	2 (3.3%)
Alcohol	1 (1.6%)
Autoimmune	2 (3.3%)
Neurological:	**2 (3.3%)**
Cerebrovascular accident	2 (3.3%)
Gastrointestinal:	**1 (1.6%)**
Inflammatory bowel disease	1 (1.6%)

Table 2: Pleural infection characteristics

Pleural characteristics	
Right side, n	36 (59%)
Moderate-Large effusion (>2 rib spaces) on ultrasound, n	56/60 (93%)
Ultrasound evidence of loculations, n	54/58 (93%)
Bacteria identified in pleural fluid culture, n	21 (34%)
Pleural fluid pH[‡], mean (SD)	6.99 (0.24)
Pleural fluid glucose[‡] mmol/L, median (IQR)	0.9 (0.5-3.4)
LDH[‡] as fold increase of serum upper limit of normal, median (IQR)	4.5 (2.5-11.0)

IQR; interquartile range, SD; standard deviation, LDH; Lactate dehydrogenase,
[‡] Results for pH, glucose and LDH were available in 47, 50 and 55 patients respectively. Three patients did not have any pleural biochemistry available.

Table 3: Microbiological of pleural fluid cultures

Aerobes	
Staphylococcus	**6 (9.8%)**
Staphylococcus aureus	2 (3.3%)
Coagulase negative *staphylococcus*	4 (6.6%)
Streptococcus	**5 (8.2%)**
Streptococcus intermedius	3 (4.9%)
Streptococcus pneumoniae	1 (1.6%)
Streptococcus agalactiae	1 (1.6%)
Enterococcus spp.	**3 (4.9%)**
	3 (4.9%)
Gram negative	**9 (14.8%)**
Pseudomonas spp.	4 (6.6%)
Acinetobacter spp.	1 (1.6%)
Coliform bacteria	4 (6.6%)
Anaerobes	
Anaerobes	**6 (9.8%)**
Actinomyces meyeri	1 (1.6%)
Clostridium perfingens	1 (1.6%)
Bacteroides spp.	1 (1.6%)
Mixed anaerobic	3 (4.9%)

Table 4: Summary of outcomes from published studies of intrapleural 10mg tPA with 5mg DNase for pleural infection

	n=	Treatment success (%)	Surgery n (%)	LOS median (IQR)	Death at 30 days††† n (%)	Pleural bleed n (%)
Current study tPA 5mg (DNase 5mg)	**61**	**93.4**	**3 (4.9)**	**7 (5-10)**	**1 (1.6)**	**3 (4.9)**
Rahman et al. (9) *NEJM 2011*	48	96†	2 (4.2)	11.8± 9.4 (mean±SD)	4 (8.3)††	2 (4.2)
Piccolo et al. (13) *Ann Am Thorac Soc 2014*	107	92.3	8 (7.5)	10 (6-17)	3 (2.8)	2 (1.8)
Mehta et al.[a] (15) *Respiration 2016*	55	92.7	4 (7.3)	13 (11-18)	3 (5.5)	0 (0.0)
Majid et al. (14) *Ann Am Thorac Soc 2016*	73	90.4	7 (9.6)	7 (5-11)	2 (2.7)*	4 (5.4)

[a] tPA/DNase was administered once daily

† Defined as the proportion of patients who did not require surgical intervention at 30 days and does not include deaths during admission (not reported)

†† Cause of death not reported in study

††† Death from any cause at 30 days. Majid et al. report deaths related to pleural infection only.

* Death likely due to pleural infection

Figure 1: Change in pleural effusion size on chest radiograph (n=55) expressed as a percentage of the hemithorax area before 5mg tPA/DNase treatment (pre-treatment) and 72 hours following the first dose of tPA/DNase (post-treatment). Six patients were excluded from analysis; three had extensive baseline changes secondary to mesothelioma and three patients did not have an CXR available at either baseline or within 48hrs post-treatment *** $p<0.001$, Wilcoxon Signed Rank Test.

Figure 2: Cumulative volume of pleural fluid drained (n=60) in the 24 hours before treatment (-24 hrs) and at 24 and 72 hours following the first dose of tPA/DNase. ***ANOVA on Ranks, $p<0.05$ by Dunn's post-hoc test.

Figure 3: Change in C-reactive protein over the course of tPA/DNase treatment. Baseline is the day prior to first dose tPA/DNase and is taken at 100%. *ANOVA on Ranks, $p<0.05$ by Dunn's post-hoc test.

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