Research Protocol

Intrapleural DNase and Tissue Plasminogen Activator in Pediatric Empyema (DTPA trial)

Principal Investigator:
Eyal Cohen, Hospital for Sick Children, University of Toronto, Toronto, Ontario

Co-Investigators:
Sanjay Mahant, Hospital for Sick Children, University of Toronto, Toronto, Ontario
Felix Ratjen, Hospital for Sick Children, University of Toronto, Toronto, Ontario
Jeffrey Hoch, St. Michael's Hospital, University of Toronto, Toronto, Ontario
Bairbre Connolly, Hospital for Sick Children, University of Toronto, Toronto, Ontario
Kevin Thorpe, St. Michael's Hospital, University of Toronto, Toronto, Ontario
Muhammad Mamdani, St. Michael's Hospital, University of Toronto, Toronto, Ontario
Ian Maclusky, Children's Hospital of Eastern Ontario, Ottawa, Ontario
Sophie Laberge, CHU Sainte Justine, Montreal, Quebec
Lucy Giglia, McMaster Children's Hospital, Hamilton, Ontario
Mark Walton, McMaster Children's Hospital, Hamilton, Ontario
Connie Yang, British Columbia's Children's Hospital, Vancouver, British Columbia

SUMMARY

Pneumonia is one of the most common reasons for hospitalization in children. In recent years, there has been an increase in the incidence of pleural empyema, defined as pneumonia with a complicated effusion leading to respiratory compromise and/or extensive loculations. With modern treatments, including antibiotics and intrapleural drainage with instillation of fibrinolytics, long-term outcomes are excellent. However, substantial short-term morbidity persists, including prolonged hospital stay with a painful chest tube and 'treatment failure' necessitating further procedures.

DNase (dornase alfa) may work synergistically with fibrinolytic drugs such as tissue plasminogen activator (tPA) leading to liquefaction of pleural collections and improved drainage. Based on an adult trial, there may be an additional benefit from the addition of intrapleural DNase to tPA, but this requires study in a pediatric population as the underlying disease (e.g. microbiology and natural history), and other, age-related biological factors may potentially affect treatment response. This randomized controlled trial aims to address the efficacy, cost effectiveness and safety of DNase combined with tPA compared with tPA alone in the management of childhood pleural empyema.

STUDY AIMS

Over-arching aim*:* The overarching aim of this research collaborative is to improve outcomes for children with pleural empyema.
Primary Research Question: In previously well children who present with pleural empyema, does intrapleural DNase with tissue plasminogen activator (tPA) via chest drain for three doses over 48 hours improve the time to hospital discharge compared with three doses over 48 hours of tPA alone?
Secondary Research Questions: Will there be differences between the groups with respect to other outcomes related to efficacy, cost and safety?

TRIAL DESIGN

Design: The study design will be a blinded, five-centre pragmatic, superiority randomized controlled trial in a parallel group, 1:1, two arm design.
Procedure: Previously healthy children (6 mo – 18 years) with pleural empyema requiring chest tube drainage will be recruited.
Interventions: At the time of chest tube insertion, subjects will be randomized to intrapleural instillation of tPA alone or tPA with DNase for three doses over 48 hours.
Outcome: Primary outcome is time to hospital discharge. Secondary outcomes include time to meeting discharge criteria, time to drain removal, duration of fever after intervention, need for ventilatory support or non-invasive ventilation, serious bleeding, need for further procedural interventions, hospital readmission, cost and mortality.
Sample Size: A sample size of 92 (46 per group) will have the power to detect a 2 day difference in length of stay between the groups. While we do not anticipate any post-randomization ineligibility, for example from misdiagnosis, we will increase the sample size by 5% to account for this possibility (total of 49 patients per group). It is anticipated that over the study period, 180 children will be admitted to the five centres who meet eligibility criteria.
Analysis: Student's t-test will assess differences in the primary outcome between groups.

FEASIBILITY

The investigators are clinical experts with a strong track record in outcomes research in pediatric empyema and methodological experts in clinical trials.

COMPLICATED PNEUMONIA (PLEURAL EMPYEMA) IN CHILDREN

Pneumonia is one of the most common reasons for hospitalization in childhood, [1] and accounts for more inpatient resource utilization (cost) than any other pediatric diagnosis outside of the newborn period.[2] Up to 50% of children hospitalized with pneumonia have an associated parapneumonic effusion.[3] Most of these effusions are small and uncomplicated and will resolve with antimicrobial treatment of the underlying infection, but in some cases, a complicated effusion can develop characterized by enlargement leading to respiratory compromise and/or extensive loculations. Such effusions, commonly referred to as pleural empyema, characteristically lead to substantial morbidity including respiratory distress, pain, and prolonged hospitalization leading to child school loss, parental work loss and stress on families. These children generally do not respond to antibiotic therapy alone and require management with a drainage procedure. In recent years, there has been a dramatic increase noted in the incidence of pediatric pleural empyema in multiple countries. [4-9] A review of Canadian data (excluding Quebec and Manitoba) found that there are now over 1,400 new cases each year. [10] Hospitalization data from the United States seems to suggest that this growth has been most apparent in young children, aged 2-4 years,[11] with Canadian data documenting a recent increase in incidence rates by over 450% in those aged 1-4 years. [10] Although the exact cause of this epidemiologic change is somewhat unclear, postulated causes include pneumococcal serotype replacement and increasing antibiotic resistance.[12]

There are important gaps in the scientific literature regarding the optimal therapy for pleural empyema in children that have been highlighted in various recent clinical practice guidelines that note that high quality evidence to support therapeutic recommendations is often lacking;[13,14] of over 50 recommendations considered in a recent review, only four (<8%) had high quality evidence.[14] This problem is pervasive in child health research. High quality clinical evidence is often based on the results of randomized controlled trials, and our research group has identified a paucity of randomized controlled trials (RCTs) in child health. Over the past 20 years adult RCTs published in leading general and subspecialty medical journals have increased substantially, while pediatric RCTs have increased only modestly.[15,16] This problem is particularly relevant in pharmaceutical (drug) trials, and has undoubtedly led to the widespread use of pharmaceutical products in children without sufficient data on efficacy or safety;[17] Despite some legislative initiatives in the United States and Europe to promote drug trials in children, extensive logistical and financial disincentives persist. Consequently, 79% of hospitalized children are treated with drugs for unapproved indications, otherwise known as 'off-label' use.[18]

THERAPY FOR PLEURAL EMPYEMA IN CHILDREN

Antibiotics: In children with pleural empyema, antibiotic coverage for likely causative organisms (*S. pneumoniae, S.aureus and S. pyogenes)* is essential. Unfortunately, a specific antimicrobial etiology is identified in less than 20% of cases, [19] so empiric therapy is commonly used. Although the potential choice of agents is wide and guided by local policies and patterns of antibiotic-resistant organisms such as penicillin-resistant *S. pneumoniae* and methicillin-resistant *S.aureus* (MRSA), recent published guidelines in both the United States [13] and Canada [20] suggest that empiric therapy with a third generation cephalosporin (ceftriaxone or cefotaxime) with the addition of either clindamycin or vancomycin if clinical, laboratory, or imaging characteristics are consistent with infection caused by *S. aureus*.

Drainage procedures: Published clinical practice guidelines support the use of drainage procedures in addition to antibiotics in managing pleural empyema in children.[13,14,21,22] These guidelines are based on aggregate reports suggesting significantly longer hospital stays, duration of antibiotic therapy and progression to surgical intervention in children treated with antibiotics alone.[23] A variety of different drainage procedures have been reported in the literature including chest tube placement with or without

fibrinolytics, repeated ultrasound-guided needle thoracentesis, video-assisted thorascopic surgery (VATS) and open thoracotomy with decortication. A systematic review [24] and cost-effectiveness analysis [25] of the relative merits of each of these procedures has been conducted by our team. Based on the best-available evidence, either of two approaches is the most effective therapy for treating childhood empyema: (a) insertion of a chest drain with regular instillation of fibrinolytics; or (b) VATS. Recent Canadian data suggests variability in practice across hospitals, although insertion of a chest drain with regular instillation of fibrinolyics may be more cost effective.[25]

Fibrinolytics: The rationale for utilization of fibrinolytics in parapneumonic effusion is its role in dividing pleural septations and loculations that interfere with drainage. A variety of different fibrinolytic agents have been described in the literature: streptokinase, urokinase, and tissue plasminogen activator (tPA). Streptokinase is rarely used as it is antigenic generates a systemic antibody response similar to that found when the drug is given systemically.[26] A randomized controlled trial in the United Kingdom comparing regular chest tube instillation of urokinase with saline for 3 days in 60 children with parapneumonic effusions found that length of stay was significantly shorter with urokinase (7.4 vs. 9.5 days).[27] Another trial from the UK comparing urokinase instillation with VATS in 60 children found no difference in the primary outcome of length of stay. [28] Urokinase is not available in North America.

Current fibrinolytic utilization is based on availability in different jurisdictions (e.g. urokinase in Europe, tissue plasminogen activator in North America). North American studies of fibrinolyic agents have utilized recombinant tPA, and, similar to urokinase, evidence suggests that it substantially improves pleural drainage. Early observational studies suggested that early administration (at the time of chest drain insertion) and frequent administration of tPA was associated with shortened hospital stays,[29] similar efficacy to urokinase,[30] and safety.[29,30] A randomized controlled trial of 36 children comparing once daily tPA x 3 doses (4 mg in 40 mL normal saline at the time of insertion and then at 24 and 48 hours) with VATS found similar hospital stays (6 vs. 6 days) and lower overall hospital charges in the tPA group. [31]

Safety data on fibrinolytics suggests no serious adverse events aside from intrapleural bleeding.[29,30] Most bleeding is mild and clinically insignificant (i.e. does not lead to a drop in hemoglobin). It is also difficult to differentiate the etiology of cases of clinically significant bleeding between the surgical procedure of insertion of the drain and the drug itself. A recent review of the data at the Hospital for Sick Children suggests a prevalence of 2% for clinically significant bleeding (leading to a drop in hemoglobin) from chest drain insertion and/or fibrinolytic instillation (unpublished data). Current guidelines support the instillation of tPA as standard of care for pleural empyema being treated with a drain.[8,10-12]

DEOXYRIBONUCLEASE (DNase)

Despite the utilization of fibrinolytics, pleural drainage can still be challenging leading to prolonged hospital stays with a painful chest tube *in situ* and 'treatment failure' whereby pleural disease persists that cannot be drained necessitating salvage procedures such as open thoracotomy. The need for subsequent procedures is somewhat centre dependent, and, in clinical trials of tPA in childhood empyema, ranges from 17%[31] to 38%,[32] but such procedures are associated with significant morbidity including pain and a variety of potential surgical complications. One potential explanation for both the inadequate drainage leading to prolonged hospital stays and the phenomenon of 'treatment failure' is the presence of extracellular uncoiled DNA liberated from dead leukocytes and other bacterial components. Such residual material may increase viscosity, permit biofilm formation, and thus interfere with drainage.[33-36] Fibrinolytic agents are not thought to reduce pleural viscosity, but recombinant human DNase has been shown *in vitro* to help decrease the viscosity by cleaving free DNA and hence liquefying parapneumonic pus.[37,38] Subsequent animal studies have demonstrated that

the combined effects of tPA and DNAse in a rabbit model of empyema is more effective than either agent alone.[39] Small human (adult) case series have also described benefit from the addition of DNase to the treatment of empyema.[40,41]

Safety data on DNase is primarily derived from its currently licensed indication, nebulization at a dose of 2.5 to 5 mg once or twice daily for the reduction of sputum viscosity in patients with cystic fibrosis. It is well tolerated with rash, voice alteration, chest pain and laryngitis as common side effects.[42]

***DNase RCT in adults*:** A pivotal randomized controlled trial led by Rahman et al. from the UKCRC Oxford Respiratory Trials Unit was recently published in the New England Journal of Medicine addressing the effectiveness of DNase in adult patients.[42] The authors conducted a blinded 2-by-2 factorial trial in which 210 adult patients with pleural infections were randomly assigned to four study treatments for 3 days: double placebo, intrapleural tPA and DNase, tPA and placebo, or DNase and placebo. The primary outcome was the change in pleural opacity, measured as the percentage of the hemithorax occupied by effusion on chest radiography on day 7 compared with day 1. Secondary outcomes included referral for surgery, duration of hospital stay and adverse events. For the primary outcome, the authors found a significantly greater reduction in pleural opacity in the tPA-DNase group compared with the placebo group (-29.5±23.3% vs. -17.2±19.6%; mean difference -7.9%; 95% confidence interval (CI), -13.4 to -2.4; p=.005). The change in pleural opacity observed with tPA alone or DNase alone was not statistically different from that observed with placebo. All secondary outcomes also pointed to superiority of tPA-DNase compared with other study arms. Hospital stay for the tPA-DNase group (11.8±9.4 days) was about 50% shorter compared with placebo (24.8±56.1 days), whereas in the DNase-only group and the tPA-only group, length of stay was similar to placebo. The frequency of surgical referral at 3 months was lower in the tPA-DNase group compared with placebo (4% vs. 16%; odds ratio for surgical referral, 0.17: 95% CI, 0.03 to 0.87, p=.03). Surgical referrals were increased in the DNase-only group (odds ratio, 3.56; 95% CI, 1.30-9.75, p=.01), and were non-significantly reduced in the tPA-only group (odds ratio, 0.29; 95% CI, 0.07 to 1.29, p=.1). Mortality rates were 8% at 3 months and 12% at 12 months, but were similar across all study groups. Inflammatory measures (C-reactive protein, systemic white blood cell count and odds of fever) were also assessed. Significant (p<.05) differences were found in mean white count on day 7 and fever on day 6 or 7 between tPA-DNase and placebo. The new treatment was not associated with any excess of adverse events. Serious adverse events described included intrapleural hemorrhage (n=2, both in the tPA-DNase group), gastrointestinal bleeding (n=2, both in the DNase group), hemoptysis (n=1, in the tPA-DNase group, and clinical deterioration (n=1, in the placebo group).

The findings of this trial can best be explained by the synergistic effects of tPA and DNase. The fibrinolytic effects of tPA may disrupt fibrinous septations that would otherwise divide the infected collection, allowing DNase to liquefy the thick pus. Similarly, the thinning of pleural fluid by DNase may provide more effective access for tPA to pleural septations.

In summary, a large well-designed trial in adult patients showed that the combination of tPA and DNase therapy improves the drainage of pleural fluid in patients with pleural infection, reduces hospital stay and the need for thoracic surgery. Data for children are lacking at the present time.

***Extrapolating from Adult DNase trial to the management of children*:** The aforementioned review of pediatric pleural empyema suggests that current therapeutic options are still suboptimal. Short-term morbidity persists associated with prolonged hospitalization and pain/discomfort from chest tubes, as well as the need for secondary salvage surgical procedures with potential complications. There may be an additional benefit from the addition of intrapleural DNase to tPA instillation into chest drains. However, extrapolating from adult studies in pleural empyema to children is problematic as pediatric pleural empyema is in many ways a different disease for the following reasons:

(a) Mortality rates differ substantially. The mortality rate in adults is thought to be 10-20%,[43 44-46] and comorbidities are common. Most children who develop pleural empyema are otherwise healthy, and mortality is extremely rare in those treated with a drainage procedure. No mortality has been described in pediatric empyema RCTs.[23,24]
(b) Epidemiologic trends showing a rising incidence in empyema have been primarily demonstrated in children. Although microbiologic confirmation is often elusive, this does suggest that the microbial etiology of pediatric empyema (e.g. pneumococcal serotypes not covered by conventional vaccines) may differ from adult patients.
(c) Therapies that have been found to be ineffective in adult patients have been effective in children. For example, intrapleural streptokinase was not found to be beneficial in an adult trial (MIST1 trial),[46] but was found to be useful for some children in a pediatric trial,[47] and in a case series.[48]

Therefore, although there is a biological rationale and clinical efficacy data from adults, currently it is not known whether the addition of DNase to tPA will provide improvement in outcomes for pediatric pleural empyema.

SUMMARY OF THE EVIDENCE TO DATE FOR PEDIATRIC PLEURAL EMPYEMA

Pleural empyema is a rising problem in children and leads to significant short-term morbidity. Rigorous evidence with pediatric data is essential to inform clinical decision-making. The proposed trial brings clinical experts with a strong track record in outcomes research in pediatric empyema together with methodological experts in clinical trials in an effort to improve the outcomes of children admitted to hospital with pleural empyema.

Although there is still some ongoing controversy, the best evidence from randomized controlled trials seems to suggest that either VATS or small bore percutaneous chest tube placement with instillation of fibrinolytics result in the best outcomes in pediatric pleural empyema as measured by hospital length of stay, although chest tube with fibrinolytic therapy may be more cost-effective. Fibrinolytics are a safe and effective therapy based on observational and randomized control trial evidence. However, persistent short-term morbidity associated with prolonged hospitalization and pain/discomfort from chest tubes, as well as the need for secondary salvage surgical procedures. Based on a well-designed study in adults, there may be an additional benefit from the addition of intrapleural DNase to tPA, but this requires study in a pediatric population as the underlying disease (e.g. microbiology and natural history), and other, age-related biological factors may potentially affect response to treatment. This randomized controlled trial is designed to address the efficacy, cost effectiveness and safety of DNase combined with tPA compared with tPA alone in the management of childhood pleural empyema.

STUDY AIMS

Over-arching aim: The overarching aim of this research collaborative is to improve outcomes for children with pleural empyema.

Primary Research Question: In previously well children who present with pleural empyema, does intrapleural DNase with tissue plasminogen activator (tPA) via chest drain for three doses over 48 hours improve the time to hospital discharge compared with three doses over 48 hours of tPA alone ?

Primary Hypothesis: Three doses of DNase-tPA, when compared to three doses of tPA via chest drain will reduce the time to discharge after chest drain insertion in children hospitalized with pleural empyema.

Secondary Research Questions: Will there be differences between the groups with respect to other outcomes related to efficacy, cost and safety? The following outcomes will be assessed:

a) Time to meeting discharge criteria.
b) Time to drain removal.

c) Duration of fever, defined as the number of days with temperature >38ºC.
d) Need for ventilator support or non-invasive ventilation following intervention.
e) Risk of serious bleed, defined as intrapleural bleeding resulting in a drop in hemoglobin of greater than 20 g/L or needing a transfusion.
f) Need for further interventions, defined as the need for further intervention such as placement of another chest drain (by any technique) or surgical intervention such as thoracotomy and decortication, video-assisted thorascopic surgery, or pneumonectomy.
g) Hospital readmission related to pleural empyema or its treatment within three months of discharge.
h) Cost of the hospitalization.
i) Mortality.
j) Amount of effusion present at the time of drain removal (exploratory outcome).

Secondary Hypotheses: DNase-tPA, when compared to tPA via chest drain, will:

a) Reduce the time of fever, time to meeting discharge criteria, time to drain removal and cost.
b) Not result in an increase in need for ventilator support or non-invasive ventilation following intervention, nor an increase in risk of serious bleed, nor any need for further intervention, nor the risk of hospital readmission, nor the risk of mortality.

TRIAL DESIGN (see Appendix 1 for trial schematic)

Design: The study design will be a superiority randomized controlled trial in a parallel group, 1:1, two arm design.

Pragmatic-Explanatory Continuum: The primary purpose of this trial is to inform clinical decision making. Therefore, according to the framework described by co-investigator Kevin Thorpe and other trial methodologists, this trial has been designed along the pragmatic end of the pragmatic-explanatory continuum.[49] Specifically, most study domains [eligibility criteria, follow-up intensity, primary outcome and primary analysis] will follow pragmatic approaches ("Does this intervention work under usual conditions?"); practitioner expertise and adherence and the flexibility of the interventions will follow approaches midway along the pragmatic-explanatory continuum ("Can this intervention work under ideal conditions?"). This protocol has been designed following the 2010 SPIRIT guidelines (Standard Protocol Items for Randomized Trials) [50] and results will be reported according to the 2008 CONSORT guidelines for pragmatic trials.[51]

METHODS

Study Setting: This study will occur at major tertiary care children's hospitals These sites were specifically chosen due to their size (they are among the largest children's hospitals in Canada), relatively close proximity, and interest in collaborative work in improving outcomes in pleural empyema. These centres also preferentially use chest drain with fibrinolytics (as opposed to VATS) as their first line treatment of choice for pleural empyema in children. Furthermore, in allcentres, most chest drain insertion is performed using an image guided percutaneous technique performed by interventional radiologists and have similar antibiotic prescribing patterns. Lastly, all centres are also implementing clinical pathways to standardize the management of this condition.

Eligibility - Inclusion Criteria:

1. age 6 months to 18 years
2. hospitalized with diagnosis of pleural empyema requiring chest tube drainage with fibrinolytics as judged by the attending physician with the following criteria:

(a) pneumonia with pleural effusion as documented on ultrasound of the chest; AND
(b) need for further intervention in addition to antibiotics based on clinical criteria [(persistent fever despite on antibiotics for at least 48 hours OR significant respiratory distress tachypnea, hypoxia) as a result of the pleural fluid collection]

Eligibility - Exclusion Criteria:
1. empyema as a result of tuberculosis, fungus or non-infectious causes (e.g. malignancy)
2. known coagulation impairment
3. suspected or proven allergy to tPA or DNase
4. chronic lung disease or other chronic illnesses (e.g. immunodeficiency or neurologic impairment)
5. child has already undergone a drainage procedure (e.g. chest drain or VATS).
6. recent administration of an investigational drug (within previous 30 days)
7. pregnancy
8. breastfeeding

Interventions: Patients will be randomized to receive either: (a) tPA [alteplase (Cathflo®), Roche] at a dose of 4 mg followed by a placebo (saline) administered intrapleural via chest drain once daily for 3 doses; or, (b) tPA at a dose of 4 mg followed by DNase [dornase alfa (Pulmozyme®), Roche] at a dose of 5 mg. Weight-based dosing will not be used for tPA or DNase, as pleural concentrations are unpredictable and variable. Pediatric studies of tPA[29 31] and guidelines[21] have utilized adult dosing. DNase will be constituted by the research pharmacies in clear liquids in a polyethylene syringe [42]. Since the stability of a tPA-DNase admixture is unknown, the drugs will be administered sequentially with a 1 hour indwelling time for each drug as described in the aforementioned adult trial. For tPA, the contents of the vial will be diluted to a total volume of 10 ml normal saline (tPA) for children less than or equal to 10 kg and 20 ml for children > 10 kg. A flush of 5 ml of normal saline will be instilled after drug administration. Following the instillation of tPA the drain will be clamped for one hour and then will be opened to drain under suction at a pressure of -20 cm H_2O for one hour. Then, either DNase or placebo will be instilled as a volume to 10 ml with normal saline for children less than or equal to 10 kg and 20 ml for children > 10 kg, followed by the same 5 ml normal saline flush, the same one hour period of clamping and the same one hour period of drainage at -20 cm H_2O. Thus in total, each cycle of tPA-study drug instillation and drainage will take 4 hours to complete. The pharmacies will prepare the two arms (DNase or placebo) in a manner such that both are identical (packaging, colour, volume, texture, and odour) to ensure blinding. The contents of the vials (tPA followed by DNase or placebo) will be instilled into the chest drain by clinicians caring for the child. The first dose of tPA will be given immediately (within 1 hour) after insertion of the chest drain by the interventional radiologist or surgeon in the procedure suite or by clinicians on the ward and DNase or placebo will be instilled on the ward. On the following day (day 1) and the subsequent day (day 2) a dose of tPA followed by the study drug will be administered in the morning, between 9 and 10 am, in a similar fashion. Thus, each patient will receive a total of three doses of either tPA or tPA-DNase over 48 hours.

Clinical Samples at the Hospital for Sick Children: At the SickKids site only, consent will be obtained to store samples of chest tube drainage from subjects. These samples will be used for future testing to determine the microbiological cause of empyema.

Concomitant Medication: Patients in either arm will not receive any intrapleural drug other than directed from this study.

Criteria for discontinuing study interventions: Patients who develop anaphylaxis or serious bleeding (requiring a blood transfusion or resulting in a hemoglobin drop of $\geq$ 20 g/L) while receiving study interventions will not receive further study drugs. Patients whose chest drains is displaced will only be replaced if clinically indicated for pleural fluid drainage and not for study drug administration alone. Outcomes for all patients who do not received the full study interventions will be analyzed in the group they were assigned to.

Standard care: In keeping with a pragmatic-type trial design, all children will receive standard care for this condition including supportive care, laboratory investigations, imaging, antibiotics, chest drain care and removal and discharge as outlined in the standard care guidelines. Although some variability is expected in care among treating physicians, rigorous randomization and blinding should ensure that confounders are equally distributed between groups. A detailed care map will be adapted from the existing clinical practice guideline co-authored by team investigators (EC and SM) together with the Canadian Pediatric Society. Specific elements in the care plan include:

a) Antimicrobial management - Patients in both treatment arms will receive standard therapy for pleural empyema in children as directed by the responsible physician and standardized guidelines. All participating hospitals currently recommend a 2nd or 3rd generation cephalosporin (cefuroxime or ceftriaxone or cefotaxime) as the empiric antimicrobial therapy with possible addition of clindamycin or vancomycin if *S. Aureus* is a suspected etiology. It is expected that antibiotic regimens will be tailored in line with local microbiology advice.

b) Drain insertion - Chest drains will be placed using an image guided percutaneous technique by interventional radiologists or by a surgeon using a standardized technique under anesthesia agreed upon by all sites. The recommended size of catheter drain used will be an 8 or 10 French pigtail catheter.

c) Drain management - Chest drain management will be at the discretion of treating physicians. Suggested management in the care pathway will include maintaining -20 cm H_2O of continuous suction and once daily flushes with 10 ml of normal saline (on days where no study drug is administered) to maintain patency.

d) Criteria for drainage removal - Similarly, although recommendations for drainage removal will be made in the care map (< 1ml/kg/d), ultimately the decision to remove the chest drain will be made at the discretion of the responsible physician likely based on a gestalt of clinical parameters (amount of drainage, appearance of the child, fever, etc.). All patients will likely be initially on a similar regime of antibiotics and then tailored based on microbiologic results.

e) *Diagnostic imaging* - There is no consensus on the type (ultrasound versus plain radiograph) or frequency of diagnostic imaging necessary after drain insertion in hospitalized children. Therefore, in contrast to the aforementioned adult RCT of DNase, follow-up radiographs will only be assessed as an exploratory outcome in the study protocol.

Outcomes - Primary outcome: *Time to hospital discharge*: This outcome is the most common outcome measure in trials of childhood empyema. This is defined as the time, measured in hours, and reported in days rounded to a single decimal point, from insertion of the chest drain to discharge from hospital. This will be assessed by a research assistant on a twice daily basis.

Outcomes - Secondary outcomes: Secondary outcomes include measures of effectiveness, harm and cost-effectiveness. Formal clinical follow-up and review of other parameters such as radiographic resolution and lung function testing will not be performed as preliminary work from this group of investigators and others suggests that long-term outcomes in childhood pleural empyema are almost universally positive. Our prospective cohort study has found that by six months, virtually all patients are asymptomatic and follow-up radiographs have normalized in 62/65 (95%) and pulmonary function tests normalize in 27/28 (96%).[52]

a. *Time to meeting discharge criteria*: This is defined as the time, measured in hours, and reported in days rounded to a single decimal point, from insertion of the chest drain to meeting discharge criteria. This will be assessed by a research assistant on a twice daily basis (9 AM and 4 PM) and defined as: no fever (temperature less than 38°C) for 24 hours, normal respiratory rate for age [using the World Health Organization (WHO) age-specific criteria (< 50 breaths/min for 2-12 months, < 40 breaths/min

for 1 to 5 years, and < 20 breaths/min for ≥ 5 years)], no hypoxia (transcutaneous oxygen saturations in room air less than 92%), and drinking fluids well.
b. *Time to drain removal*: This is defined as the time, measured in hours, and reported in days rounded to a single decimal point, from drain insertion to drain removal.
c. *Duration of fever after intervention*: This is defined as the duration, measured in hours, and reported in days rounded to a single decimal point, of fever (defined as temperature >38ºC taken by any method) from insertion of the chest drain until resolution. This will be recorded by a research assistant daily.
d. *Need for ventilatory support or non-invasive ventilation following the intervention.*
e. *Serious bleeding*: This is defined as intrapleural bleeding resulting in a drop in hemoglobin of greater than 20 g/L or needing a transfusion.
f. *Need for further interventions*: This is defined as the need for further intervention such as placement of another chest drain (by any technique) or surgical intervention such as thoracotomy and decortication, video-assisted thorascopic surgery, or pneumonectomy.
g. *Hospital readmission*: Any hospital readmission after discharge from hospital for initial treatment for pleural empyema within three months related to pleural empyema or its treatment (e.g. harm). All hospital readmissions will be categorized as empyema-related or not.
h. *Cost of the hospitalization*: An economic evaluation will compare the relative costs of DNase-tPA with tPA alone in previously well children who present with pleural empyema, using patient-level data from the trial. We will conduct the analysis from a hospital's perspective because hospital administrators will be making reimbursement decisions for this intervention. Since empyema is an acute condition and the long-term consequences are negligible, the time horizon of the analysis will be the length of hospital stay. Since the primary effectiveness outcome is length of hospital stay or time to hospital discharge, which can be expressed in monetary terms (i.e. hospitalization cost), we will only compare the costs of the two strategies to avoid double-counting the effectiveness. The cost of each patient includes the cost of intervention (DNase-tPA or tPA alone) and costs incurred during the hospital stay ("hospitalization cost"). We will obtain the hospitalization costs of each patient from case costing data from hospital financial departments. We will calculate mean cost per patient by intervention group, based on initial intervention assignment, and incremental cost using simple linear regression:

$$C_i = \alpha_i + \beta t_i + \varepsilon_i$$

Where C_i is the cost for each patient i, α is the intercept term, t is an intervention dummy term (t=1 if the patient received DNase-tPA and t=0 for tPA alone), and ε is the stochastic error term. The regression coefficient β estimates the incremental cost of DNase-tPA compared with tPA alone. The regression statistics will show mean cost per patient by intervention group and the uncertainty around the mean estimates. We will also conduct sensitivity analysis to assess the robustness of the results.
i. *Mortality*: this will include mortality from any cause during the hospitalization for empyema.

Exploratory outcome – chest radiography: In the aforementioned DNase trial in adults, changes in pleural opacity, measured as the percent of the hemithorax occupied by effusion on day 7 compared with day 1 was a primary outcome. In pediatric empyema, this outcome is problematic because: (a) many children will be discharged prior to day 7; (b) hemithorax size differs substantially across different sized children; and, (c) requiring an additional chest radiograph may be a substantial disincentive for parental caregiver to provide consent participate particularly given the rising concerns of ionizing radiation in developing children. Further, radiographic changes are considered surrogate measures of clinical changes as they often lag behind clinical improvement. There is currently no standard as to the timing of radiographs in hospital, but it is our experience that virtually all children will have a radiograph performed shortly before and/or shortly after chest tube removal. The

radiograph closest to the time of drain removal will be reviewed by a blinded study radiologist (BC) to determine the percentage of hemithorax occupied using a 5 point ordinal scale utilized in previous studies ranging from no fluid present to fluid occupying >75% of the most affected hemithorax. Given that radiographic improvement is time dependent, we do not have any *a priori* hypotheses about the results of this outcome across groups (i.e. those with the worst outcomes (chest drain duration) may potentially have better radiographs at the time of drain removal).

Sample size: The primary endpoint is time to hospital discharge after intervention. The mean time to hospital discharge after chest tube insertion and fibrinolytics varies. Published studies with treatment arms that have included chest drain with fibrinolytics as the primary mode of insertion reported hospital days after the intervention from 6 to 15 days.[23,24] Hospital length of stay tends to be shorter in published RCTs compared with observational studies of empyema, and the most recently published RCT using an identical tPA dosing regimen to that which we are proposing described a mean (SD) stay of 6.8 (2.9) days. [31] The desired power for the current proposal is 90% to detect a difference of 2 days in the mean time to hospital discharge between the treatment arms. Based on discussions with clinical experts in the field, hospital administrators and parents of children with pleural empyema, it is believed that a 2 day difference between treatment groups is a minimal clinically meaningful difference, and this has been used in previous empyema trials. [27] The adult DNase trial found about a 50% difference in length of stay between treatment and placebo groups. [42]

Sample size was thus calculated assuming a type 1 error rate of 0.05 (2 sided), power (1-ß) of 90%, a standard deviation of 2.9 days for each group to detect a difference of 2 days to be 46 per group. Calculations were performed based on a t test of independent groups. While we do not anticipate any post-randomization ineligibility, for example from misdiagnosis, we will increase the sample size by 5% to account for this possibility (total of 49 patients per group). There will be no other adjustment to the sample size requirements due to loss to follow-up for the primary outcome as the primary outcome is assessed in hospital where the research coordinator will be able to ensure complete data collection.

Recruitment: All study patients will be initially identified by research assistants who will review both (a) all new admissions to the relevant inpatient units in each study centre twice daily; and, (b) all referrals for chest drainage insertion at each centre. It is anticipated that most drains at both sites will be inserted by interventional radiologists, although a minority may be inserted by general surgeons. Physicians and radiologists/surgeons will also be asked to notify the site research assistant and/or site investigator of children admitted with pleural empyema who may be eligible for the study. Patients will be approached, eligibility criteria confirmed, consent obtained, and enrolled after the decision to refer for chest drainage but before chest drain insertion occurs.

Randomization – Sequence generation, allocation concealment and implementation: After informed consent is obtained, patients will be randomized into treatment groups using a random allocation sequence facilitated by the Applied Health Research Centre (AHRC), which will be the coordination and management centre for the trial and administered by the hospital research pharmacies. Randomization will be stratified by centre. Blocking will be used to ensure that the two comparison groups are about the same size throughout the trial for each center and for the trial as a whole (about 49 per group). An allocation ratio of 1:1 with random block size will be used within each stratum (centre). This will help to ensure that clinicians, investigators, or outcome assessors will not decipher the block size. A computer-based pseudo-random number generator will be used to create treatment allocation tables for each study center. After patient eligibility has been confirmed and consent obtained and just as the patient leaves the inpatient unit to the radiology suite or operating room for chest drain insertion, the site investigator, or his/her delegate, will assign the patient a unique study identification (ID)

number in sequential order. The study ID will correspond with the randomization table held in the research pharmacy for dispensing blinded DNase or placebo. The biostatistician will maintain a secure master list of the randomization codes and the assigned treatments will be checked against the master list at the end of the study.

Blinding: Patients, their parents or other caregivers, site investigators, research assistants and coordinators, treating physicians (pediatricians, radiologists, and interventional radiologists or surgeons), treating nurses, and data managers will be blinded to the treatment allocation. Group allocation will be concealed until the final data analysis is performed. Intervention drugs will be blinded by the research pharmacy. Both arms will be constituted by the research pharmacies in clear liquids in a polyethylene container [42] in a manner such that both are identical (packaging, colour, volume, texture, and odour) to ensure blinding. After obtaining the treatment number from the central randomization centre, the study pharmacist will retrieve the corresponding vial and one of its treatment number labels will be attached to the patient's case report form (CRF).

Unblinding Procedures: We do not anticipate any circumstances that would require unblinding as knowledge of study arm is not anticipated to affect any treatments for patients.

Data collection methods: Data collection for outcome measures will be mainly collected in hospital by the Research Assistant (RA). At baseline, the following data will be obtained: age, sex, duration of antibiotic treatment prior to chest drainage insertion (days), duration of fever prior to chest drain insertion (days), hypoxia in room air (oxygen saturations less than 92% in room air prior to intervention), microbiologic identification of causative agent (blood culture results, throat swab, pleural fluid culture, pleural fluid PCR), ultrasound of chest: pleural effusion size >10 mm <10 mm, stage of empyema on ultrasound of chest: Stage 1: anechoic nonseptated fluid; Stage 2: echoic fluid without septation; and Stage 3: septated fluid and Stage 4: septations with solid appearing components comprising more than one third of the effusion.[53] All ultrasounds will be reviewed by a blinded study radiologist (BC). In addition, patients will receive a follow-up phone call from the RA at 3 months enquiring about any possible readmissions and ongoing symptoms of fever, shortness of breath and/or exercise intolerance.

Data management: The Applied Health Research Centre (AHRC) of the Li Ka Shing Knowledge Institute of St. Michael's Hospital will serve as the data management centre under the direction of co-investigator Muhammad Mamdani. AHRC employs state-of-the-art web based data management software, Medidata RAVE™ (5.6.3), a secure encrypted web based clinical trial data management system which is fully configurable and incorporates sophisticated data validation rules to ensure high quality data capture. RAVE™ allows for remote web-based data entry directly from the hospital sites, facilitating real time data access.

Statistical Analysis:

Baseline characteristics: Patient characteristics and descriptive variables will be presented for each treatment arm: age, sex, duration of antibiotic treatment prior to chest drain insertion (days), duration of fever prior to chest drain insertion (days), hypoxia, defined as saturations in room air less than 92% (yes/no), bacterial identification and subtype, pleural effusion size on ultrasound (>10 mm, <10mm), stage of empyema on ultrasound (frequency). For continuous variables, means and standard deviations (SD) or medians (inter-quartile ranges) will be presented. For categorical variables, proportions will be presented.

Primary outcome: Data will be analyzed according to intention to treat principles for the primary outcome (e.g. patients who do not receive all three doses of study drug will be analyzed in the group they were assigned to). Exceptions to this principle will only include any patient who dies in hospital and will be excluded from the analysis of time to hospital discharge. Given that the primary outcome and other acute secondary outcomes are obtained during hospitalization, it is anticipated that there will be no missing data for these outcomes with the possible, but unlikely exception of in-hospital death, which is rare in childhood empyema. For the follow up outcomes at 3 months, the proportion of patients who follow up will be presented for each treatment arm, and patients who are lost to follow up will be stated but omitted from the analysis. The primary outcome, time (in days) to hospital discharge, will be described as the difference between the two means with the 95% confidence intervals. The student's t-test (independent two sample test assuming equal group size and variance), will be used to detect a difference between the two treatment groups. If the resulting data is inappropriate for a t-test (increasing variance with the mean is the biggest concern since the test is quite robust to non-normality in the population), a suitable transformation or non-parametric test may be used. If feasible, a secondary analysis of the primary outcome will analyze time to discharge, treating death as a competing risk, using methods for survival data. Since randomization is stratified between five centres, the analysis will adjust for this stratification by including centre as a covariate in a multiple linear regression model, to obtain adjusted treatment effects. Additionally, a treatment by centre interaction will be tested to see if the treatment effect differs between centres. Subgroup analyses will also be conducted to explore any potential differences in outcomes by age or sex.

Secondary outcomes and exploratory outcome: For the secondary and exploratory outcomes that are continuous variables (e.g. duration of drain insertion, hospital stay after intervention to meeting discharge criteria, duration of fever after the intervention, amount of hemithorax occupied by pleural effusion in radiographs) the difference between the two means with the 95% confidence intervals will be presented. The student's t test will be used to detect a difference between the treatment groups. Dichotomous outcomes (serious bleeding, need for further interventions, need for ventilatory support, mortality) will be described as the absolute number and proportion. A Fisher's exact test will be used to detect a significant ($p<0.05$) difference between the two treatment arms. The treatment effect will also be presented as the relative risk with 95% confidence intervals. These analyses will be viewed as hypothesis generating, and therefore, no correction for multiple testing is planned.

Data Monitoring and Safety: Data monitoring will be conducted with a Data Monitoring Committee (DMC) composed of a pediatric hospitalist, a respiratory physician and an interventional radiologist. The DMC will be completely independent of the investigators, and will be provided with clinical information from the trial case report forms for death, surgical events and adverse events, including those that were described in the adult DNase trial (hemoptysis, gastrointestinal bleeding, chest pain, nausea, transient confusion and rash). The DMC will be able to request additional information from clinicians.

Adverse Event Reporting: All serious unexpected adverse events will be reported to the REB; as per SickKids REB guidelines. All serious adverse drug reactions to the study medication will be reported to Health Canada within 15 calendar days or for death or life-threatening events, within 7 calendar days. In the latter case, a follow-up report will be filed within 8 calendar days. Adverse reactions will be managed according to the standard clinical management practices. All adverse events and adverse reactions will also be reported to the PI within 24 hours.

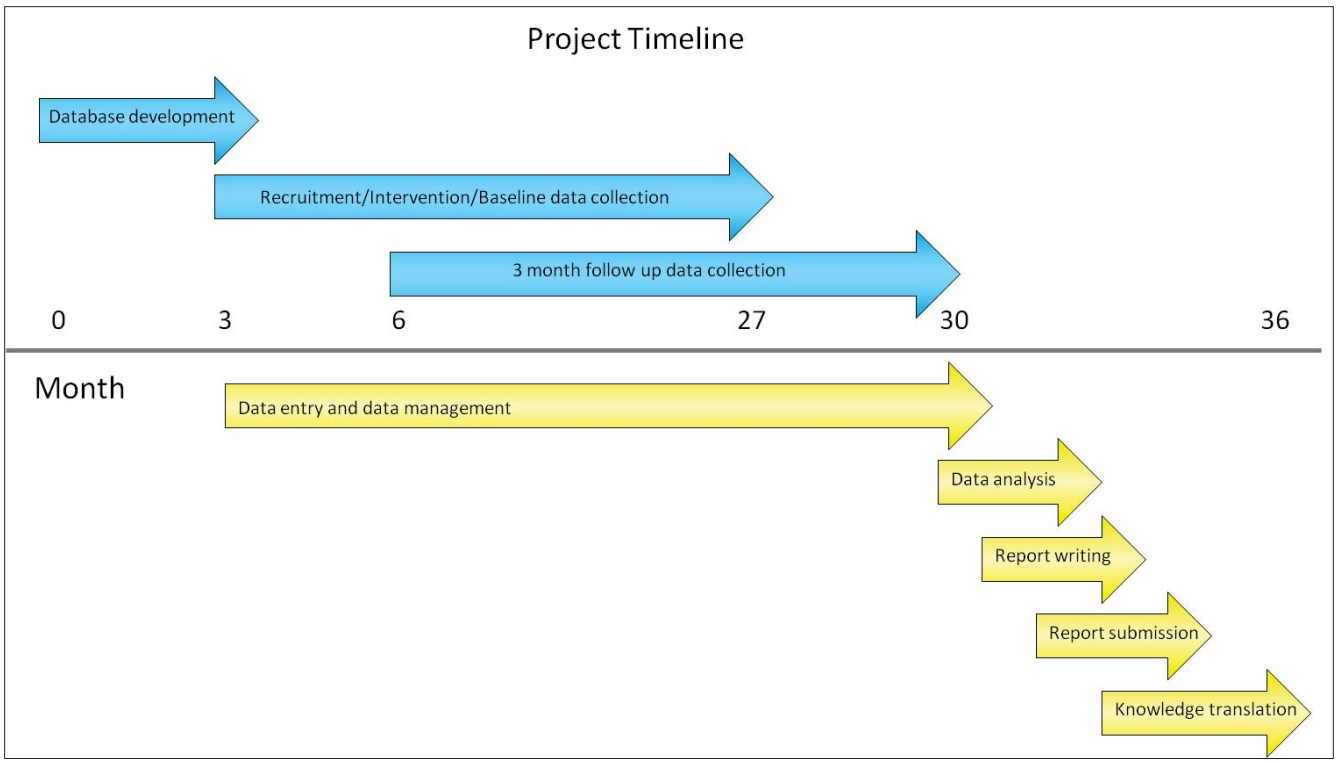
Quality Assurance: This project will be monitored by The Applied Health Research Centre (AHRC) during the data collection phase of the project. The aim is to ensure that all researchers are maintaining

the highest ethical, scientific and safety standards for all study participants, and are in compliance with all relevant policies, provincial and federal legislation, and international guidelines such as ICH- Good Clinical Practice. All studies are categorized according to the Continuing Review Matrix based on the type of study and the level of risk (I to IV) to research subjects and the Clinical Research Monitor will review at minimum 10% of the research subjects' records for study eligibility, informed consent, adherence to study protocol, reporting of adverse drug reactions and adverse events, and data quality including computer database security and storage of records. Findings of the will be presented to the Research Ethics Board and lead PI in a written report, and specific recommendations arising from the report will be implemented in a timely manner.

ETHICAL CONSIDERATIONS: Informed consent will be obtained from parental caregivers. Given the young age of children with empyema (average age of 4 years), it is anticipated that most will not be able to consent/assent to participate. Informed consent/assent will be obtained from all those who are able to provide it. Potential known adverse events from study interventions are mainly local as the drugs are not systemically absorbed and include intrapleural bleeding, rash, voice alteration, chest pain and laryngitis. Aside from receiving the study interventions, participation will require parents to agree to be contacted by research personnel for a short (< 5 min) phone call three months post-discharge.

Feasibility and Timelines: Internal data from decision support services at the three participating hospitals indicates that in fiscal year 2010-11, there were a total of 92 children with chest drains inserted for pleural empyema (25 at Sainte Justine, 33 at CHEO and 34 at SickKids), and, in 2009-10, 98 children received a chest tube (28 at Sainte Justine, 37 at CHEO and 33 at SickKids). Based on a review of the patients who had chest drains inserted at SickKids from 2008-2011, less than 5% of these will be ineligible for this trial (e.g. due to a comorbid condition such as cerebral palsy). Thus, it is anticipated that a total of 180 potentially eligible patients will be available for recruitment, at three of the five sites, over two years for this clinical trial.

Our previous experience with this population indicates a willingness to participate in research. In an observational study of long-term outcomes in pediatric empyema requiring participants to complete detailed follow-up questionnaires and attend an appointment for physical examination, spirometry and radiography for six months, 88/94 (94%) consented to participate, of whom 6 (7%) were lost to follow-up at one month and a further 8 (9%) at 6 months.



KNOWLEDGE TRANSLATION (KT) ACTIVITIES

KT activities will be facilitated by the leadership of team investigators locally, nationally and internationally. Locally, findings will be presented to clinical groups and incorporated into the empyema Clinical Practice Guidelines atall sites. Nationally, findings will be presented at the Canadian Pediatric Society's Annual Meeting focused on the Hospital Pediatrics Section whose current President is Dr. Mahant and whose President-elect is Dr. Cohen. Internationally, we will present findings at the Pediatric Academic Society's Annual Meeting, the largest international pediatric research meeting, facilitated by the Pediatric Research in Inpatient Settings (PRIS) group, an international hospitalist research and KT organization that is co-led by Dr. Mahant. Given the broad interest in the topic, we anticipate publication of findings in a high-impact general medical or pediatric journal.

IMPACT OF STUDY

Pleural empyema in children has been dramatically increasing in prevalence over the past 10 years and has important short-term morbidity. Unlike in adults, long-term outcome is very favorable and treatment interventions are aimed at reducing the duration of illness. Chest drainage with fibrinolytics has been shown to be an effective therapy in children but duration of illness is still long. This study will examine the effectiveness of adding DNase to see if it results in clinically meaningful improvement in outcomes. The study brings together a group of clinician-investigators with a track record of scholarly work focused on expanding the evidence base for managing this common condition together with researchers with expertise in clinical trials methodology. The results will answer an important clinical question and guide implementation of the most effective therapy for children with pleural empyema.

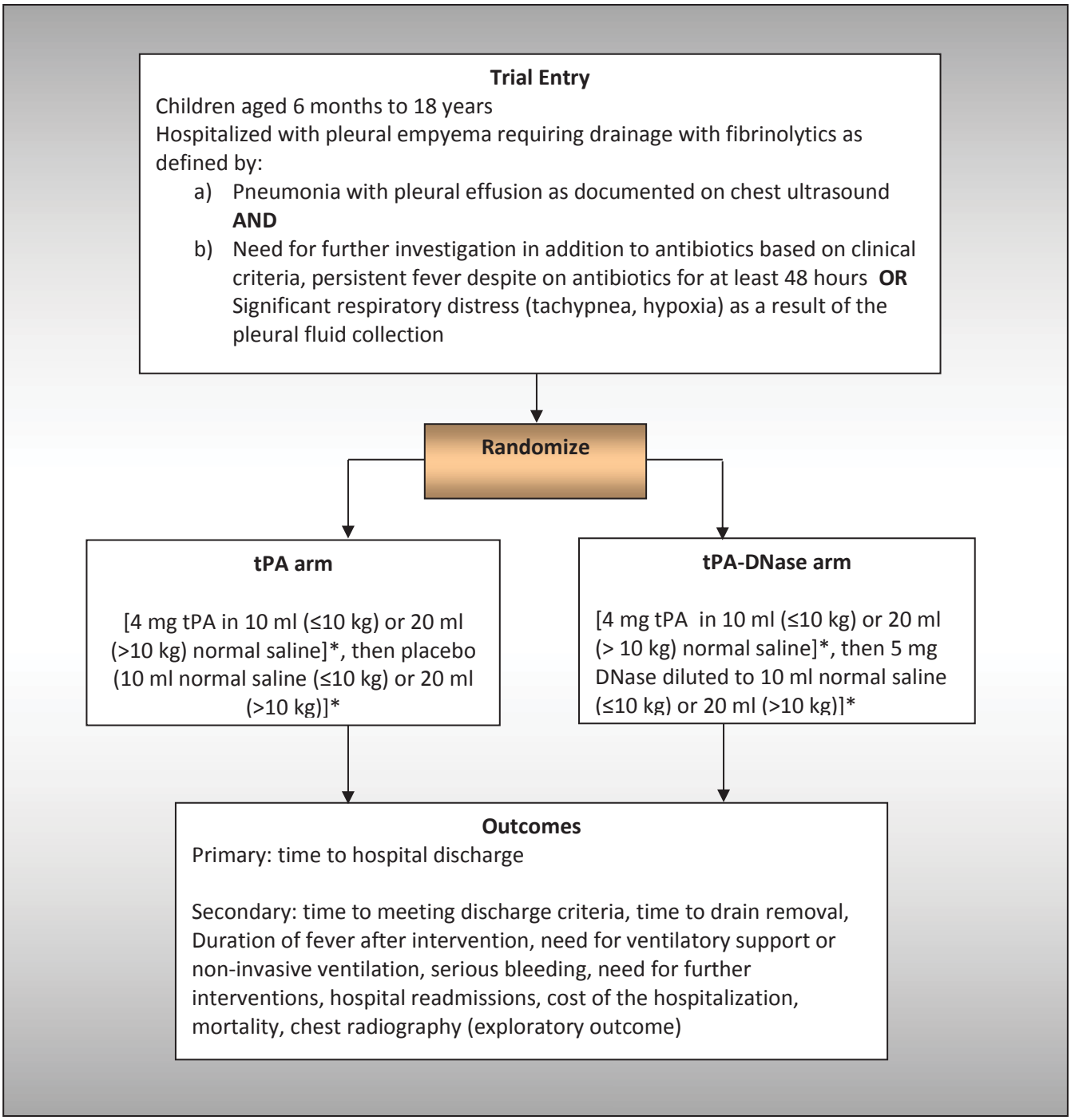
References

1. Rudan I, Tomaskovic L, Boschi-Pinto C, Campbell H. Global estimate of the incidence of clinical pneumonia among children under five years of age. Bulletin of the World Health Organization 2004;82:895-903.
2. Srivastava R, Keren R, Luan X, et al. A strategy for prioritizing pediatric inpatient comparative effectiveness research. E-PAS 2011; 41451 2011.
3. Lichenstein R, Suggs AH, Campbell J. Pediatric pneumonia. Emergency medicine clinics of North America 2003;21:437-51.
4. Obando Santaella I, Sanchez Tatay D, Mateos Wichmann I, Torronteras Santiago R, Leon Leal JA. [Increase in the incidence of parapneumonic pleural effusions]. An Pediatr (Barc) 2006;64:176-7.
5. Playfor SD, Smyth AR, Stewart RJ. Increase in incidence of childhood empyema. Thorax 1997;52:932.
6. Hendrickson DJ, Blumberg DA, Joad JP, Jhawar S, McDonald RJ. Five-fold increase in pediatric parapneumonic empyema since introduction of pneumococcal conjugate vaccine. Pediatr Infect Dis J 2008;27:1030-2.
7. Goldbart AD, Leibovitz E, Porat N, et al. Complicated community acquired pneumonia in children prior to the introduction of the pneumococcal conjugated vaccine. Scandinavian journal of infectious diseases 2009;41:182-7.
8. Rees JH, Spencer DA, Parikh D, Weller P. Increase in incidence of childhood empyema in West Midlands, UK. Lancet 1997;349:402.
9. Thompson A, Reid A, Shields M, Steen H, Taylor R. Increased incidence in childhood empyema thoracis in Northern Ireland. Ir Med J 1999;92:438.
10. Finley C, Clifton J, Fitzgerald JM, Yee J. Empyema: an increasing concern in Canada. Can Respir J 2008;15:85-9.
11. Li ST, Tancredi DJ. Empyema Hospitalizations Increased in US Children Despite Pneumococcal Conjugate Vaccine. Pediatrics 2009.
12. Spencer D, Iqbal S, Hasan A, Hamilton L. Empyema thoracis is still increasing in UK children. BMJ 2006;332:1333.
13. Bradley JS, Byington CL, Shah SS, et al. The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2011;53:e25-76.
14. Balfour-Lynn I, Abrahamson E, Cohen G, et al. BTS guidelines for the management of pleural infection in children. Thorax 2005;60(Supple 1):i1-21.
15. Cohen E, Uleryk E, Jasuja M, Parkin PC. An absence of pediatric randomized controlled trials in general medical journals, 1985-2004. Journal of clinical epidemiology 2007;60:118-23.
16. Cohen E, Goldman RD, Ragone A, et al. Child vs adult randomized controlled trials in specialist journals: a citation analysis of trends, 1985-2005. Archives of pediatrics & adolescent medicine 2010;164:283-8.
17. Caldwell PH, Murphy SB, Butow PN, Craig JC. Clinical trials in children. Lancet 2004;364:803-11.
18. Shah SS, Hall M, Goodman DM, et al. Off-label drug use in hospitalized children. Archives of pediatrics & adolescent medicine 2007;161:282-90.
19. Weinstein M. Management of parapneumonic effusions: current practice and controversies. Current Pediatric Reviews 2006;2:259-63.
20. N Le Saux JRCPS, Infectious Diseases and Immunization Committee. Pneumonia in healthy Canadian children and youth: Practice points for management. Paediatr Child Health 2011;16:417-20.
21. Chibuk TK, Cohen E, Robinson JL, Mahant S, Hartfield DS, Canadian Paediatric Society HPS. Paediatric complicated pneumonia: Diagnosis and management of empyema. Paediatr Child Health 2011;16:425-7.

22. Kokoska ER, Chen MK. Position paper on video-assisted thoracoscopic surgery as treatment of pediatric empyema. J Pediatr Surg 2009;44:289-93.
23. Avansino J, Goldman B, Sawin R, Flum D. Primary operative versus nonoperative therapy for pediatric empyema: a meta-analysis. Pediatrics 2005;115:1652-9.
24. Mahant S, Cohen E, Weinstein M, Wadhwa A. Video-assisted thorascopic surgery vs chest drain with fibrinolytics for the treatment of pleural empyema in children: a systematic review of randomized controlled trials. Archives of pediatrics & adolescent medicine 2010;164:201-3.
25. Cohen E, Weinstein M, Fisman D. What is the most cost-effective therapy for pediatric empyema? Pediatrics 2008;121:1250-7.
26. Laisaar T, Pullerits T. Effect of intrapleural streptokinase administration on antistreptokinase antibody level in patients with loculated pleural effusions. Chest 2003;123:432-5.
27. Thomson A, Hull J, Kumar M, Wallis C, Lynn IB. Randomised trial of intrapleural urokinase in the treatment of childhood empyema. Thorax 2001;57:343-7.
28. Sonnappa S, Cohen G, Owens C, et al. Comparison of urokinase and video-assisted thorascopic surgery for treatment of childhood empyema. Am J Resp Respir Crit Care Med 2006;174:221-7.
29. Weinstein M, Restrepo R, Chait PG, Connolly B, Temple M, Macarthur C. Effectiveness and safety of tissue plasminogen activator in the management of complicated parapneumonic effusions. Pediatrics 2004;113:e182-e5.
30. Wells RG, Havens PL. Intrapleural fibrinolysis for parapneumonic effusion and empyema in children. Radiology 2003;228:370-8.
31. St Peter SD, Tsao K, Harrison C, et al. Thoracoscopic decortication vs tube thoracostomy with fibrinolysis for empyema in children: a prospective, randomized trial. J Pediatr Surg 2009;44:106-11; discussion 11.
32. Kurt B, Winterhalter K, Connors R, Betz B, Winters J. Therapy of parapneumonica effusions in children: video-assisted thorascopic surgery versus conventional thoracostomy drainage. Pediatrics 2006;118:e547-53.
33. Hall-Stoodley L, Nistico L, Sambanthamoorthy K, et al. Characterization of biofilm matrix, degradation by DNase treatment and evidence of capsule downregulation in Streptococcus pneumoniae clinical isolates. BMC microbiology 2008;8:173.
34. Thomas VC, Thurlow LR, Boyle D, Hancock LE. Regulation of autolysis-dependent extracellular DNA release by Enterococcus faecalis extracellular proteases influences biofilm development. Journal of bacteriology 2008;190:5690-8.
35. Izano EA, Amarante MA, Kher WB, Kaplan JB. Differential roles of poly-N-acetylglucosamine surface polysaccharide and extracellular DNA in Staphylococcus aureus and Staphylococcus epidermidis biofilms. Applied and environmental microbiology 2008;74:470-6.
36. Eckhart L, Fischer H, Barken KB, Tolker-Nielsen T, Tschachler E. DNase1L2 suppresses biofilm formation by Pseudomonas aeruginosa and Staphylococcus aureus. The British journal of dermatology 2007;156:1342-5.
37. Light RW, Nguyen T, Mulligan ME, Sasse SA. The in vitro efficacy of varidase versus streptokinase or urokinase for liquefying thick purulent exudative material from loculated empyema. Lung 2000;178:13-8.
38. Simpson G, Roomes D, Heron M. Effects of streptokinase and deoxyribonuclease on viscosity of human surgical and empyema pus. Chest 2000;117:1728-33.
39. Zhu Z, Hawthorne ML, Guo Y, et al. Tissue plasminogen activator combined with human recombinant deoxyribonuclease is effective therapy for empyema in a rabbit model. Chest 2006;129:1577-83.
40. Simpson G, Roomes D, Reeves B. Successful treatment of empyema thoracis with human recombinant deoxyribonuclease. Thorax 2003;58:365-6.

41. Fujiwara K, Yasumitsu T, Nakagawa K, Shiono S, Fukuhara K. [Intrapleural streptokinase-streptodornase in the treatment of empyema and hemothorax]. Kyobu geka The Japanese journal of thoracic surgery 2002;55:1115-9.
42. Rahman NM, Maskell NA, West A, et al. Intrapleural use of tissue plasminogen activator and DNase in pleural infection. The New England journal of medicine 2011;365:518-26.
43. Farjah F, Symons RG, Krishnadasan B, Wood DE, Flum DR. Management of pleural space infections: a population-based analysis. The Journal of thoracic and cardiovascular surgery 2007;133:346-51.
44. Ferguson AD, Prescott RJ, Selkon JB, Watson D, Swinburn CR. The clinical course and management of thoracic empyema. QJM : monthly journal of the Association of Physicians 1996;89:285-9.
45. Davies CW, Kearney SE, Gleeson FV, Davies RJ. Predictors of outcome and long-term survival in patients with pleural infection. American journal of respiratory and critical care medicine 1999;160:1682-7.
46. Maskell NA, Davies CW, Nunn AJ, et al. U.K. Controlled trial of intrapleural streptokinase for pleural infection. The New England journal of medicine 2005;352:865-74.
47. Singh M, Mathew JL, Chandra S, Katariya S, Kumar L. Randomized controlled trial of intrapleural streptokinase in empyema thoracis in children. Acta paediatrica 2004;93:1443-5.
48. Rosen H, Nadkarni V, Theroux M, Padman R, Klein J. Intrapleural streptokinase as adjunctive treatment for persistent empyema in pediatric patients. Chest 1993;103:1190-3.
49. Thorpe KE, Zwarenstein M, Oxman AD, et al. A pragmatic-explanatory continuum indicator summary (PRECIS): a tool to help trial designers. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne 2009;180:E47-57.
50. Chan AW, Tetzlaff J, Altman dG, Gotzsche PC, Horobjartsson A. The Spirit Initiative: Defining
Standard Protocol Items for Randomized Trials. Enhancing the Quality and Transparency of Health Research. [http://www.equator-network.org/resource-centre/library-of-healthresearch-reporting/reporting-guidelines-under-development/reporting-guidelines-underdevelopment/. Accessed January 11, 2012. (Accessed at
51. Zwarenstein M, Treweek S, Gagnier JJ, et al. Improving the reporting of pragmatic trials: an extension of the CONSORT statement. BMJ 2008;337:a2390.
52. Cohen E, Weinstein M, Mahant S, et al. The natural history of complicated pneumonia: A prospective observational study. . E-PAS 2011;1645.5.
53. Jaffe A, Calder AD, Owens CM, Stanojevic S, Sonnappa S. Role of routine computed tomography in paediatric pleural empyema. Thorax 2008;63:897-902.

Appendix 1: Trial Schematic



* followed by 5 ml normal saline flush