# Outcome Prediction and Alternative Interventions in Pleural Infection

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## ALTERNATIVE FORMAT SUBMISSION

This thesis is presented as a Publication format thesis to allow incorporation of the published works. The introduction (Chapter 1) consists of a literature review adapted from a number of first author review articles and book chapters published during the period of my PhD registration. Chapters 2, 3 and 5 are a collection of fully published works in peer reviewed journals. Chapter 2 additionally contains a separate unpublished small study in Appendix A2.4. Chapter 4 is presented in traditional thesis format. Finally, Chapter 6 is a commentary linking the thesis chapters, outlining their coherence and significance, as well as introducing future work.

# THESIS ABSTRACT


## Introduction

Pleural infection is common with considerable healthcare burden, requiring prolonged antibiotics and multiple interventions comprising chest tube drainage, intrapleural therapy or thoracic surgery. Patient outcomes remain poor and current treatment pathways are insufficient. Using mixed methods, this thesis evaluates outcome prediction markers and the potential for redrawing the existing treatment pathway toward earlier escalation of therapy, understanding patient priorities, and assessing the complications of existing therapies.

## Methods

Analysis of prospectively collected biological pleural fluid samples with matched radiology and clinical outcome data from a large multicentre observational cohort study was conducted to explore radiological and biological outcome predictors. A multicentre randomised controlled trial was conducted to explore the feasibility of early use of combination intrapleural fibrinolytic and enzyme therapy (IET) or surgery. A qualitative study using semi-structured interviews was conducted to understand the participant experience in pleural infection trials and identify patient priorities. A retrospective analysis of a large IET treated cohort was performed to analyse bleeding complications.

## Results

Plasminogen activator inhibitor-1 (PAI-1) was identified as the first biological predictor of mortality in pleural infection. PAI-1 plays an important role in the development of sonographic septations, but their presence does not predict clinically important outcomes. The MIST-3 study demonstrated feasibility and patient acceptability of early randomisation to IET or surgery, with modifications to the study protocol required to improve compliance. MIST-3 and its qualitative sub-study highlighted important insights into trial design and patient centred outcomes. IET carries low incidence of bleeding complications and predictors of increased bleeding risk were identified.

## Conclusion

The treatment pathway in pleural infection has remained largely unaltered for almost two decades. This thesis has explored the potential role of radiological and biological outcome prediction to personalise therapy, and evaluated the potential for earlier intervention in the patient pathway improve outcomes relevant to patients and clinicians.

## COVID-19 STATEMENT

The SARS-CoV-2 pandemic occurred in March 2020, a month after successful completion of my Confirmation in February 2020. The pandemic had a significant impact on the originally planned workstreams as well as the studies themselves. The incidence of pleural infection, the disease representing the main focus of this thesis, saw a marked reduction in incidence and subsequent hospital admissions as discussed in the introduction chapter. Personally, it also resulted in me having to return to NHS service at the start of the pandemic to support front line respiratory services.

In brief, the workstream relating to radiological (chest radiograph and CT) biomarkers of pleural infection was planned to occur in collaboration with the academic radiology unit at Oxford (my host institution) which had to redirect its focus toward urgent public health (COVID-19) studies. An ultrasound sub-study assessing the utility of intrapleural contrast enhanced ultrasound had to end prematurely due to concerns about asking participants to have prolonged face-to-face ultrasound assessments and the specific contrast enabled ultrasound machine was only secured on loan for a limited period and had to be returned to the manufacturer. The details of these studies are included as an appendix to Chapter 2.

After consultation with my supervisors, this workstream was therefore replaced by Chapter 5 which was a retrospective analysis of the largest international cohort of pleural infection patients treated with intrapleural fibrinolytic and enzyme therapy, assessing bleeding complications. This was completed in collaboration with colleagues in the United States and published with myself as joint first author.

The MIST-3 feasibility RCT (Chapter 3) and its qualitative study (Chapter 4) both did not meet their target recruitment numbers but were still completed and analysed successfully.

## AUTHOR DECLARATION

I declare that the work in this thesis represents an original contribution to the field of research. The works herein were conducted and are presented in accordance with the requirements of the University of Sheffield's Code of Practice for Research Degree Programmes. The studies/workstreams presented are the candidate's own work and any assistance or collaboration is specifically indicated in the text.

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## ACKNOWLEDGEMENTS

First and foremost, I would like to dedicate the work within this dissertation to my late father, Osama, who sadly passed away after a short battle with metastatic pancreatic cancer during the first year of my PhD. He was a kind, loving father, a Consultant Nephrologist, my best friend and my role model in life. He instilled in me a strong ethos of the pursuit of education and knowledge during my formative years and has been my constant drive throughout this PhD. I hope he would have been proud of my achievements to date. I will always remember him printing my first publication (a review article on pleural infection!), reading it cover to cover and calling me the following day to discuss it!

Secondly, my supervisor Professor Najib Rahman who continues to be an inspiration, friend and mentor to me. His passion, drive and achievements as a respiratory academic have undoubtedly been a massive spur in me achieving what I have done to date. He has trusted me to deliver, empowered me the with the freedom to grow and shape my own thoughts and directions in the study ideas we discuss whilst always being generous with his time and offering guidance and critique with kindness and humility whenever it was needed or asked. He has opened up doors and opportunities that will undoubtedly shape the rest of my career. When colleagues ask my advice on undertaking a PhD, I always say choose your supervisor wisely and I could not have dreamed for a better one with Naj, to whom I am so indebted.

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world of laboratory research and was incredibly supportive. I was also lucky enough to work with the highly experienced staff in the Oxford Respiratory Trials Unit and must specifically thank Emma Hedley, who was always willing to offer guidance in the set up of my studies and helped me navigate the various aspects of research bureaucracy.

Last, but not least, my amazing wife, Fatma, who has sacrificed so much personally bearing the lion's share of domestic and parental duties to allow me to pursue my ambitions. Without her love, patience and understanding, this simply would have not been possible. My mum for her prayers and blessings, my younger brother and sister for being my 'hype men' and to my beautiful children, Ahmed and Zainab, currently 8 and 4, the true blessings in my life, thank you for being the most delightful distractions from the pressures of academia.

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# ABBREVIATIONS

CI ..... Chief Investigator

CRF ..... Case Report Form

CT ..... Computed Tomography

DNase ..... Deoxyribonuclease

F ..... French (conventional chest tube size unit)

ICU ..... Intensive Care Unit

IET ..... Intrapleural Enzyme Therpay

IL-6 ..... Interleukin-6

IL-8 ..... Interleukin - 8

IPFT ..... Intrapleural Fibrinolytic Therapy

LOS ..... Length of Stay

MCP-1 ..... Monocyte Chemoattractant Protein-1

MIST ..... Multicentre Intrapleural Sepsis Trial

MPE ..... Malignant Pleural Effusion

NIH ..... National Institute of Health

NIHR ..... National Institutte of Health Research

PAI-1 ..... Plasminogen Activator Inhibitor-1

PILOT ..... Pleural Infection Longitudinal Outcomes Study

PIS ..... Patient Information Sheet

RCT ..... Randomised Controlled Trial

RfPB ..... Reasearch for Patient Benefit

scuPA ..... Single Chain Urokinase Plasminogen Activator

TNF-α ..... Tumour Necrosis Factor alpha

tPA ..... Tissue Plasmingen Activator

TSC ..... Trial Steering Committee

UK ..... United Kingdom

VATS ..... Video Assisted Thoracoscopic Surgery

VEGF ..... Vascular Endothelial Growth Factor

# CHAPTER 1

# INTRODUCTION

This chapter is an amalgamation of several in-depth literature searches undertaken by myself throughout my PhD solely for my thesis, but also published review articles (1-4), book chapters (5-6) and a Society Taskforce Clinical Statement (7), as primary author, detailed below:

1. Bedawi EO et al. Recent developments in the management of pleural infection: A comprehensive review. Clin Respir J. 2018 Aug;12(8):2309-2320. doi: 10.1111/crj.12941.

2. Bedawi EO, et al. Pleural infection: a closer look at the etiopathogenesis, microbiology and role of antibiotics. Expert Rev Respir Med. 2019 Apr;13(4):337-347. doi: 10.1080/17476348.2019.

3. Bedawi, E.O. et al A New Approach to Pleural Infection: Let It Be?.Curr Pulmonol Rep 8, 112–122 (2019). https://doi.org/10.1007/s13665-019-00230-1

4. Bedawi EO et al Advances in pleural infection and malignancy. Eur Respir Rev. 2021 Jan 13;30(159):200002. doi: 10.1183/16000617.0002-2020.

5. EO Bedawi & NM Rahman – Pleural Effusion: Infection (Para-pneumonic and Empyema); Encyclopaedia of Respiratory Medicine, 2nd Edition; Edited by Sam Janes; Elsevier 2020

6. EO Bedawi & NM Rahman – Pleural infection: moving from treatment to prevention; ERS Monograph; Pleural Diseases; European Respiratory Society; March 2020

7. Bedawi EO et al ERS/ESTS statement on the management of pleural infection in adults. Eur Respir J. 2022 Oct 13:2201062.

# 1. **INTRODUCTION**

Pneumonia (infection/inflammation of the lung(s), predominantly at the level of the alveoli) affects more than 200,000 patients per year in the UK. It is responsible for more hospital admissions and bed days than any other lung disease in the UK (Chalmers et al., 2017). Up to half of patients with pneumonia develop pleural effusion(s) (excess fluid in the pleural space, between the normally contiguous layers of visceral and parietal pleural membranes lining the lungs and chest wall respectively), and this in itself is associated with a 3-6 fold increase in mortality (Dean et al., 2016). While the majority of these 'parapneumonic' effusions will resolve with antibiotic treatment alone, around 15% progress to 'pleural infection', implying that they require specific treatment. There are over 15,000 new cases of pleural infection seen each year (Davies et al., 2010).

In a recent study evaluating the epidemiology of pleural infection in England, the median patient age was 62 years with a bimodal distribution, and there was a male predominance (approximately 2:1) (Arnold et al., 2021). Studies have shown a doubling of incidence in the last decade and this has been evident particularly amongst the elderly, as well as people who are immunocompromised (Arnold et al., 2021; Bobbio et al., 2021; Mummadi et al., 2021). Although 70% of pleural infection occurs in patients with identified risk factors (diabetes, immune suppression), however 30% of cases occur in otherwise healthy individuals (Cargill et al., 2019).

Outcomes remain poor, with 30-day mortality rates of 4% (Cargill et al., 2019) and a one year mortality rate of approximately 14% that has been consistently reported in the last two decades (Arnold et al., 2021; Cargill et al., 2019; Davies et al., 1999), and others having a slow recovery and long-term sequelae such as restricted lung function.

The average hospital length of stay (LOS) associated with pleural infection is 14-19 days (Arnold et al., 2021; Cargill et al., 2019; Rahman et al., 2011). This places a significant burden on patients, their families, and the health service. There are no robust figures to reflect the current economic burden on the health service in the UK. Based on resource use from large, multicentre randomised trials, the estimated inpatient costs alone are around £90million a year (taking into account inpatient bed days at £68million, 15% requiring intrapleural fibrinolytics at £3.5million, 20% requiring

video assisted thoracoscopic surgery at £12million, each patient receiving at least one CT scan £5.25million and the standard initial 7 day course of antibiotics (Davies et al., 2010) costing £1.2million). This figure is likely to be higher given the analysis of Davies et al. was over 13 years ago.

Three recent major International guidelines (European Respiratory Society/European Society of Thoracic Surgeons, British Thoracic Society, and American Association for Thoracic Surgeons) all advocate prompt hospital admission for chest tube drainage of the infected pleural collection and early administration of appropriate antibiotics as the mainstays of treatment (Bedawi et al., 2022b; Roberts et al., 2023; Shen et al., 2017). However, the largest prospective observational study of adult pleural infection (n=543), the PILOT study (Corcoran et al., 2020a), demonstrated that this so-called 'standard care' is insufficient in approximately 34% of patients, who will go on to require further treatment . A proportion, estimated at 20%, will receive intrapleural enzyme treatment as an additional treatment, in cases where there is poor drainage and slow clinical and radiological resolution of infection (Rahman et al., 2011). A further 15% (Arnold et al., 2021) of all patients with pleural infection end up requiring referral for surgical management due to failure of initial medical treatment after 3-5 days of inpatient care. In recent years, newer "keyhole" surgery (Video Assisted Thoracoscopic Surgery, VATS, using 3 small incisions in the chest under general anaesthetic) is used in patients who have failed initial treatment, as it is less invasive than traditional surgery such as thoracotomy (Shen et al., 2017) (which requires an up to 25cm incision in the chest under general anaesthetic). This treatment pathway has thus far been based on expert consensus and there are no head-to-head randomised controlled trials informing patient selection, timing or whether either treatment is superior.

## 1.1 EPIDEMIOLOGY OF PLEURAL INFECTION

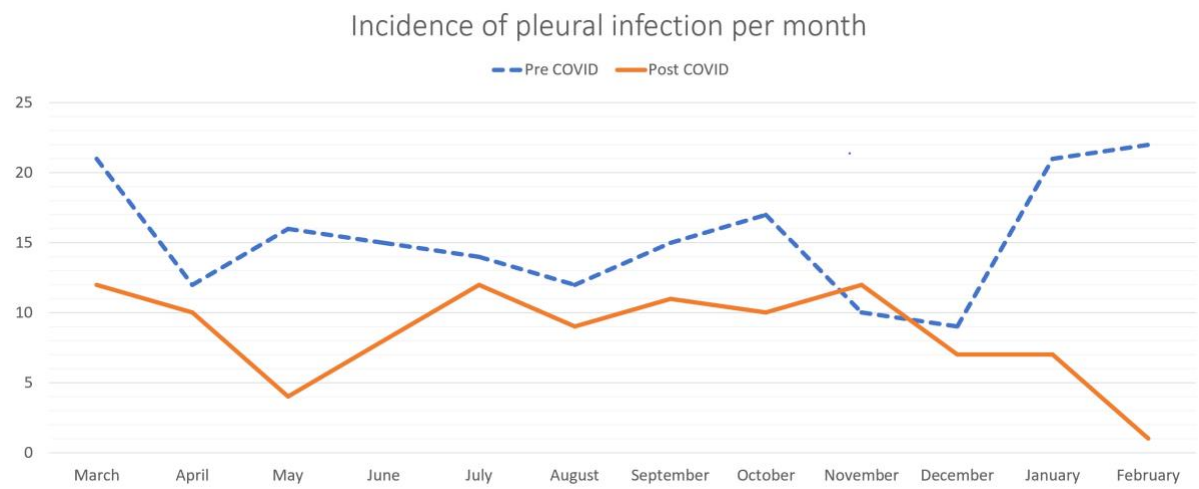
In the early 21st century, a plethora of evidence emerged demonstrating a rise in the rates of pneumococcal disease (caused by the Gram positive *Streptococcus pneumoniae*) with resultant increases in the incidence of pneumonia and pleural infection (Burgos et al., 2011; Farjah et al., 2007; Grijalva et al., 2011). Studies have suggested that widespread vaccination programmes might have caused a replacement phenomenon with non-vaccine serotypes becoming increasingly

responsible for disease (Byington et al., 2010). The prevalence of non-PVC7 (7-valent pneumococcal conjugate vaccine) serotypes has been particularly evident in countries that introduced PCV7 into the paediatric immunisation program, particularly serotypes with predilection for invading the pleural space. Most of these serotypes, namely serotypes 1, 19A, 3, and 7F, are targeted by PCV13 (13-valent pneumococcal conjugate vaccine), which was registered for paediatric vaccination from 2009 and adult vaccination from 2011 (Fletcher et al., 2014). Early studies on the consequent effects on pleural infection incidence have been inconclusive (Chacon-Cruz et al., 2016; Thomas et al., 2013) and data from several larger epidemiological studies are eagerly awaited. Nonetheless, the change in the epidemiology of pleural infection is not wholly explained by non-vaccine pneumococcal serotypes alone, as there is also an increase in non-pneumococcal pleural infection as well as pleural infection without an identified pneumonia.

A huge rise in pleural infection cases is well documented following the influenza pandemic in 1918 (Mozingo, 1918) and the epidemiological study from Arnold et al. also found that for 9 of the 10 years studied, the highest annual point incidence of influenza nationally coincided with the highest admission rate for pleural infection (Arnold et al., 2021). During the COVID-19 pandemic a reduction in empyema admissions was observed in particular in the paediatric population, and in adults there was a reduction in pneumococcal aetiology with an increase on polymicrobial infection, perhaps related to delayed presentation (Chan et al., 2023). A similar observation was noted by myself during the first wave of the pandemic so I conducted a small, but to my knowledge, the only published study examining the impact of COVID-19 on the incidence and profile of pleural infection in the UK (Bedawi et al., 2022a). The study was conducted while my studies in this thesis were on hold due to the direct impact of the COVID-19 pandemic, and the final publication is included as an appendix (A1.1). In brief, using pleural infection diagnoses data prospectively recorded on Pleural Multidisciplinary Team (MDT) databases from 5 geographically diverse, and hence representative, specialist pleural units across the UK, I found a 32.6% decrease in pleural infection cases in the year following the start of the pandemic, compared to the year prior (Figure 1.1). Compared to previous influenza pandemics, it is noteworthy that during the COVID- 19 peaks, antibiotics were used to cover likely secondary bacterial infections, and this may have helped prevent empyema complications. The potential role of public health measures in reducing pleural infection incidence is

intriguing and one that has not been specifically explored in the existing literature. It is highly likely that decreased social mixing, isolation of older, more vulnerable patients with additional comorbidity, often at increased risk of pleural infection, as well as social distancing measures have had in combination a beneficial impact. There was a notably higher proportion of purulent and culture positive infections in the post-COVID cohort, which I hypothesised may have been a result of poorer access to prior antibiotics in the community as there was a notable reluctance from patients to access their general practitioner and a reduction in face-to-face assessments.

**Figure 1.1: Incidence of pleural infection per month pre and post COVID-19 pandemic – reproduced from Bedawi et al., The Impact of the COVID-19 Pandemic on Pleural Infection incidence: a UK multicentre retrospective analysis. ERJ Open Res 2022**

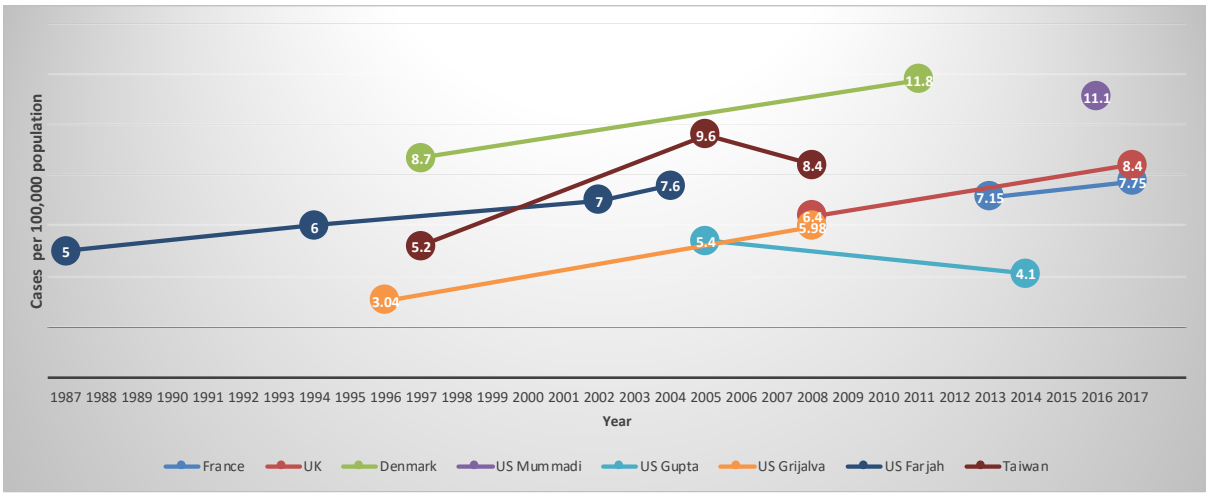



***Pre-COVID: March 2019 - February 2020**
***Post-COVID: March 2020 – February 2021**

Nonetheless, there has been an irrefutable increase in the incidence of pleural infection as shown by pooled incidence data from the 8 largest epidemiological over the last 2 decades (Figure 1.2). An ageing population may explain the increasing incidence of pleural infection in older patients with comorbidities who are living longer; such individuals have an increased risk of aspiration of oropharyngeal commensals (Kanellakis et al., 2022), and recent studies using anaerobic cultures and/or PCR-based diagnostics (e.g. (Dyrhovden et al., 2023) suggest this may have been under-recognised . The use of more specific imaging such as Computed Tomography (CT) and thoracic ultrasound (TUS) at the 'front door' of hospital admission is likely to have contributed to more accurate and timely diagnoses, as well as increased use of

improved microbiological diagnostics (blood culture bottles vs standard culture alone, as well as molecular techniques including 16S Polymerase Chain Reaction). This is not to underestimate the role of increased awareness of and vigilance for pleural infection amongst clinicians, increasing involvement of specialist pleural services as well as growing research initiatives.

**Figure 1.2: Trends in incidence of pleural infection in different countries from the world literature – reproduced from Bedawi et al., ERS/ESTS Statement on the Management of Pleural Infection in Adults ERJ 2022**




## 1.2 RISK FACTORS FOR PLEURAL INFECTION

About 60% of cases of pleural infection are related to a primary pneumonic process, therefore risk factors for pleural infection are assumed to be similar to those for pneumonia (Corcoran and Rahman, 2016). A large single centre prospective observational study of 1269 patients admitted with community acquired pneumonia used multivariate regression to identify predictors independently associated with development of complicated parapneumonic effusion or empyema (Chalmers et al., 2009). These included:

- albumin <30 g/l adjusted odds ratio (AOR) 4.55 (95% CI 2.45 to 8.45, p<0.0001)
- sodium <130 mmol/l AOR 2.70 (1.55 to 4.70, p = 0.0005)
- platelet count >400x$10^9$ /l AOR 4.09 (2.21 to 7.54, p<0.0001)
- C-reactive protein >100 mg/l AOR 15.7 (3.69 to 66.9, p<0.0001) and

- a history of alcohol abuse AOR 4.28 (1.87 to 9.82, p = 0.0006)
- intravenous drug use AOR 2.82 (1.09 to 7.30, p = 0.03)

Non-steroidal anti-inflammatory drugs (NSAIDs), commonly used as analgesics or antipyretics early in the course of pneumonia, have been shown to exert multiple effects on different components of innate and adaptive immunity and thus interfere with host response to acute infection (Hussain et al., 2012). Although not based on randomized controlled data, the use of NSAIDs in community-acquired pneumonia has been shown to be independently associated with a more complicated course and increased rate of pleuropulmonary complications, including CPPE and empyema (Basille et al., 2017; Voiriot et al., 2011). This is probably through an initial blunted systemic response leading to a delay in presentation and diagnosis and hence late initiation of adequate antibiotic therapy.

## 1.3 DEVELOPMENT OF PLEURAL INFECTION

### 1.3.1 TRANSITION FROM SIMPLE TO COMPLICATED PARAPNEUMONIC EFFUSIONS

The majority of pleural infection is thought to be the result of the formation and evolution of a parapneumonic effusion across three progressive stages (Molnar, 2007), although many have hypothesised that this is an over-simplification, and newer insights are needed as much of the evidence is from older studies using animal models. Initially, it is proposed that the direct invasion of microorganisms within the lung parenchyma leads to breakdown of local host defences and provocation of intra-alveolar inflammatory exudates. Next, the resultant parenchymal inflammation causes an increased permeability of the visceral pleural membranes and leakage of interstitial fluid. The mesothelial cell lining is further disrupted, by neutrophil migration and by pleural mesothelial cells releasing pro-inflammatory cytokines including tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8) into the pleural space. Notably, these mediators are found in higher concentrations in effusions of an infectious aetiology compared to effusions due to malignancy and heart failure (Strieter et al., 1994). Finally, anatomical distortion of the visceral pleural mesothelial lining follows, creating intercellular ‘gaps’ and increasing permeability, allowing accumulation of pleural fluid (Broaddus et al., 1994, 1992; Kroegel and Antony, 1997).

This initial exudative phase is analogous to simple parapneumonic effusions, with no detectable bacteria and hence a normal glucose level with no acidity (pH > 7.2). Prompt antibiotic therapy at this stage is likely to result in treatment of the pneumonia and resolution of the effusion (Light et al., 1980). It is noteworthy that the presence of an effusion in patients with pneumonia, even the so-called 'simple' parapneumonic, is known to be associated with a 3-6 fold increase in mortality (Dean et al., 2016).

Whilst recent pleural infection research has focused on the benefit of drainage once bacterial invasion occurs, the ideal approach is to stop parapneumonic fluid formation in the first place, an 'upstream' approach. Targeting specific candidates, such as MCP-1, or using a general approach to dampen pleural inflammatory responses have been explored. In a large study (n=3,602) of pneumonia patients, those taking inhaled steroids were less likely to develop parapneumonic effusions (Odds ratio 0.42) (Sellares et al., 2013) and an RCT of 60 children with parapneumonic effusion showed that high dose intravenous dexamethasone significantly improved recovery time (Tagarro et al., 2017). A lower incidence of pleural infection in patients with COPD has been described (Chalmers et al., 2009) and could possibly be attributed to long-term inhaled corticosteroids, although this is unproven.

To address this question prospectively and specifically in adult pleural infection, the STOPPE trial was a pilot, multicentre double-blind, placebo controlled randomised trial in a population of adults with pneumonia and pleural effusion (Fitzgerald et al., 2019b). Patients were randomised 2:1 to IV dexamethasone (4mg b.d. for 48 hours) vs placebo. The investigators screened 374 and randomised 80 patients (dexamethasone: n=51 vs placebo: n=28). There were no significant differences in terms of time to relapse from clinical stability, chest radiograph appearances at one month, resolution of inflammatory markers, hospital length of stay or antibiotic use. When one considers that only 30% of uncomplicated parapneumonic effusions develops to true pleural infection, the study was probably significantly under-powered to detect a difference in any of the aforementioned secondary outcomes. The administration of IV dexamethasone did not cause lasting harm but there were a significant number of hyperglycaemia adverse events.

Therefore, with no current evidence-based intervention to cease progression from the simple parapneumonic effusion (SPPE) stage, inflammation persists. In addition to the

increase in inflammatory mediators such as TNF-α, this has been associated with depression of the normally high local fibrinolytic activity, in part through a rise in Plasminogen activator inhibitor-1 (PAI-1) and, to a lesser extent, PAI-2 (Idell et al., 1991). As a consequence of the reduced fibrinolytic activity, fibrin deposition occurs over the visceral and parietal pleura, dividing the pleural space by strands of fibrin mesh known as septations, which compartmentalise the fluid into pockets or locules. The degree of elevation of PAI-1 levels seen at this stage appears to correlate with residual pleural thickening (Chung et al., 2013). This may explain why patients who enter this fibrinopurulent phase and are diagnosed with complex parapneumonic effusion, require urgent drainage to prevent detrimental effects on lung function, as well as to achieve sepsis control. The bacterial metabolism and neutrophil phagocytic/metabolic activity that occurs in this phase leads to increased lactic acid production, reflected in a drop in pleural fluid pH and glucose, biochemical hallmarks of pleural infection (Light et al., 1973; Sahn et al., 1983). Additionally the level of lactate dehydrogenase (LDH) rises due to its release by neutrophils and mononuclear cells involved in pleural inflammation (Saint-Rémy et al., 1986). If sepsis control is not achieved prior to further progression, the fluid becomes frankly purulent secondary to bacterial and inflammatory cell death and lysis.

The final ‘organising’ stage is characterised by proliferation of fibroblasts and pleural scarring. Non-expandable lung (NEL) may ensue due to visceral pleural fibrosis, likely to result in significant lung function impairment. Platelet-derived growth factor (PDGF) (Mutsaers et al., 2006) and transforming growth factor beta (TGF-β) (Kunz et al., 2004) have been have been implicated in this process. The clinical significance of this phase has been suggested to mark the point at which surgical intervention becomes a pre-requisite for successful outcomes, since medical treatments such as intrapleural enzyme therapy (IET) are unlikely to have any therapeutic effect on collagenous fibrous tissue. The caveat here is that there is marked inter-patient variability in the timescale of progression to this stage (Landreneau et al., 1996). This is of particular importance in the elderly, who often present with a more indolent ‘slow burning’ infection and hence a trial of ‘medical’ management may still be worthwhile in this setting.

The rate of progression through these stages is likely influenced by the patient’s own immunity and the virulence of the infecting organism. Whether or not progression is

truly linear as outlined above is also unclear, as not all patients will develop purulent collections, and many end up with heavily loculated collections, which may be with or without such purulence. It is plausible that a combination of bacterial factors and host fibrinolytic responses result in varying degrees of septation formation as a defence mechanism to wall off infection. Key unknowns in this area are whether the development of septations is necessary for the development of an empyema, or whether instead a certain degree of septation can prevent development of empyema, resulting in a densely loculated collection without free-flowing pus. Interestingly, the clinical course that ensues after treatment at the organising phase is also variable. While some patients may undergo gradual resolution of pleural thickening, recovering fully at 12 weeks (Neff et al., 1990), others may develop a low-grade chronic inflammatory state and longstanding lung function deficits (Hamm and Light, 1997).

### 1.3.2 WHERE DOES THE INFECTION ARISE?

Recent bacterial studies and animal models have demonstrated several potential routes of microbial penetration into an otherwise sterile pleural space, and have found that empyema is not always secondary to a classical pneumonic process (Corcoran et al., 2015). The trigger is often aspiration of oropharyngeal bacteria with development (or not) of pneumonic changes. The reasons why in some cases secondary bacterial invasion occurs and the factors that contribute to the development of an infected pleural space are poorly understood.

An understanding of the mechanisms that contribute to pleural injury has been impeded by the lack of a suitable and survivable murine model resembling human disease that permits investigation of the pathogenesis of pleural infection (Cvijanović et al., 2014). The majority of animal models of pleural infection have used direct intrapleural inoculation, which bypasses the stage of bacterial infiltration from the lungs to reach the pleural space. A mouse model using intranasal inoculation of *S. pneumoniae* (serotype 2 strain D39) demonstrated evidence of bacteria and necrosis within the mesothelial cell layer within 24 hours, and formation of adhesions at 48 hours (Wilkosz et al., 2012). Whilst this progression is far quicker than that seen in humans, it does suggest that translocation of bacteria through mesothelial cells is an important invasion route, at least for *S. pneumoniae*. In the same study, direct intrapleural inoculation of *S. pneumoniae* resulted in a rapid septicaemia, suggesting

that the pleural space itself is permissive for bacterial replication to overwhelm local immune defences. This was observed to a much lesser extent when the bacteria were injected intravenously, suggesting that (indirect) haematogenous spread of bacteria into the pleural space is a less likely route of pleural infection seeding, but again, this could be organism and model specific. The rapid and inevitable development of empyema in this mouse model may suggest that humans have efficient mechanisms that often prevent pleural infection, possibly relating to pre-existing immunity from previous colonisation, infection, or vaccination.

The *Streptococcus milleri* group of bacteria are facultative anaerobic commensals of the oropharynx. They are amongst the most frequent isolates from community-acquired pleural infection and yet, they are rarely identified as causing pneumonia. This could explain why a surprisingly high proportion of cases of empyema have no radiological evidence of pneumonia, as was seen in 12% and 30% in the MIST-1 (Multicentre Intrapleural Sepsis Trial-1) and MIST-2 cohorts respectively (Jaffe et al., 2008) (Franklin et al., 2021). This may suggest that perhaps a more elderly patient population with increasing risk factors for aspiration may be contributing to the rising incidence of empyema; lung infection sufficient to constitute a diagnosis of pneumonia may not be required for such organisms to enter the pleural space. The role of aspiration in the development of pleural infection is likely to be more significant than often appreciated, judging by the presence and polymicrobial nature of oropharyngeal bacteria in pleural infection samples (Kanellakis et al., 2022). It is important to note that whilst aspiration is often associated with elderly patients and hospital acquired infections, our recent systematic review (Hassan et al., 2019a) found anaerobic isolates to be relatively common even in community acquired infections and in younger patients. This might be related to poor dental hygiene, as an under-recognised risk factor for pleural infection (Corcoran et al., 2015), with spread to the pleura potentially via the haematogenous route. To date, evidence for the following non-pneumonic pleural infection routes has been hypothesised and/or reported (Corcoran et al., 2015; McCauley and Dean, 2015; Smith et al., 1991)

a) *Haematogenous spread* of bacteraemia in the context of systemic infections such as endocarditis or discitis.

   Some groups of patients may be more predisposed to this, such as patients with liver cirrhosis in the presence of a hepatic hydrothorax, so called ‘spontaneous bacterial empyema’ (SBEM). The mechanism may relate to

altered gut microbiota with enhanced translocation of bacteria and bacterial products across the intestinal epithelium to mesenteric lymph nodes and the systemic circulation via the portal vein (Giannelli et al., 2014; Sturm et al., 2023). It is important to note that in cases of true SBEM, treatment comprises intravenous antibiotics +/- albumin and generally, thoracostomy tubes should be avoided unless the fluid is grossly purulent, since chest tube drainage may result in life threatening fluid depletion, protein loss and electrolyte imbalance (Tu and Chen, 2012).

b) *Translocation through visceral pleural defects* or fistulae in the context of lung cancer, post radiotherapy or postoperatively.

c) *Penetrating injury across the parietal pleura* in the context of trauma or chest tube insertion.

d) *Mediastinal spread* in cases of oesophageal rupture (Boerhaave's syndrome) or surgery.

e) *Transdiaphragmatic spread* in the context of intraabdominal infection +/- alcoholic cirrhosis.

## 1.4 MICROBIOLOGY

### 1.4.1 PLEURAL FLUID MICROBIOLOGY – CONVENTIONAL TECHNIQUES

Understanding the route of bacterial entry into the pleural space may shed light on the patterns of microbiology that are observed. Our current limited ability to identify offending organisms in pleural infection poses a significant challenge to clinicians. This limitation may result from infected pleural effusions being acidic, hypoxic and nutritionally deplete, with subsequently low bacterial concentrations, although bacterial numbers may be notably higher in purulent collections (Porcel et al., 2014). Prior antibiotic treatment and causal agents that are difficult to isolate in standard laboratories due to stringent growth requirements may also be contributory. The inoculation of pleural fluid into BACTEC blood culture bottles (Menzies et al., 2011) has been shown to improve the diagnostic yield and this has been incorporated into routine clinical practice.

Maskell et al. conducted the first detailed pleural fluid microbiological study, analysing pleural fluid prospectively collected from 434 pleural infections as part of the largest pleural infection RCT to date, MIST-1 (Maskell et al., 2005). The pleural fluid samples underwent standard culture as well as screening for bacteria by amplification and sequencing of bacterial 16S ribosomal RNA gene. In what is now considered to be a landmark paper in the microbiology of pleural infection (Maskell et al., 2006a), the investigators observed for the first time that the bacteriology of pleural infection is inherently different from that of pneumonia and requires different treatment. Reflecting known differences in the bacteriology of community and hospital acquired infections, Maskell et al., as well as data from other studies (Marks et al., 2012; Park et al., 2016a) have identified differences between these categories of pleural infection (Table 1), with Streptococcal isolates (*S. viridans* and *S. pneumoniae*) predominating in community-acquired cases but increased frequency of *Staphylococcus aureus* and gram-negatives in the hospital acquired group. They also demonstrated interesting trends in survival based on bacterial subsets with the lowest mortalities seen in streptococcal subsets (*S. pneumoniae, S. intermedius group*) and in those infections in whom no pathogen was identified (culture negative). One year mortality was significantly increased in those with gram-negative bacteria, *S. aureus* and mixed aerobes.

**Table 1.1. Isolated bacteria from pleural infections split according to infection setting. Data is collated from three studies: Maskell et al., 2006, Marks et al., 2012 and Park et al., 2016.[-] From Bedawi et al Clin Resp Journal 2018.**

<table>
<tr><th></th><th colspan="2">Community-acquired</th><th colspan="2">Hospital acquired</th></tr>
<tr><td>Organism group</td><td colspan="4"></td></tr>
<tr><td>Gram positive</td><td colspan="2">65%</td><td colspan="2">51%</td></tr>
<tr><td>Gram negative</td><td colspan="2">17%</td><td colspan="2">38%</td></tr>
<tr><td>Anaerobes</td><td colspan="2">18%</td><td colspan="2">11%</td></tr>
<tr><td rowspan="5">Most common organisms</td><td colspan="2">Viridans streptococci 25%</td><td rowspan="2">Staphylococcus aureus</td><td>Methicillin-resistant 31%</td></tr>
<tr><td colspan="2">Streptococcus pneumoniae 23.8%</td><td>Methicillin sensitive 11%</td></tr>
<tr><td rowspan="2">Staphylococcus aureus</td><td>Methicilin-resistant 4%</td><td colspan="2">Enterobacteriaceae 13.6%</td></tr>
<tr><td>Methicillin sensitive 11.4%</td><td colspan="2">Viridans streptococci* 9%</td></tr>
<tr><td colspan="2">Enterobacteriaceae 7.5%</td><td colspan="2">Pseudomonas species 6.5%</td></tr>
<tr><td colspan="2">Pseudomonas species 3.2%</td><td colspan="2">Klebsiella species 6%</td></tr>
</table>

In the first systematic review of adult pleural infection (Hassan et al., 2019a), which to date remains the largest in the world literature (n=10,245 from 75 studies) our group found the average diagnostic yield of bacterial cultures in pleural fluid was 56%, meaning that in almost half of patients the infecting organism(s) remain unknown, and antimicrobial treatment is entirely empirical. The analysis by the publication year indicates an increased role for gram positive bacteria in last few years. In addition, *S. aureus* has overtaken the viridans *streptococci* as the most common isolate, and the proportion of methicillin resistant staphylococcal isolates has increased from 48 to 58%.

Subgroup analyses from our recent systematic review also demonstrated a clear geographical variation in the organisms causing pleural infection. These differences are likely to be complex and multifactorial. For example, in tropical regions, the profile was strongly gram positive, and patients affected by pleural infection in these regions were notably younger with higher rates of human immune deficiency virus (HIV), both features likely to be implicated in the higher incidence of pneumococcal pneumonia/pleural infection. In the subtropics, a dominance of gram negative isolates was seen. The distinctive abundance of *Klebsiella spp* and *Pseudomonas spp* infections is unexplained but was predominantly influenced by data derived from two comprehensive studies originating from the Indian subcontinent (Mohanty et al., 2007; Sonali, 2013).

### 1.4.2 PLEURAL BIOPSY TISSUE – CONVENTIONAL TECHNIQUES

Pleural tissue biopsy has been widely adopted in endemic areas for a long time for suspected tuberculous effusions, as well as evaluating other causes of pleural disease. In the AUDIO study, Psallidas et al. performed ultrasound- guided pleural biopsies in patients diagnosed with pleural infection at the time of chest drain insertion. The material obtained was sent in 0.9% saline for microbiological examination, alongside standard pleural fluid culture and gram stain, in addition to inoculation of pleural fluid in BACTEC blood culture bottles, as well as standard blood cultures. Patients did not have to have evidence of pleural thickening on ultrasound. The results showed an increase in microbiological yield to 45% compared to pleural fluid and blood cultures in the same study (20% and 10%, respectively) (Psallidas et al., 2018). This increased detection of bacteria in pleural tissue is intriguing and may add to our

understanding of the pathobiology of pleural infection. In their discussion, the authors hypothesized that an improved blood supply of the pleural membrane and better nutrition may provide a more favourable environment for the bacteria to be located, rather than within the pleural fluid. Interestingly, pleural biopsy culture positivity was not affected by prior antibiotic administration; this may simply reflect limited antibiotic penetration into the pleural tissue, or perhaps the importance of other features of the pathogenesis such as biofilm formation in this condition. Of note, this was a pilot, feasibility study involving just 20 patients but has set the scene for larger clinical studies to evaluate the use of this technique as an additional test to form part of the standard workup of pleural infection.

### 1.4.3 PLEURAL FLUID MICROBIOLOGY – NEXT GENERATION SEQUENCING

Although the systematic review showed that the incidence of polymicrobial pleural infection identified by conventional microbiological methods was in the region of 23%, in a recent metagenomic study of pleural infection, The Oxford Pleural Infection Metagenomics Studies or TORPIDS (Kanellakis et al., 2022), used state of the art 16S rRNA next generation sequencing (NGS) to rigorously analyse the microbial diversity of pleural infection using prospectively collected pleural fluid samples (n=263) from the largest prospective observational study in pleural infection, the PILOT study (Corcoran et al., 2020a). This study identified pathogens in a much higher proportion of pleural samples than conventional microbiology. Importantly, in this study we found that pleural infection was in fact predominately polymicrobial – approximately 80% of cases. Moreover, we found an abundance of anaerobes, likely as a result of the limitations of culture-based pathogen detection methods used in the majority of the reported studies in the systematic review.

In addition, the TORPIDS study demonstrated distinct microbial patterns between monobacterial and polymicrobial disease. Whilst nutritionally fastidious anaerobes, Gram-negative and bacteria of the *S. anginosus* group are predominant in polymicrobial samples, *S. pneumoniae* was the most prevalent pathogen in community acquired monobacterial infection. A speculative explanation for this finding is that *S. pneumoniae* biofilms do not favour symbiosis with other bacterial species, either due to strong competition for available nutrients or because *S. pneumoniae* have sufficient

virulence factors, hence do not require infection 'partners' to partake in bacterial co-infection. This novel finding is potentially useful in antibiotic rationalising and may suggest that when S. pneumoniae is cultured in pleural fluid, the spectrum can be narrowed.

In keeping with a previous study, (Dyrhovden et al., 2019), the most abundant anaerobic pleural anaerobic pathogens identified by 16S rRNA NGS in the TORPIDS study are commonly found in the oral cavity and dental microbiome. As noted above, this suggests that aspiration of oropharyngeal and oral/dental pathogens plays a more significant role in the pathogenesis of pleural infection than was previously assumed and may in part explain the observed polymicrobial predominance.

## 1.5 DIAGNOSIS OF PLEURAL INFECTION

### 1.5.1 **CLINICAL PRESENTATION** – adapted from Bedawi & Rahman, Pleural Infection: Moving from Treatment to Prevention; ERS Monographs 87 – Pleural Diseases, European Respiratory Society 2020

The diagnosis of pleural infection can often be delayed and challenging, with clinician awareness being key. 'Classical' biochemical parameters are not absolute for diagnosis. Fever and rigours in the presence of an effusion in the context of a non-resolving pneumonia makes matters straightforward. However, there is also a pattern of presentation, frequently seen in the elderly, of a more indolent illness with malaise, anorexia, and weight loss. In the presence of a pleural effusion, these patients are, understandably, often mistakenly enrolled onto diagnostic pathways such as those for suspected malignancy. The delayed recognition of pleural infection in this often frail cohort of patients, inevitably confers a negative effect on treatment success and subsequent recovery (Meyer et al., 2018; Towe et al., 2019). It is also important to identify younger patients who are at greater risk of developing complex parapneumonic effusion associated with pneumonia, even if an effusion is not initially present (or does not meet diagnostic criteria for pleural infection), as these patients require close monitoring. As discussed in greater detail above, risk factors independently predictive of this occurrence include diabetes, immunosuppression, gastroesophageal reflux disease (GERD), alcohol excess, intravenous drug use and, the often overlooked, poor oral hygiene.

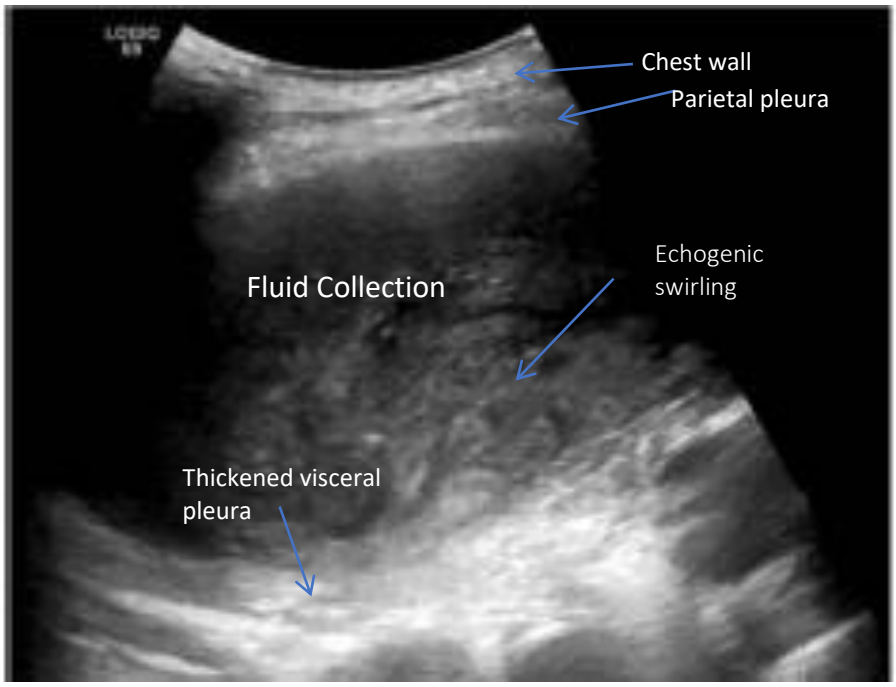
### 1.5.2 **IMAGING –** adapted from Bedawi et al., 'A New Approach to Pleural Infection: Let it Be?' Current Pulmonology Reports 2019

As clinical examination is often unhelpful in pleural infection, imaging plays a key role in the evaluation and subsequent management (Heffner et al., 2010). An initial postero-anterior chest radiograph (CXR) may demonstrate a pleural-based opacity, signifying a possible underlying effusion, however small fluid collections may not be visible and a CXR often does not indicate the cause of an effusion. Integration with other clinical features may increase the diagnostic accuracy, for example history of productive cough, fever and elevated inflammatory markers may indicate an infective aetiology. The detection of a pleural opacity should now always be promptly followed by thoracic ultrasound evaluation, and often additionally by computed tomography.

Ultrasound allows identification of free fluid and quantification of fluid volume, differentiates effusion from solid mass as well as excluding other radiographic mimics of pleural effusion, such as lung collapse. Findings such as echogenicity (Figure 1.3) can infer a likely inflammatory component suggestive of a complex parapneumonic effusion, although recent data from our unit has demonstrated that even with advanced, modern ultrasound machines, this is not always true (Asciak et al., 2019). Sampling of echogenic effusions can occasionally reveal transudates (by Light's criteria) and previous data has demonstrated that up to 27% of exudative effusions are anechoic (Yang et al., 1992).

Ultrasound is currently the most sensitive tool for assessing septations in the pleural space (Soni et al., 2015), and increased ultrasound practice has led to greater recognition of the presence of septations. There is a sparsity of data on the incidence of the presence of septations on initial ultrasound assessment of pleural infection, but this is estimated to be over 50% of cases at time of presentation (Mayo and Doelken, 2006). Particularly when combined with other features such as echogenicity, pleural fluid septations are highly suggestive of an exudative effusion and, in the appropriate clinical context, pleural infection. They are also important in guiding diagnostic sampling; of interest, a small case series of 7 patients found a diagnostically significant variation in pleural fluid pH between locules in septated parapneumonic effusions (Maskell et al., 2004).

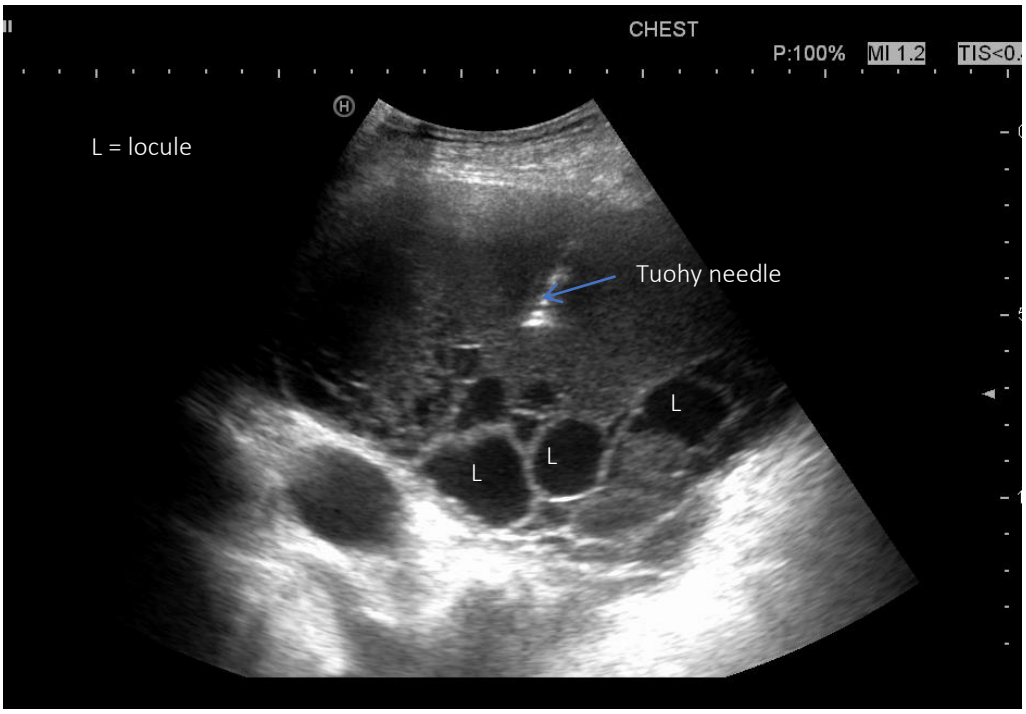
**Figure 1.3 – Ultrasound image demonstrating large echogenic pleural collection, with 'echogenic swirl' sign consistent with empyema.**




Additionally, bedside ultrasound permits assessment of the extent of septation. Through the nature of their 'floating' movement (or lack thereof) it is possible to predict whether these are likely to be communicating, or indicative of fixed, walled-off pockets known as 'loculations' (Figure 1.4), that may negatively impact on fluid drainage. This may be relevant in facilitating earlier discussions surrounding more aggressive interventions with intrapleural fibrinolytics or surgery (see 'Outcomes' Section 1.7 below).

The detailed assessment that can be achieved with ultrasound has meant that computed tomography (CT) is usually not needed in the initial routine assessment and management of pleural infection. The CT signs regarded as typical for pleural infection include thickening and contrast enhancement of the parietal pleura, increase in the thickness and attenuation of the adjacent extra-pleural fat, and enhancement of both the visceral and parietal pleura ('split pleura sign') (see figure 5), presence of multiple bubbles in the effusion (signifying anaerobic 'gas-producing' bacteria), and pleural septations. These signs have good sensitivity, but low specificity for pleural infection (Porcel, 2018a).

**Figure 1.4. Real time ultrasound guided drain insertion via a hollow Tuohy needle into a 'loculated' and infected pleural space (locules designated by 'L').**



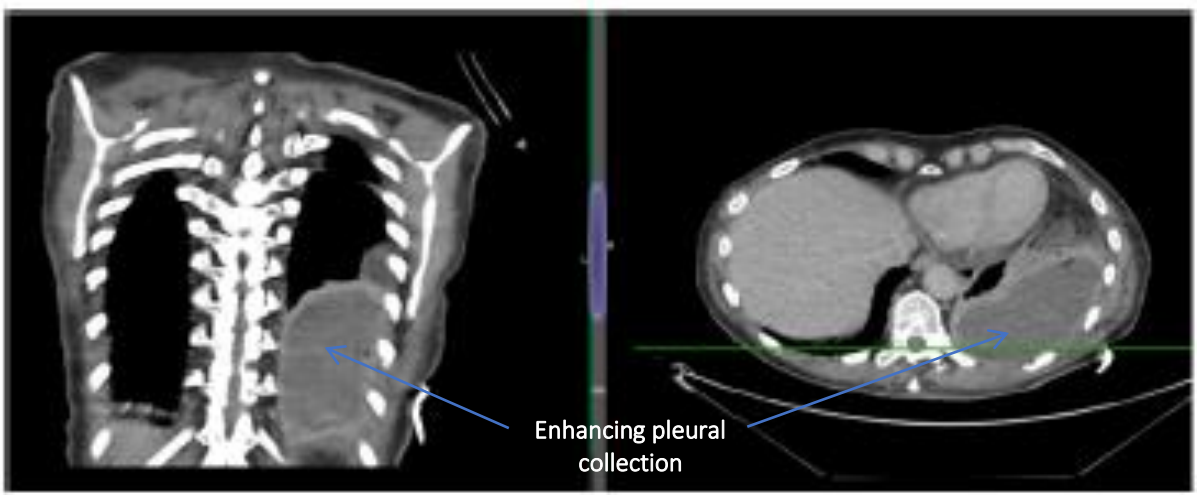

A recent study comparing chest radiograph, CT and US appeared to demonstrate the latter to outperform CT in ruling in pleural infection. US had a sensitivity and specificity of 69.2% and 90% respectively, compared to chest CT sensitivity of 76.9% and specificity of 65% (Svigals et al., 2017). The positive likelihood ratio of US to diagnose CPPE was significantly higher than those for CT and chest radiograph (6.92, 2.20 and 1.54 respectively; $p<0.05$) (Svigals et al., 2017).

However, in cases of persistent pleural sepsis beyond the initial 48 hours, evaluation with a contrast-enhanced CT scan can be invaluable, for example in revealing mal-positioned chest tubes in complex pleural collections, parenchymal lung abscesses, an adjacent subdiaphragmatic abscess as well as bronchopleural fistulas. The latter is particularly relevant in the context of surgery and intrapleural fibrinolytics, and therefore a CT is recommended when either of these is being considered for a more holistic evaluation of the thorax, mediastinum and the subdiaphragmatic region.

To date, the literature does not define a role for Magnetic Resonance Imaging (MRI) in adult pleural infection, although its role as a radiation-free non-invasive imaging modality is being explored in paediatric pleural infection, where further cross-sectional imaging is specifically required (Konietzke et al., 2020; Sodhi et al., 2021). Of note,

most of the aforementioned CT features have MRI correlates, such as the increased extrapleural fat attenuation, which may be seen as increased signal on fat suppressed T2 weighted (T2W) images. Infectious pleural effusions have a typical fluid appearance of low signal on T1W and high signal on T2W images. MRI outperforms CT in visualisation of septations (Helm et al., 2010).

**Figure 1.5. Large enhancing, loculated collection on CT consistent with pleural infection**




### 1.5.3 DIAGNOSTIC SAMPLING AND CONVENTIONAL PLEURAL FLUID BIOMARKERS – adapted from Bedawi et al., ERS/ESTS Statement on the Management of Pleural Infection ERJ Feb 2023

Pleural fluid analysis is vital to achieving the correct diagnosis and guiding the most appropriate subsequent intervention. In the presence of a clinical history or biochemical picture compatible with infection, current guidelines (Roberts et al., 2023; Shen et al., 2017) recommend using pleural fluid pH<7.2 [or in the absence of pH, a combination of glucose concentration <40 mg/dL (2.2 mmol/L) with a lactate dehydrogenase [LDH] >1000 IU/L (Fitzgerald et al., 2019a) as the most important predictor of chest tube drainage. The same groups agree that the presence of pus and/or microorganisms on Gram stain or culture of pleural fluid should necessitate chest tube drainage.

Several factors can affect both biochemical and cytological features of pleural fluid. Residual lidocaine or heparin in the syringe can falsely lower the pH, whilst either air in the syringe, a delay in analysis or pleural fluid protease-producing organisms can lead to a false elevation in pH (Cheng et al., 1998). While most cytological

examinations of pleural infection fluid will show ‘acute inflammation’ with neutrophil predominance, it should be noted that early antibiotic administration can convert pleural fluid characteristics into a lymphocyte predominant picture (Ferreiro et al., 2017).

A single ‘pH’ cut-off in a condition that represents a progression along a spectrum does present potential flaws. Hence, the latest iteration of the BTS guidelines has revised this approach, highlighting that whilst a pH>7.38 indicates a very low risk of pleural infection requiring chest tube drainage, a pH between 7.16 and 7.38 represents an intermediate risk and these patients should have chest tube drainage considered in the presence of a high LDH (>900 IU), especially if they have large pleural effusions, low pleural fluid glucose, pleural contrast enhancement on CT or septation on ultrasound.

In the absence of pleural fluid low pH or macroscopic purulence, Porcel et al suggested that a pleural fluid CRP>100mg/l can aid diagnosis of CPPE and aid decision to drain but this had poor correlation with fluid pH, and was based on retrospective analysis (Porcel et al., 2016a). The utility of serum procalcitonin (PCT) in the diagnosis of pleural infection was evaluated in a large prospective trial and concluded that this was not superior to serum CRP and WCC for the diagnosis of bacterial pleural infection (Dixon et al., 2017). A recent narrative review of serum PCT (sPCT) in pleural infection found sPCT sensitivity and specificity for diagnosing pleural infection ranged from 69-83% and from 80-94%, respectively. The authors concluded that the current evidence does not support the routine use of serum PCT for diagnosis or as a predicting factor for drainage in pleural infection (de Fonseka and Maskell, 2018) although it may have some utility in specific complex scenarios, such as distinguishing an infected versus inflammatory malignant pleural effusion, where a lower threshold for initiating antimicrobial therapy may be warranted in the setting of a raised sPCT (Bedawi et al., 2022b).

## 1.6 MANAGEMENT OF PLEURAL INFECTION

### 1.6.1 **GENERAL MEASURES –** adapted from Bedawi & Rahman, Pleural Effusion: Infection (Parapneumonic and Empyema); Encyclopaedia of Respiratory Medicine 2e; Elsevier 2019

Although the primary focus of treatment in pleural infection is early antibiotics and drainage of the infected fluid collection, attention to basics such as fluid status and urine output should not be overlooked. Pleural infection is associated with a significant catabolic state and consideration of nutrition early in the course of illness is therefore of paramount importance and has been recognized as a determinant of poor outcomes since the first World War (Nwiloh et al., 1989). These patients are also at an increased risk of venous thromboembolism and should receive adequate prophylaxis with low molecular weight heparin, unless contraindicated (Davies et al., 2010).

### 1.6.2 **ANTIBIOTICS –** adapted from Bedawi et al., Pleural infection: a closer look at the etiopathogenesis, microbiology and role of antibiotics, Expert Review of Respiratory Medicine 2019

In addition to bacterial sensitivity, an important consideration in pleural infection is adequate delivery of antibiotics to the infected pleural cavity. In general, antibiotic levels in pleural fluid are believed to be similar to those in the serum, but most studies in humans have involved patients with diseases other than pleural infection (Taryle et al., 1981). Using a rabbit model of empyema, Teixera et al. suggested that infected pleural fluid antibiotic levels are lower than serum levels, due to the decreased permeability of thickened pleura (Teixeira et al., 2000). The acidic environment created by the infected, protein-rich fluid is also likely to affect antibiotic penetrance (Hughes and Van Scoy, 1991). However, in contrast to the findings of Teixera et al., it has also been suggested that the effects of acute infection, involving inflammation, vasodilation, oedema and increased membrane permeability, should increase antimicrobial penetration to this space (Valcke et al., 1990). Another possibility is that antibiotic penetration varies according to the underlying pathophysiology of pleural fluid formation, such that in cases of secondary pleural infection associated with pneumonic inflammation induces greater permeability of the visceral pleura, antibiotic penetration is increased, and these patients may respond more favourably to such treatment.

In the study by Teixera et al., equilibration of serum and pleural fluid levels occurred very rapidly with penicillins and metronidazole and, and along with our knowledge of the associated microbiology, this explains widespread first-line use of these agents. Ceftriaxone, clindamycin, and vancomycin followed in serum/pleural equilibrium in that order. Very poor penetration was seen with gentamycin, confirmed by other studies and in keeping with recommendations against use of aminoglycosides in international guidelines (Davies et al., 2010). Using a similar rabbit model to that employed by Teixera et al., other agents including clindamycin, levofloxacin and moxifloxacin have also been studied. Based on the correlation between pleural fluid and serum antibiotic levels, this would suggest that these may be appropriate alternatives for susceptible organisms (Liapakis et al., 2004). Other commonly used agents including ertapenem and linezolid (particularly in the context of hospital acquired infection and MRSA) were also found to penetrate well into empyemic pleural fluid (Saroglou et al., 2010).

Extrapolating conclusions such as this from rabbit data has its limitations due to the difference in visceral pleural thickness (thinner pleura in rabbits), the mechanism of induction of empyema and variation in microbiology between a human and a rabbit. More recent studies of antibiotic concentrations in human pleural infection are lacking as pleural infection research has disproportionately focused on pleural drainage (Lau et al., 2022).

Initial selection of agent should depend on whether the patient is likely to have a community or hospital acquired infection, given the variation in microbiology and anticipated cover required. This should then be correlated with local hospital policies and antibiotic resistance patterns. In community-acquired infection, treatment with an aminopenicillin will cover the common causative organisms, but a pencillin/beta-lactamase inhibitor such as co-amoxiclav, or metronidazole should also be given due to the frequent co-existence of penicillin resistant aerobes (including *S. aureus*) and anaerobic bacteria. Clindamycin, alone, or in combination with ciprofloxacin or a cephalosporin is likely to provide good alternatives for patients with penicillin allergy (Davies et al., 2010). In the setting of hospital acquired or post-surgical infection, vancomycin and piperacillin/tazobactam will cover the added risk of MRSA and *Pseudomonas spp*. Vancomycin and meropenem may be indicated if there is a history or suspicion of extended spectrum beta-lactamase producing organisms (Shen et al., 2017)

### 1.6.3 **CHEST TUBE DRAINAGE –** adapted from Bedawi & Rahman, Pleural Effusion: Infection (Parapneumonic and Empyema); Encyclopaedia of Respiratory Medicine 2e; Elsevier 2019

In modern management of pleural infection, the traditional proverb 'the sun should never set on a parapneumonic effusion' still very much applies (Sahn and Light, 1989). As soon as pleural infection is diagnosed, a chest tube should be inserted without delay and indeed, delays to drainage beyond 2 days have been associated with worse outcomes (Meyer et al., 2018).

There is still a preference amongst some clinicians to insert larger chest tubes, but this practice does not have a robust evidence base and is not reflected in recent guidelines. Retrospective analysis of prospective data from the MIST 1 trial (n = 405) (Maskell et al., 2005) showed that initial drain size did not influence any of the predefined outcomes, including mortality, requirement for surgery, length of hospital stay, residual chest radiograph changes or lung function tests at 3 months (Rahman et al., 2010). This study included patients with multi-septated as well as frankly purulent effusions. This would seem logical when one considers the associated physiology, which demonstrates that pleural fluid flow is related to the balance between the negative transthoracic suction pressure and the compliance of the underlying lung. Larger bore drains may allow fluid to flow faster and be less prone to blockage (which can be overcome by regular flushing of smaller catheters), but eventual successful drainage is unlikely to be changed. Smaller bore drains (<15F) have the added benefit of ease of insertion, and analysis of the MIST-1 data by chest tube size demonstrates that these smaller drains do not negatively impact outcomes and are significantly more comfortable (Rahman et al., 2010). A recent systematic review and meta-analysis concluded that small bore tubes are sufficient for the initial management of pleural infection (Mei et al., 2023) and recent guidelines are in agreement with this (Roberts et al., 2023).

Rather than questioning drain size, given advances in the understanding of the underlying biology and development of intrapleural therapies (see sections 1.6.4 and 1.6.5 below), the emphasis is now on optimal placement under ultrasound guidance, securing placed tubes with bespoke dressings and sutures given the considerable rate of drains falling out in clinical practice (Asciak et al., 2018), plus attention to connecting a 3-way tap and prescribing regular saline flushes (e.g. 30 mL t.d.s) to maintain

patency (Bedawi et al., 2022b). Standard criteria for removal should also apply, including drainage of <100 mL in a 24 h period and adequate radiological resolution. Whilst previous guidelines have recommended assessing drainage success at day 5-7 for consideration of further management (Davies et al., 2010), current recommendations are for assessing medical treatment failure within 48 hours with prompt consideration of intrapleural fibrinolytic therapy or surgery.

### 1.6.4 'MEDICAL TREATMENT FAILURE'

The combination of antibiotics and drainage via a chest tube is referred to in national guidelines as 'standard medical therapy' or 'standard care'. This may fail due to a number of reasons:

1. The presence of thick infected pleural fluid which cannot easily drain down the pleural catheter. Infected fluid is thick due to DNA liberated from dead leukocytes that increases its viscosity.
2. The presence of locules which partition the fluid into separate and undrainable pockets. Locules are due to the development of fibrinous septations within the infected collection.
3. The presence of resistant collections of infecting organisms in bacterial structures known as 'biofilms'. Biofilms are described as a community of micro-organisms attached to a surface, producing extracellular polymeric substance (EPS). The organisms exhibit an altered phenotype compared with the corresponding planktonic cells, and the EPS is a complex matrix made up of both fibrin and free DNA, which both serves as a storage facility for nutrients and entraps other microbes and non-cellular materials. Biofilm bacterial cells withstand host immune responses and are much less susceptible to antibiotics than their non-attached individual planktonic counterparts.

One of the major methodological flaws of previous comparative (medical vs surgical) studies (Bilgin et al., 2006; Oğuzkaya et al., 2005; Wait et al., 1997) was the absence of objective criteria to determine whether or not standard care had been successful, i.e. true 'medical treatment failure'. The 2010 BTS guidelines addressed this and recommended that medical treatment failure in pleural infection be defined as the presence of residual and clinically significant pleural collection (based on chest radiograph, ultrasound and/or CT) plus at least one of the following:

- Clinical evidence of ongoing sepsis as manifested by fever, tachycardia, or hypotension.
- A serum CRP that fails to fall by more than or equal to 50% compared to baseline.
- A lack of significant response in peripheral blood WCC.

In more recent studies, objective medical treatment failure criteria have been considered. The recent PILOT study, the largest prospective observational pleural infection outcome study (n=546), encouraged clinicians to using objective medical treatment failure decisions before escalating to the treatment of their choice; surgical referral or intrapleural therapy and probably provides the best estimate of true medical treatment failure rate, which is 34% (Corcoran et al., 2018).

### 1.6.5 INTRAPLEURAL FIBRINOLYTIC MONOTHERAPY

The theory that the increasing amount of fibrin and density of the septations within the infected pleural space may be the reason behind failure of standard medical treatment, sparked interest in intrapleural fibrinolytic therapy (IPFT). This was described as early as 1949 by Tillett et al. using streptokinase, but resulted in significant immunological side effects (Tillett and Sherry, 1949). Whilst these problems were likely to have been due to contamination of the streptokinase during production, this approach was abandoned for decades. Purified streptokinase and urokinase then became available in the late1980's with variable clinical uses (for example to treat myocardial infarction). A number of small studies over the next 2 decades demonstrated some clinical benefit of instilling these agents intrapleurally in empyema. In 2004, a meta-analysis concluded that while there was potential benefit from IPFT in pleural infection, this was insufficient to recommend its routine use in clinical practice, mainly attributed to small sample sizes and heterogeneous study designs (Cameron and Davies, 2004). Following on from this, Diacon and colleagues conducted the first RCT of intrapleural streptokinase (n=53) with purely clinical primary outcome measures; clinical treatment success and need for referral to surgery (Diacon et al., 2004). They reported that after 7 days, streptokinase-treated patients had a higher clinical success rate (82% versus 48%, $p$=0.01) and fewer referrals for surgery (43% versus 9%, $p$=0.02). However, this study had 2 important limitations. Firstly, the failure rate in the control arm was unusually high at 50% [versus 25-35% in the literature at that time (Chin and Lim,

1997; Davies et al., 1997)]. Secondly, they referred patients for surgery either due to ongoing sepsis or lack of radiological improvement at day 7. Again, previous literature (and clinical judgement) would suggest that both criteria are required for consideration of surgery and in fact, in the same study, Diacon et al. showed that in the absence of ongoing sepsis, residual radiographic opacity resolved spontaneously with no long-term sequelae.

The results of the MIST-1 trial were published in 2005, challenging pre-existing assumptions and concluding that intrapleural administration of streptokinase did not improve mortality, the rate of surgery, or the length of the hospital stay among patients with pleural infection (Maskell et al., 2005). Compared to the Diacon paper, the MIST-1 study population was older with more significant comorbidity and this may have contributed to its negative results. In an attempt to be pragmatic and reflective of 'real life' practice, the management decisions in MIST-1 were made by the clinicians at the bedside, while the interventions in the Diacon study were guided by a predefined protocol. With the benefit of the current knowledge of the importance of imaging, it is impossible to determine what proportion of the MIST-1 study participants had sonographic septations or extensive pleural thickening. As the study recruited 'all comers', this is likely to have led to a heterogeneous sample spanning all stages of empyema formation. Thus there is still uncertainty as to whether IPFT alone might be beneficial in some subgroups and certainly, in some parts of Europe and the developing world due to limited access but recent guidelines are now clear that monotherapy should not be used in the treatment of pleural infection (Bedawi et al., 2022b; Chaddha et al., 2021; Roberts et al., 2023)

### 1.6.6 COMBINATION INTRAPLEURAL ENZYME THERAPY (IET)

Whilst streptokinase may help break down septations, it was not postulated to alter fluid viscosity, nor does it prevent formation of bacterial biofilms in the infected pleural space. Data from cystic fibrosis patients demonstrates that nebulised DNase is effective in reducing viscosity of sputum and enhancing airway clearance (Jaffé and Bush, 2001). Two laboratory studies, assessing the effects of fibrinolytic and DNase on samples of purulent pleural fluid from pleural infection, were published in 2000, suggesting that DNase, in combination with a fibrinolytic, could be effective therapy (Light et al., 2000; Simpson et al., 2000). The MIST-2 randomised trial, published in

2011, recruited 210 patients over 3 years from 11 UK centres (Rahman et al., 2011). It was designed as a double-dummy, double-placebo RCT with 4 arms comparing placebo, tPA (tissue plasminogen activator) alone, DNase alone and combination tPA and DNase (Figure 1.6). The primary outcome was absolute reduction in CXR opacification, using a validated digital measurement protocol, to exclude interpretation bias.

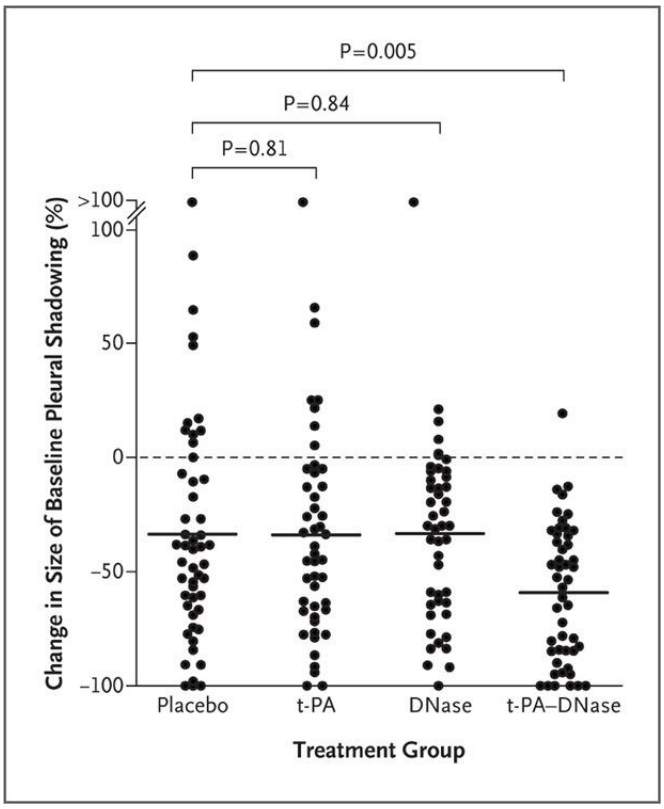
Secondary outcomes included duration of hospital stay, referral to surgery, and death. The study showed combination therapy had a statistically significant benefit on several of these measures – e.g. reduced need for surgery (OR for surgery 0.17, 95% CI 0.03 to 0.87) – less than 5% required surgery - and reduced hospital stay (6.7 days, 95% CI 12.0 to 1.9, p = 0.006) compared to placebo. Mortality rates, however, did not differ significantly between groups.

The study confirmed that neither fibrinolytic alone (as in MIST-1) or DNase in isolation were better than placebo. This led to the conclusion that the addition of DNase had a significant synergistic effect, primarily through a combination of adhesion/septation lysis (tPA) as well as reduction of viscosity (DNase). Additional benefits have been hypothesized including biofilm degradation by DNase and a potential 'lavage' component through a class effect of fibrinolytic-induced enhanced pleural fluid formation mediated by mesothelial cell-derived monocyte chemotactic protein (MCP)-1 (Lansley et al., 2015).

It is also important to note that to date, there is no data that IET reduces mortality, or that it is superior to surgery in resolving pleural thickening and in turn, improving lung function and improving time to recovery and quality of life.

The limited number of patients in the combination arm (n=52) meant that routine use could not be recommended in all patients, but until larger trials are published, it is a useful option in cases where standard of care chest drainage has failed and where patients are not suitable surgical candidates. Similarly, as concluded by a recent Cochrane meta-analysis, larger cohorts are needed to consolidate the safety profile (Altmann et al., 2019), although case series data have shown no safety concerns and high efficacy (Piccolo et al., 2015).

**Figure 1.6. Change in Area of Pleural Fluid on Chest Radiography on Day 7 versus Day 1, According to Study Group. From Intrapleural Use of Tissue Plasminogen Activator and DNase in Pleural Infection (MIST-2), Rahman et al, NEJM 365:518-526 Copyright © (2011) Massachusetts Medical Society. Reprinted with permission.**




Side effects are infrequent and generally mild. Pain requiring escalation of analgesia, particularly following the first treatment dose, is the most commonly reported symptom in 15-20% of patients but compliance and tolerance are generally favourable (Mehta et al., 2016; Piccolo et al., 2014).

Prior to the publications demonstrating safe and effective use, there was concern regarding risk of bleeding associated with IPFT/IET. Systemic bleeding from intrapleural tPA/DNase is exceedingly rare, likely due to low systemic absorption and the short half-life of tPA. In addition, lower doses are used in this context, compared to those used intravenously in thrombolysis. In 344 cases from five published series, significant pleural bleeding (defined as requiring blood transfusion) was reported only in 11 (3.2%) cases, all of which were managed conservatively with none fatal (Komissarov et al., 2018). Nonetheless, a recent Cochrane meta-analysis concluded that there is a paucity of studies specifically evaluating the safety of IET and that further studies are needed to consolidate this.

The dosing regimen for intrapleural tPA/DNase used in MIST2 was empirically based on earlier case reports. Some recently published pilot data has looked at the effectiveness of dose de-escalation of the MIST-2 protocol, and suggests that halving the dose of tPA (to 5mg twice daily intrapleurally) with the same 5mg dose of DNase is both safe and effective (Popowicz et al., 2017). This regime is yet to be tested in a RCT setting. Despite a similar bleeding incidence of approximately 6%, such a regime may help alleviate some of the concerns surrounding the use of IPFT, as well as making it more cost-effective. A small prospective observational study of 38 patients looked to simplify the regime, comparing concurrent vs sequential intrapleural instillation of tPA and DNase and hence removing the 1hr interval between each administration, and reported similar treatment success (Kheir et al., 2018). Another study changed the frequency to once daily in 55 patients and was able to treat 92.7% successfully, without the need for surgical intervention (Mehta et al., 2016). A recent case series published a successful experience of treating 10 patients with multiloculated empyema by sequential delivery of 2 or more courses of intrapleural tPA/DNase to non-communicating pleural pus collections within the same hemithorax, with no incidence of haemorrhage, escalation in analgesic requirement or adverse reactions (Biswas et al., 2016).

An attractive idea in this era of personalized medicine is the question of whether the dose of IPFT can be individualized. Samples of pleural fluid from the MIST-2 study were found to have highly variable fibrinolytic potential prior to treatment, and it has been suggested, though not yet clinically tested, that patients with reduced plasminogen activator activity theoretically require higher doses of fibrinolytics (Lee et al., 2016); novel biomarkers indicating ‘fibrinolytic targets’ to guide such individual regimens are required.

### 1.6.7 SURGICAL MANAGEMENT OF PLEURAL INFECTION

Despite reasonably high success rates with medical therapy and increased use of IET, surgery continues to play an important role in the management of pleural infection. While there is significant inter-patient variability, patients presenting later in the evolution of empyema with an organised/fibrotic pleural cortex or ‘rind’, are less likely to achieve a full recovery and lung re-expansion without surgical intervention.

In recent years, newer “keyhole” surgery (Video Assisted Thoracoscopic Surgery, VATS, using 3 small incisions in the chest under general anaesthetic) is used in patients who have failed initial treatment, as it is less invasive than traditional surgery such as thoracotomy (Shen et al., 2017) (which requires an up to 25cm incision in the chest wall).

Early surgery has been advocated in pleural infection (Shen et al., 2017) based on two randomised studies which compared standard care (antibiotics and chest tube drainage) to early VATS (Bilgin et al., 2006; Wait et al., 1997). Both demonstrate earlier hospital discharge with VATS but are underpowered (90 patients total) and have important methodological weaknesses (e.g. unclear criteria for medical failure, lack of objective decision-making criteria). In addition, there is likely significant selection bias, as the patients undergoing surgery were younger (median age 43 vs 60 years) and less co-morbid than the average patient with pleural infection (Rahman et al., 2011; Wait et al., 1997). Results from these studies have therefore not been adopted into clinical practice. Recent randomised trials of thoracic surgery in the UK have demonstrated the ability of the surgical community to effectively randomise, including patients with higher ages than those noted above (Lim et al., 2022) (69 years old, 93% completion rate of surgery).

Delays in surgical intervention are a predictor of conversion of thoracoscopic to open surgery (Bedawi et al., 2022b; Schneiter et al., 2008; Towe et al., 2019); it is therefore very plausible that *earlier* surgical intervention may be beneficial. However, surgery is associated with significant risks including the risk of general anaesthetic, a current 10% rate of conversion of VATS to a larger operation (thoracotomy), and 20% of patients undergoing thoracotomy experience long term chest pain that requires treatment with analgesics. In addition, there is no evidence which addresses *when* patients failing initial therapy should be referred for surgery, despite SIGN methodology searches via national guidelines (Roberts et al., 2023); currently patients are generally referred between 3 and 7 days post initial treatment.

A recent review of the existing retrospective case series data on early surgical (VATS) treatment demonstrates a low treatment failure rate (8.4%) in a large number of patients (n=719) (Ricciardi et al., 2022). A recent meta-analysis of VATS versus open thoracotomy decortication for patients with empyema (Pan et al., 2017) demonstrates

a relapse rate (indicative of surgical failure) of 7.2%. These two studies give an approximate surgical failure rate of 8% (defined as readmission, need for another pleural procedure or another operation), however this has not been tested in RCT format.

Current guidelines advocate the use of surgery in cases of 'medical treatment failure', or when an advanced fibrotic state is suspected with extensive pleural thickening (Roberts et al., 2023). These are usually the more advanced cases where VATS debridement is more likely to fail and necessitate further surgical options such as thoracotomy and decortication. The limitations of VATS at this stage are largely due to difficulty accessing the pleural space through thick parieto-visceral adhesions using a thoracoscope, or inadequate pleural decortication to achieve lung re-expansion (Subotic et al., 2018). The reported complication rate after VATS decortication varies from 9% to 40% (Jagelavicius et al., 2017), the most frequent complications being prolonged air leak, bleeding, recurrence or persistence of the disease, surgical wound infection and a residual pleural space (Subotic et al., 2018). The 30-day post-operative mortality ranges from 2-6% (Lardinois et al., 2005) and it should again be noted that there remains a significant conversion rate to thoracotomy for VATS cases. Again, this seems to argue for the potential for earlier surgical intervention to improve outcomes.

Studies have also looked at whether any preoperative radiological features, intraoperative findings or pleural fluid microbiology can predict operative success or risk of conversion, but these have had conflicting results and therefore this literature remains inconclusive (Cassina et al., 1999; Lardinois et al., 2005; Roberts, 2003; Stefani et al., 2013; Striffeler et al., 1998). Delays in surgical intervention have been shown to be the most common predictor of conversion to open thoracotomy (Lardinois et al., 2005; Stefani et al., 2013). It is noteworthy that the guidelines (Davies et al., 2010) recommending timing of surgery after medical treatment failure, i.e. evidence of worsening infection or ongoing sepsis, were based on low quality evidence. To date, there is no robust randomised clinical trial data to inform patient selection or timing of surgery. These uncertainties constitute a significant knowledge gap.

### 1.6.8 ALTERNATIVE MANAGEMENT STRATEGIES – AMBULATORY/CONSERVATIVE MANAGEMENT, MEDICAL THORACOSCOPY, INTRAPLEURAL SALINE IRRIGATION, AND THE ROLE OF INDWELLING CATHETERS IN PLEURAL INFECTION –

adapted from Bedawi et al., ERS/ESTS Statement on the Management of Pleural Infection ERJ Feb 2023

Small parapneumonic effusions that are <5 cm on an erect lateral chest X-ray (Metersky, 2003) or <2.5 cm on CT scan (Moffett et al., 2011) can generally be managed without thoracentesis (chest tube drainage), although where diagnostic sampling is feasible, this will likely be helpful to confirm diagnosis and ascertain microbiology. A recent retrospective study confirmed that some patients with small pleural collections can be managed successfully with antibiotics alone with slightly higher but statistically insignificant infection-related mortality rate (Porcel et al., 2016b). This suggests that for very small or difficult to access pleural infection collections, it is possible in selected patients to treat with antibiotics alone without drainage of fluid, although regular review is recommended.

In some centres, iterative or repeated therapeutic thoracenteses are used as standard first line treatment (Porcel et al., 2016b). Four case series (Jouneau et al., 2015; Letheulle et al., 2014; Simmers et al., 1999; Storm et al., 1992) of patients (n=250) with CPPE or empyema who underwent iterative thoracocenteses were summatively analysed in a review of minimally invasive management of pleural infection, and a 76% successful treatment rate was reported with repeated thoracocentesis (Porcel, 2018b). The advantages proposed by advocates of this technique are that the patients are more mobile than they would be with a chest drain *in situ*, different locules may be targeted at each aspiration procedure, and that there is a possibility of outpatient management reducing hospital stay and cost (Jouneau et al., 2015). One recently published retrospective comparative study of two successive cohorts of patients with CPPE or pleural empyema in whom repeated thoracentesis with intrapleural urokinase (n=52) vs. intrapleural urokinase plus DNase (n=81) was applied as the first line treatment, showed failure rates of 17% and 19% respectively (Luque Paz et al., 2021). It would seem a reasonable option for lower risk patients without evidence of systemic sepsis and small-moderate volume effusions; however, to date there is no RCT data to support this as a first line option and it is currently not recommended by any guidelines (Davies et al., 2010; Shen et al., 2017). Importantly, the associated

healthcare resource utilisation and the potential increased risk of repeated procedure-related complications have not been adequately studied.

If fibrinolytics are contraindicated (e.g. due to previous allergic reaction), pleural saline irrigation has been shown to be a potentially useful therapeutic option. In 2015, Hooper et al. conducted the first RCT of pleural irrigation with normal saline versus standard care alone in patients with pleural infection. The administration regimen consisted of 250 ml 0.9% sodium chloride into the pleural space; the tube was then clamped for an hour before being open to free drainage. This was repeated 3 times a day for a total of nine irrigations and demonstrated a superior resolution of CT pleural fluid volume (primary outcome) over the course of the treatment compared to standard care alone, as well as a reduction in surgical referrals (secondary outcome) (Hooper et al., 2015). It is noteworthy that the 50% surgical requirement in the control group is very high compared to other RCTs, and this was an unblinded study. Two retrospective studies (Guinde et al., 2021; Porcel et al., 2017) have also demonstrated that intrapleural saline irrigation may be useful in the management of pleural infection but further studies are required in larger multicentre RCT settings.

Medical thoracoscopy is well established in the management of pleural effusion, however, its role in pleural infection is less clearly defined. Advocates of medical thoracoscopy have demonstrated success rates of 79.3% - 97.7% in multi-loculated organising empyema (Hardavella et al., 2017; Ohuchi et al., 2014; Ravaglia et al., 2012; Tacconi et al., 2010). A recent meta-analysis of non-randomised studies reported a pooled treatment success rate of 85% when utilised as first-line therapy or after failure of chest tube, with a complication rate of 9% (Mondoni et al., 2021). Higher success rates were associated with bacteriological negative effusions and administration of adjuvant intrapleural fibrinolysis (Mondoni et al., 2021). A recent RCT of medical thoracoscopy versus intrapleural fibrinolytic therapy showed a shorter LOS post intervention associated with the thoracoscopy arm (Kheir et al., 2020). The small numbers within the trial and the limitations of the primary outcome require further studies to establish the true role of medical thoracoscopy in empyema. The Studying Pleuroscopy in Routine Pleural Infection Treatment (SPIRIT) feasibility randomised trial (ISRCTN98460319) has not been published, but widely presented at national conferences and pleural research meetings, and demonstrated failure of feasibility of this approach in the context of UK thoracoscopy services.

Whilst more commonly used in the setting of malignancy, in the context of pleural infection, indwelling pleural catheters (IPCs) are relevant in two ways; firstly catheter-related pleural infection as a complication of IPC insertion, and secondly IPCs as a therapeutic option for the outpatient management of chronic pleural infection, especially with trapped lung. In a recent Modified Delphi Consensus Statement on the management of IPCs, two types of infectious complications were defined: local IPC-related infections (including catheter associated cellulitis, exit site infection, tunnel tract infection) and IPC-related pleural space infection (Gilbert et al., 2020; Miller et al., 2021). In a large multicentre retrospective review of 1,021 patients treated with IPC, pleural space infections developed in 50 (4.9%) patients with an overall mortality risk of 0.3% (Fysh et al., 2013), significantly lower than standard pleural infection. In another large multicentre series (n=1318), Wilshire et al recently found a similar infection rate (6-7%) but importantly also showed that the risk of IPC-related infection did not appear to be increased by antineoplastic therapy use or an immunocompromised state. In multivariable competing risk analyses they found longer IPC *in-situ* duration to be associated with a higher risk of infection (Wilshire et al., 2021).

IPC-related infections generally tend to occur around 6 weeks post insertion (Fysh et al., 2013; Wilshire et al., 2021), which goes against them being directly procedure-related, however studies investigating the mechanisms leading to pleural space infections in this group are lacking (Lui et al., 2016). They are most frequently reported in association with *S. aureus* organisms, followed by *P. aeruginosa* but to date, there are no studies specifically evaluating the bacteriology and significance of bacterial colonisation in this cohort [52]. Most patients can be successfully treated with oral antibiotics (3-4 weeks) and attaching the catheter to an underwater seal drainage bottle for continuous drainage, without need for IPC removal or replacement (Fysh et al., 2013; Gilbert et al., 2020). Although this condition rarely requires surgical intervention (Porcel et al., 2020), early discussion with thoracic surgical teams is usually conducted if the patient is receiving systemic chemotherapy. An additional chest drain and surgical intervention is sometimes considered especially if there is evidence of undrained collections contributing to systemic sepsis (Gilbert et al., 2020). Longer antibiotic courses are frequently required and intrapleural enzyme therapy

(IET) via the IPC is another therapeutic option for patients who are not surgical candidates (Gilbert et al., 2020; Miller et al., 2021).

Recurrent or chronic pleural infection creates difficult management issues, especially in those with trapped lung and where there is no surgical option. Small studies and case series have shown IPC's to be a potentially useful treatment strategy for achieving longer term sepsis control in those candidates who are not fit for surgery or those who decline it (Davies et al., 2008; Saqib et al., 2017).

## 1.7 OUTCOME PREDICTORS AND MEASURES IN PLEURAL INFECTION

Data from a large Danish cohort (Meyer et al., 2018) found that a delay in instituting pleural drainage by more than 2 days from diagnosis was associated with both a worse 30-day and 90-day mortality. Delayed surgical referral has been shown to be associated with risk of conversion to thoracotomy and worse outcomes, with each additional preoperative hospital day (up to 5 days) being associated with 1.2x increased risk of mortality per day (Towe et al., 2019). The current practice of sequential progression of therapies from chest tube drainage to intrapleural therapies to consideration of surgical intervention, in a 'one size fits all' pathway may be to the detriment of certain patients. Despite significant advances in the assessment and management of pleural infection over the course of the last two decades, major improvements in outcomes have been lacking. It would be of great value if clinicians were able to identify patients with a worse prognosis at presentation, to better evaluate the risk associated with these more aggressive therapies, potentially even intervening earlier in the course of the illness.

Challenges of predicting clinical outcome include the variable presentation and the variable speeds at which patients progress (or not) through the various stages in the pathophysiology. Some patients with short history and apparently mild illness at presentation progress rapidly, requiring rescue therapy within 2-3 days. Conversely, in a study of thoracoscopy in patients with apparently chronic pleural infection, over half were found to have no evidence of intrapleural scar tissue, and hence were still at the fibrinopurulent stage of their infection (Landreneau et al., 1996).

Traditionally used predictors of outcome in pleural infection such as fluid purulence have not been confirmed in clinical studies specifically designed to assess their use (Davies et al., 1999), and large randomised data (MIST-1) showed no association between fluid purulence and poor clinical outcome. As detailed above, the significance of septations is uncertain and does not seem to predict outcomes based on current studies. Despite studies looking at novel biomarkers in pleural infection (Dixon et al., 2017; Ozsu et al., 2013; Porcel et al., 2009; Wu et al., 2017), and clinically important factors influencing its measurement relating to sample collection method (Rahman et al., 2008), to date, pleural fluid pH level of less than 7.2 (cut-off agreed by most international guidelines) continues to be the single most important and widely-used indicator of need for drainage. However, pleural fluid pH was not shown to independently have significant outcome prediction value in studies specifically looking at risk stratifying pleural infection patients at presentation (Rahman et al., 2014). Better biomarkers and/or more complex scoring systems are urgently needed.

### 1.7.1 QUALITATIVE / QOL OUTCOMES IN PLEURAL INFECTION

There is a paucity of data regarding quality of life (QOL) and functional ability of patients during and following pleural infection. A previous study of adult pleural infection patients identified forced expiratory volume over one second to return to normal in 58% (60/104) of patients at a median of 62 months follow-up (Casali et al., 2009). An additional trial specifically reviewed time to return to work in the surgical management of pleural empyema, noting a return to work at a median of 34 days within the thoracotomy group and a median of 25 days within the video assisted thoracoscopy group (Cardillo et al., 2009).

Previous trials in pneumonia demonstrate that full symptomatic recovery may take up to 4 weeks following infection, with these infections having a deleterious impact on the QOL of elderly patients up to a year following discharge (Mangen et al., 2017; Metlay et al., 1998).

Another publication noted median return to work at 15-24 days, but 18-50% of patients hadn’t returned to their baseline after four weeks (Pick et al., 2019). None of these relate directly to pleural infection. The often subacute presentation and catabolic state that ensues with pleural infection in combination with a significantly greater burden of treatment compared to pneumonia suggests that time to, and indeed completeness,

of recovery are likely to be substantial. Due to the limited studies, clinicians are limited in their ability to have an informed discussion with patients and their relatives, and this is an important area of unmet research need in the literature. The MIST-3 study, which will inform the largest body of work of this thesis will aim to add to this.

### 1.7.2 OUTCOME PREDICTION - CLINICAL

The RAPID score was developed as the clinical outcome prediction tool specifically for pleural infection (Rahman et al., 2014) and is the most rigorous published evidence to date at a prognostic model that can help predict a patient's outcome from pleural infection at presentation. It was derived after 22 baseline characteristics were examined on the MIST-1 cohort (Maskell et al., 2005) and subsequently validated on the MIST-2 cohort (Rahman et al., 2011). Five characteristics [Renal (i.e. urea), Age, Purulence (with non-purulent fluid being associated with higher risk), Infection source (community vs hospital) and Dietary factors (i.e. albumin)] were found to be strongly independently associated with poor outcome (Table 1.2). Each patient's score ranged between 0-7 and could be categorised into low, medium, and high risk giving a broad estimation of 3-month mortality.

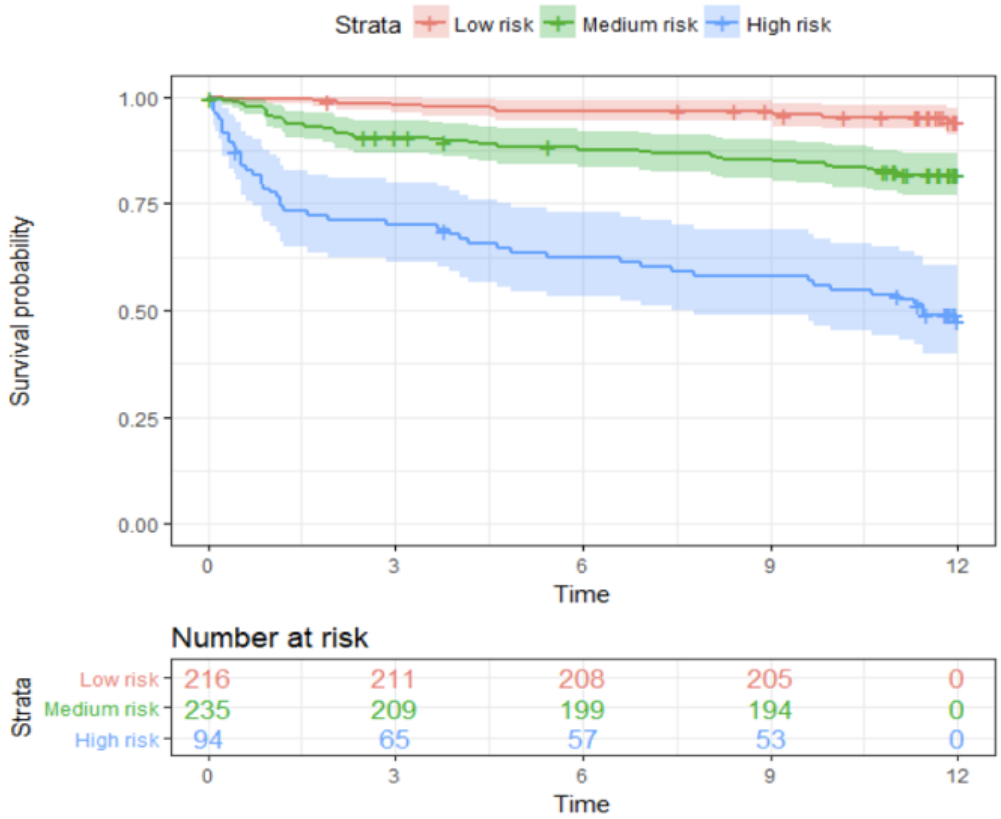
**Table 1.2 – the RAPID score**

| Parameter | Measure | | Score |
|---|---|---|---|
| Renal | Urea | <5mmol/L<br>5-8 mmol/L<br>>8 mmol/L | 0<br>1<br>2 |
| Age | Age | <50 years<br>50-70 years<br>>70 years | 0<br>1<br>2 |
| Purulence of fluid | Purulent<br>Non-purulent | | 0<br>1 |
| Infection Source | Community acquired<br>Hospital acquired | | 0<br>1 |
| Dietary Factors | Albumin | >27mmol/L<br><27mmol/L | 0<br>1 |
| | | | |
| Risk categories | **Score 0-2**<br>**Score 2-4**<br>**Score 5-7** | | **Low risk**<br>**Medium-Risk**<br>**High Risk** |

The RAPID score recently underwent prospective external validation in the international multicentre observational (PILOT) study (n=546) (Corcoran et al., 2020b),

where patients were treated according to standard guidelines and local practice. PILOT demonstrated robust clinical ability of the RAPID score to stratify patients into different categories according to increasing risk of three-month mortality (Figure 1.7).

**Figure 1.7 – Kaplan Meier survival plot based on RAPID stratification (taken from the PILOT study) (Corcoran et al ERJ 2020 with permission)**




One interesting observation from the PILOT study was the higher rate of surgical referral in the low-risk group (19%) compared to the high-risk group (5.9%). No significant differences were observed in rates of intrapleural therapy between the 3 groups, but the overall rate of intrapleural fibrinolytic therapy in this study was low, making it difficult to draw conclusions.

Despite the PILOT study specifically excluding patients with an expected survival of less than 3 months due to pre-existing comorbidity, the majority of deaths occurred within the first three months following diagnosis of pleural infection, as has been seen in previous studies (Davies et al., 1999; Maskell et al., 2005), suggesting that mortality is disease-specific and potentially amenable to improvement.

Since the PILOT study (Corcoran et al., 2020b), RAPID has been assessed in a number of single centre, retrospective studies in the USA, New Zealand and Japan, which have all further validated its clinical applicability, association with mortality (Touray et al., 2018; White et al., 2015; Wong and Yap, 2016; Yamazaki et al., 2019), treatment costs (Touray et al., 2018), and more recently its use has been explored in surgical selection of patients (Liou et al., 2023).

Whilst the RAPID score represents a major step forward in the ability to prognosticate patients with pleural infection, it cannot yet direct clinical care or decision making. The main goal now should be to incorporate it into future prospective studies assessing the safety and efficacy of new treatment paradigms – perhaps using less invasive, ambulatory strategies in the RAPID ' low risk' population (Porcel, 2018b) and early invasive treatment such as surgery or IET in the high risk groups. RAPID may also be used to inform clinicians' discussions of the likely outcome from pleural infection at presentation and the balance of risk or benefit from any planned medical or surgical intervention.

Examining the literature for other non-RAPID clinical prognostication tools in pleural infection, the Charlson Comorbidity Index (CCI) has been shown to be a reasonable predictor of outcome in 3 pleural infection cohorts (Bobbio et al., 2021; Shen et al., 2012; Søgaard et al., 2014). Other clinical factors shown to be associated with adverse outcomes in pleural infection may also be helpful in overall prognostication and rationalisation of further/earlier intervention in individual cases. These include malignancy, alcohol excess and cardiovascular disease (Cargill et al., 2019), the latter having also been associated with prolonged LOS in the RAPID study. An important caveat here is that, in contrast to the RAPID criteria, the majority of these are derived from hospital episode statistics from administrative databases that are flawed by coding inaccuracies and thus represent a lower level of evidence.

### 1.7.3 OUTCOME PREDICTION - MICROBIOLOGY

Bacteria can contribute to the process of pleural organisation through formation of biofilms, bacterial aggregates embedded in a matrix comprising polysaccharides, lipids, proteins including fibrin and extracellular DNA, and hence potentially amenable to degradation by fibrinolysins (Jørgensen et al., 2016). Various studies have found

that the outcome of pleural infection is affected by the causative organism and the setting of infection. Although yield using conventional techniques is low, culture-positivity in its own right has been associated with higher mortality (Brims et al., 2019). In patients referred for decortication, pleural fluid culture positivity was associated with longer duration of hospital stay and worse surgical outcomes (Okiror et al., 2014). It is particularly of interest that PAI-1 levels (see Section 1.8 below) are higher in pleural effusions from gram positive bacteria, compared to gram negative and uncomplicated culture negative parapneumonic effusions (Lee et al., 2018)

In a study examining the bacteriology of 164 culture-positive pleural infections, isolates from the *Streptococcus* genus were the most common, but when specifically looking at patients who required ICU admission, the most common isolate was *Klebsiella pneumoniae* (Lin et al., 2008). In another study involving patients with community acquired pleural infections, non-*S. milleri* pleural sepsis was associated with longer durations of hospitalization (Lindstrom and Kolbe, 1999). Analysis of the bacteriology of patients from the MIST1 trial (Maskell et al., 2006b) revealed that one-year mortality was significantly worse for patients with infections caused by *S. aureus* or mixed aerobic infection as opposed to infections caused by *S. milleri* or mixed anaerobic infection. The same study looked at the source of infection and its effect on patient outcomes and found that mortality was higher with HA infection (commonly caused by the earlier groups of organisms) in comparison to CA infections (typically caused by the latter group) (Maskell et al., 2006b). Another study also found that HA infection was a risk factor for worse 30-day mortality in patients with pleural infection (Park et al., 2016b).

Association between bacterial patterns and 1-year survival was amongst the primary outcomes of the recent TORPIDS study (Kanellakis et al., 2022), discussed in Section 1.4.3 above. The presence of anaerobes or bacteria of the *S. anginosus* group *(S. anginosus, S. intermedius, S. constellatus)* was associated with better patient survival. The presence or dominance of *S. aureus* was linked with lower survival, while dominance of *Enterobacteriaceae* was associated with higher risk of death perhaps due to being more resistant to antibiotic therapy. Given that *S. aureus* was recently found to be the most common organism isolated regardless of study or setting with increasing prevalence of methicillin resistance (Hassan et al., 2019b), there is likely to be a role for earlier escalation of therapy and vigilant follow up in this patient group.

### 1.7.4 OUTCOME PREDICTION - RADIOLOGICAL

Defining radiological parameters predicting outcomes have been challenging to study in pleural infection, as studies to date have been largely small, retrospective and have demonstrated that radiology tends to predict clinician behaviour rather than true outcome from pleural infection (Porcel et al., 2016a). One study of n=84 patients focused specifically on predicting IET failure using statistical modelling and machine learning, and found the presence of pleural thickening and necrotising pneumonia or lung abscess to be associated with IET failure (Khemasuwan et al., 2018).

One of the largest and more recent studies was in a French nationwide retrospective cohort study (n=25,512 empyema hospitalisations) specifically assessing the epidemiology and prognostic factors. They identified the CT evidence of a bronchopleural fistula (present in 31% of their large cohort) to be independently predictive of mortality (OR 2.09 99%CI 1.88-2.32) (Bobbio et al., 2021).

Sonographic septations (discussed in more detail in Section 1.5.2) have been of great interest due to the widespread and commonplace use of thoracic ultrasound. Their presence tends to be assumed to be linked to worse outcomes and upfront intrapleural and/or surgical therapy but the evidence truly associating septations with worse clinical outcomes is sparse (Bedawi et al., 2018).

### 1.7.5 BIOLOGICAL – NOVEL BIOMARKERS

Current guidelines advocate early diagnostic sampling of pleural fluid using a pH <7.2, glucose <2.2mmol/l and LDH>1000IU/l to be consistent with a diagnosis of pleural infection. To date, the pleural fluid pH remains the best predictor of the requirement for chest tube drainage, but this is based on a meta-analysis of 7 studies incorporating just n=251 patients that never underwent prospective validation. Moreover, pleural fluid pH has no ability to predict the requirement for fibrinolytics or surgery.

Studies have attempted to identify methods of earlier diagnosis or other means of rationalising pleural fluid drainage. Numerous biomarkers, such as inflammatory cytokines (tumour necrosis factor-alpha/TNF-α, interleukin-8/IL-8, and IL-1β), enzymes (neutrophil elastase, myeloperoxidase/MPO, and metalloproteinases/MMPs), C-reactive protein (CRP), and soluble triggering receptor

expressed on myeloid cells-1 (sTREM-1), have been evaluated but not proven superior to traditional criteria (Alegre et al., 2002; Alemán et al., 2003; Iglesias et al., 2005; Porcel et al., 2009, 2004).

Other markers such as Interleukin-18 have been found to be linked to the intensity of neutrophilic pleural inflammation in patients with pleural effusions and up-regulated in the pleural space of patients with empyema, but clinical applicability remains unclear (Rovina et al., 2013). Recently, Wu et al. conducted comprehensive proteome profiling of pleural fluid from simple and complicated parapneumonic effusions to assess the performance of four novel proteins – BPI (bactericidal permeability-increasing protein), NGAL (neutrophil gelatinase-associated lipocalin), AZU1 (azurocidin-1) and calprotectin. BPI, a neutrophil granule protein with antimicrobial activity against bacteria, was found to be superior to LDH, glucose and pH in the diagnosis of CPPE with an AUC value of 0.966. Furthermore, the combination of pleural fluid BPI levels with LDH levels improved the sensitivity to 100% for identifying CPPE (Wu et al., 2017). The authors did not evaluate how using BPI would change management e.g., decision to drain or influence outcomes, so the benefits in a clinical setting are yet to be proven. The finding that BPI levels were twice as high in patients with empyema, makes this a potentially interesting area to explore in the clinical setting against pleural aetiologies that can occasionally mimic pleural infection biochemically, such as malignant pleural effusion and inflammatory pleuritis, when the clinical context is unclear, and diagnosis cannot be supported further by negative microbiology.

Recently, Arnold et al. demonstrated that pleural fluid suPAR (soluble urokinase plasminogen activator receptor) more accurately predicted the need for more invasive management compared to conventional biomarkers, as assessed by referral for intrapleural fibrinolytic therapy or thoracic surgery (Arnold et al., 2020). suPAR is the soluble form of uPAR (urokinase type plasminogen activator receptor), which, once bound to endogenous uPA (urokinase), catalyses the conversion of plasminogen to plasmin (a potent fibrinolytic). To make a firm statement about the clinical relevance of suPAR will require an external prospective validation cohort with predetermined criteria for intrapleural fibrinolytic therapy and/or surgery. However, this study adds credence to the role of baseline pleural fluid biomarkers of fibrinolytic activity, perhaps through regulation of the development of pleural loculation, in predicting clinically important outcomes.

Apart from suPAR, other new inflammation-and fibrinolysis related biomarkers such as PAI-1 and its activity have been implicated in the pathogenesis of pleural injury outcomes (Florova et al., 2015; Komissarov et al., 2018). The following section will review PAI-1, which will form an important focus of this thesis.

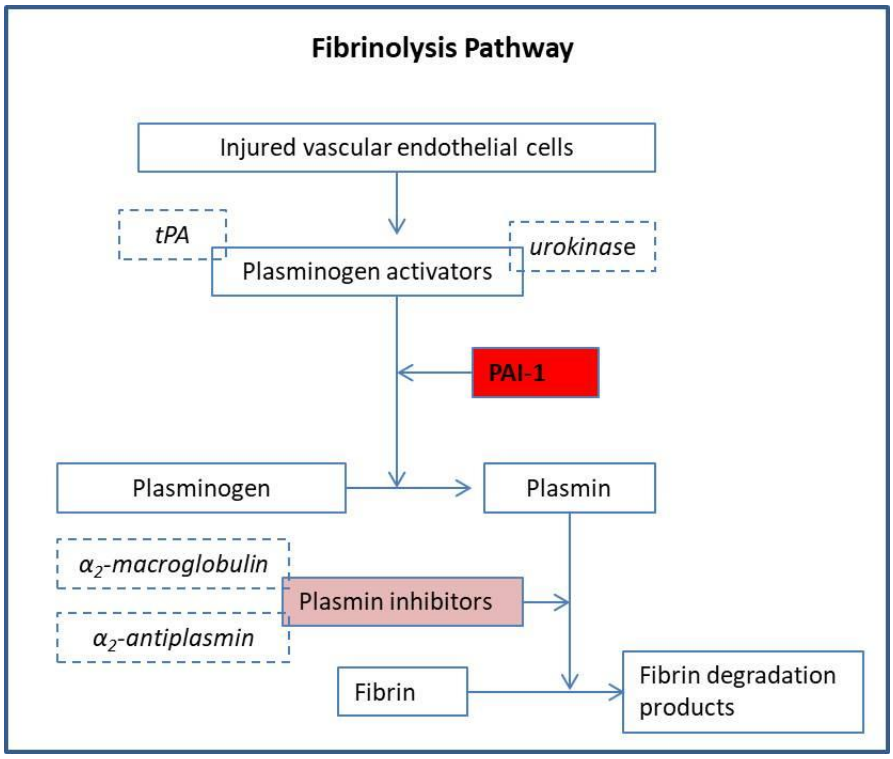
## 1.8 PLASMINOGEN ACTIVATOR INHIBITOR 1 (PAI-1)

### 1.8.1 OVERVIEW AND GENERAL BIOLOGY

The plasminogen activator system (PAS) has been implicated in a number of physiological and pathological processes beyond coagulation and fibrinolysis, including inflammation, malignancy and wound healing (Kruithof, 2008). A key step in the PAS is conversion of plasminogen to plasmin by plasminogen activators (Fay et al., 2007). Plasmin, a serine protease, is the main enzyme in the PAS and plays a key role in the fibrinolysis pathway. It is generated from its precursor plasminogen by two types of plasminogen activators, tissue-type plasminogen activator (t-PA) and urokinase-type activator (u-PA), which are responsible for the cleavage of plasminogen to form plasmin targeting a specific Arg-Val peptide bond located within the protease domain (Kruithof, 2008). The activity of both of these is regulated by the specific plasminogen activator inhibitors (PAIs), the principal and most important of which is Plasminogen Activator Inhibitor 1 (PAI-1) (Figure 1.8) (Cesari et al., 2010).

PAI-1 is one of the most studied biomarkers of the fibrinolysis system. It is also known as endothelial plasminogen activator inhibitor, or serpin E1. PAI-1 is a single chain glycoprotein member of the superfamily of serine-protease inhibitors (or serpins) that in humans is encoded by the *SERPINE1* gene. Its expression in plasma (as well as that of tPA) has been shown to positively correlate with ageing (Hashimoto et al., 1987; Yamamoto et al., 2005) as well as being significantly enhanced in a variety of clinical conditions involving increased inflammation.

**Figure 1.8 - Summary flow chart of the fibrinolysis pathway**




Whilst endothelial cells are an important source of PAI-1, PAI-1 is also produced by megakaryocytes (and is abundant in circulating platelets), smooth muscle cells, monocytes/macrophages, fibroblasts, adipocytes, peritoneum, cardiac myocytes, liver cells and mesothelial cells (Zorio et al., 2008). After release into the bloodstream, PAI-1 is present either in an active form or, more frequently, complexed with either t-PA or vitronectin (a relatively thermostable glycoprotein, which is able to stabilise and convert PAI-1 into an active form) (Kohler and Grant, 2000). The increased expression of PAI-1 *in vivo* suppresses fibrinolysis, consequently leading to the pathological fibrin deposition and tissue damage (Aso, 2007).

PAI-1 is also considered an acute phase reactant, closely influenced by inflammatory cytokines (IL-6, IL-1, TNF-alpha), growth factors (TGF-beta), and hormones (insulin, glucocorticoids, adrenaline) (Cesari et al., 2010). Circulating plasma PAI-1 concentrations have shown to be, in part, genetically determined, while also having several metabolic determinants including insulin resistance, body mass index and plasma lipids (Cesari et al., 2010). PAI-1 levels tend to be higher in males (Krishnamurti et al., 1988) and also vary by race/ethnicity, seen to be lowest in Afro-Caribbean and highest amongst the Chinese (Lutsey et al., 2006). Differences in body

composition and adipose tissue distribution may account for a large part of this variability. (Cesari et al., 2010). Diurnal variation in PAI-1 secretion has also been well-documented, shown to be highest in the morning resulting in a reduction of fibrinolytic activity in healthy individuals, and importantly, in patients with coronary artery disease (classically associated with an early morning increased risk of acute coronary syndrome) (Angleton et al., 1989).

### 1.8.2 PAI-1 IN PLEURAL INFLAMMATION

A number of factors including the variable response of different patients to pleural infection, the efficacy of the local immune response, stage of disease at treatment onset and co-morbidities could influence outcomes of chest tube drainage or response to IET.

Development of pleural fluid septations is thought to be initiated by the activation of the coagulation cascade, resulting in deposition of fibrin to form fibrin sheets. The importance of pleural fibrin deposition in the pathogenesis of pleural disease was first demonstrated by Idell and colleagues (Idell et al., 1991) through a series of clinical and experimental observations. They hypothesised that the local equilibrium between procoagulant and fibrinolytic activities is disrupted to favour fibrin deposition in exudative pleural inflammation. They proceeded to characterise procoagulant and fibrinolytic activities (using reverse fibrin gel enzymography) in pleural exudates from patients with pneumonia, lung cancer or empyema and transudates from patients with congestive heart failure. Concentrations of PAI-1 in exudative pleural fluids were increased up to 913-fold but no plasminogen activator inhibitor activity was demonstrated in transudates. There was a significant difference between PAI-1 increases in parapneumonic effusions ($p=0.123$) and empyema ($p=0.014$), but interestingly empyema fluids did not consistently express comparably increased pro-coagulant activity (as had been previously reported (Glauser et al., 1975), consistent with the heterogeneity of empyema. Additionally, increments of PAI-1 antigen in the empyema fluids were not correlated with changes in plasma samples, which were relatively low and generally within normal levels reported in plasma. These data were amongst the first to indicate that local pleural PAI-1 and plasminogen activator activity are likely to have a key role in pleural inflammation/infection states.

Fifteen years later, Chung et al. performed a prospective study using ultrasound and pleural effusions of various aetiologies, concluding that compared with free-flowing effusions, fibrinolytic activity was significantly depressed in loculated effusions (Chung et al., 2005). They suggested that a higher intensity of pleural inflammation in loculated effusions may enhance the release of TNF-$\alpha$, IL-1$\beta$, and TGF-$\beta$, which could theoretically increase the local levels of PAI-1, resulting in an imbalance of PAI-1 and t-PA in pleural spaces with fibrin deposition and loculation of pleural effusions. This was shortly followed by another study comparing patients with empyema (n=30) versus non-complicated parapneumonic effusions (n=21), where the median and range of PAI-1 concentrations in loculated empyema fluids was found to be significantly greater than that of free-flowing non-complicated pleural fluids (Iglesias et al., 2005). Hence, PAI-1 does appear to have strong correlation with pleural adhesion formation in the setting of empyema. Levels of pleural fluid PAI-1 activity have been shown to inversely correlate with pleural fluid fibrinolytic activity and it has been suggested that the inhibitory capacity of pleural fluid in a given patient may inform personalised dosing of IET (Tucker and Idell, 2013).

This hypothesis becomes even more compelling when one considers the data from animal models. Komissarov et al. hypothesised that the type of infection that a given subject experiences may affect the severity of pleural loculation. They inoculated their rabbit model with intrapleural *Pasteurella multocida* or *S. pneumoniae.* The animals responded with an inflammatory pleural effusion and the collections were reminiscent of loculated pleural effusions seen in humans (Komissarov et al., 2016). The pleural effusions had increased PAI-1 levels that correlated with increased PAI-1 activity and these concentrations far exceeded those seen in the pleural effusions of rabbits with tetracycline (TCN)-induced pleural injury previously studied by the same group (Florova et al., 2015). Interestingly and in keeping with the higher PAI-1 levels, the doses of IPFT (tPA) that they had used to attenuate pleural collections in the TCN-induced pleural injury rabbits were ineffective and considerably higher doses were required to treat empyema, with additional variability between in the *P. multocida* and *S. pneumoniae*-induced empyemas. The authors concluded that PAI-1 and associated activity levels were responsible for the differential effects of fibrinolysins on clearance of the fibrinous pleural collections in this model.

In the study using the rabbit model with tetracycline-induced pleural inflammation, as levels of intrapleural PAI-1 and its activity increased, the endogenous PA activity fell in a linearly correlated manner in animals treated with tPA. These findings suggest the possibility that PAI-1 is a legitimate target to improve IPFT dosing and strategy in what is currently lacking as a precision medicine approach in pleural infection. It is important to note that PAI-1 plays an important regulatory role in normal healing and control of fibroblast elaboration of collagen (Marudamuthu et al., 2015) thus the aim is to attenuate levels without achieving absolute PAI-1 deficiency. One study using a murine model found that whilst overexpression of PAI-1 augments intrapleural fibrin deposition, PAI-1 deficiency promotes profibrogenic alterations of the mesothelium that exacerbate pleural organisation and lung restriction (Tucker et al., 2016)

PAI-1-neutralising monoclonal antibodies have been tested as adjunctive therapy to potentially allow reduction in the dose of IPFT and lessen bleeding risk (Florova et al., 2015). Similarly, single-chain urokinase-type plasminogen activator (scuPA), a proenzyme fibrinolytic, has been tested in rabbit models with promising results, suggesting that the PAI-1 resistance and durability of intrapleural scuPA may be advantageous, and may play a substantial role in the future of IPFT. scuPA is currently undergoing a phase 1 dose escalation clinical trial testing in patients with loculated empyema (NIH 1U01HL121841-01A1).

### 1.8.3 FIBRIN DEPOSITION, SEPTATIONS AND LOCULATIONS IN PLEURAL INFECTION – what do they mean in clinical practice?

During the evolution and progression of an empyema, intrapleural accumulation of inflammatory cytokines and rising levels of fibrinolysis inhibitors such as tissue plasminogen activator inhibitor (PAI) occurs. This depression of fibrinolytic activity results in fibrin deposition that coats the visceral and parietal pleural surfaces. As stage 2 of empyema development progresses, fibroblast proliferation along the established fibrin matrix creates dense inelastic septations within and around the pleural cavity. Along with collagenous thickening, these septations eventually divide the space into separate pockets, in what is hypothesized to be an attempt to wall off residual infection (Corcoran et al., 2015; Lee et al., 2016).

It is assumed that septations are associated with poor drainage and worse outcomes (Chen et al., 2000). This assumption has never been proven in a large, prospective trial, specifically in patients with pleural infection. Moreover, although the terms 'septated' and 'loculated' effusions are often used interchangeably, septations do not always equate to loculated (i.e., pocketed, non-communicating) effusions, and theoretically, the latter are probably more of a barrier to drainage. Importantly, some clinicians use the presence of septations on ultrasound as an indication to avoid attempting drainage, to avoid 'unnecessary' complications of chest tube insertion, as they assume there will be a low likelihood of success. Others use septations as a reason to refer patients directly for a surgical procedure. Our understanding of the biology of the infected pleural space, the reasons underlying medical treatment failure and the success of combination therapy suggests that this may be an oversimplification. The variation in practice highlights the lack of current understanding of the true meaning and implications of septations in pleural infection.

The association of septations with clinically important outcomes remains unclear. I conducted a scoping literature search (Figure 1.9) and identified only six papers that have examined the presence of sonographic septations at diagnosis and assessed their association with clinically important outcomes (Table 1.3). Several studies suggest that septations are of clinical importance but disagree on their implications. The studies were mostly single centre, retrospective and had methodological problems. One study showed that patients with septations had increased LOS and frequency of chest tube drainage without increase in mortality, but was limited by a lack of objective decision making criteria and blinding (Chen et al., 2000).

Another study of 140 patients showed septations were associated with higher rate of ICU admission, but the septated group was significantly older with more underlying comorbidity (Chen et al., 2009). A separate study with similar conclusions had wide confidence intervals (95%CI 2.18-79.65) and showed no significant difference in outcomes between unilocular and multilocular effusions (Huang et al., 1999). A study of 50 patients correlating ultrasound appearances with severity of infection demonstrated that septations do not indicate a more advanced stage of infection, nor did their presence predict a worse outcome (Kearney et al., 2000). A recent study in 2016 suggested loculations were an independent predictor of surgical treatment but had no clinical patient outcome linked to their analysis (Chang et al., 2016).

**Figure 1.9 – Summary flow diagram of literature search on septations and clinical outcomes**

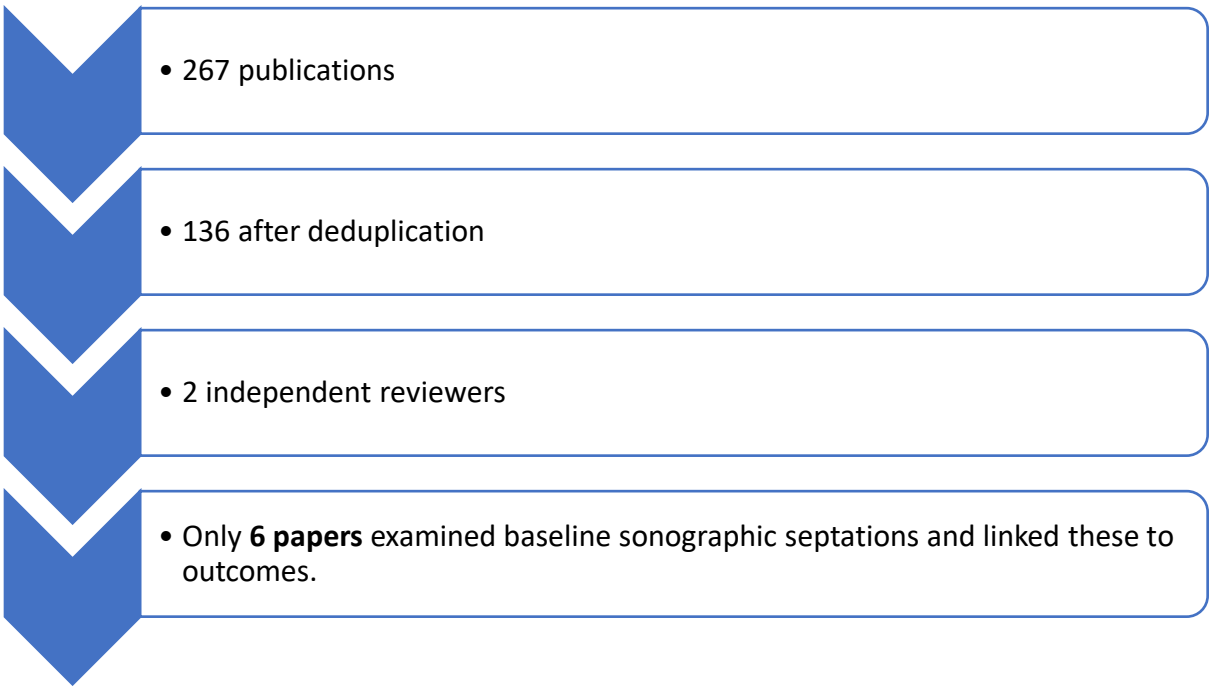



**Table 1.3 – Studies exploring association between sonographic septations and clinical outcomes.**

| Author | Year | Type of study | n | Outcomes |
|---|---|---|---|---|
| **Kearney** | 2000 | retrospective | 50 | surgery |
| **Chen** | 2000 | retrospective | 163 | LOS, surgery, mortality |
| **Chen** | 2009 | retrospective | 141 | LOS, surgery, ICU, mortality |
| **Y F Lai** | 2009 | prospective | 87 | RPT, loss of lung function |
| **Chang** | 2016 | retrospective | 276 | LOS, surgery |
| **Bongiolatti** | 2017 | retrospective | 64 | LOS, surgery |
| **n= number of patients** | | | | |
| **LOS = length of stay** | | | | |
| **RPT = residual pleural thickening** | | | | |

I therefore concluded that the assumption that septations are associated with poor drainage, and in turn, poor clinical outcomes, is not evidence based. It is important to note that previous large pleural infection trials, MIST-1 (Maskell et al., 2005) and MIST-2 (Rahman et al., 2011), did not address septations, as they were conducted prior to the era where ultrasound has now become standard-of-care, and thus septations were not addressed in the RAPID model . Understanding the clinical significance of septations is this an important topic of clinical research in pleural infection, given their ease of assessment using bedside ultrasound and clinical utility, and one that will form an important focus of this thesis.

## 1.9 CONCLUSION

The incidence of pleural infection is rising, and it will continue to be a condition that respiratory clinicians will have to contend with as part of their daily practice. A large part of the challenges it poses are the heterogeneity in its presentation, pathogenesis, and the population it affects. Individualising treatment through robust risk stratification and prognostication at a patient level as well as phenotyping their individual infection are likely to help us bring the condition to the current era of precision medicine seen in other respiratory subspecialties. The criteria to identify patients for surgery and IET, and the timing of these procedures remain uncertain. This can only be achieved through better insights into predictors of poor outcome with fibrinolytic therapy, and earlier identification of those patients who may benefit from a more pro-active approach earlier on in the course of their illness.

## 1.10 HYPOTHESIS AND AIMS

Within this thesis, I have attempted to address an **overarching hypothesis**, which is that the management of pleural infection can be *improved* with earlier and more aggressive escalation of treatment and *personalised* using multimodality outcome prediction to identify those with the most aggressive disease and/or at risk of worse clinical outcomes. The thesis will address the following project aims:

1. To strengthen the evidence base for the safety of IET as a treatment option in pleural infection.
2. To identify/characterise accessible radiological and/or biochemical biomarkers in pleural infection that prognosticate clinically relevant outcomes.
3. To undertake the MIST-3 study feasibility study of recruitment to early intervention (intrapleural enzyme treatment or surgery) versus standard care.
4. To conduct a qualitative analysis of semi-structured patient and carer interview data within the MIST-3 study.

My **specific objectives** are:

Workstream 1

- To analyse prospectively collected data on sonographic septations detected at ultrasound early in disease to determine if these potentially represent a valid prognostic indicator using paired clinical outcome data.
- To analyse PAI-1 levels in pleural fluid (using stored samples from 250 patients in the PILOT study) as a biological driver of septations and predictor of clinical outcomes in pleural infection
- To correlate improvement in CXR appearance with clinical outcomes using retrospective data from the MIST-2 study.

Workstream 2

- To recruit 75 patients to the MIST-3 feasibility randomised controlled study.
- To explore the feasibility of recruitment and early randomisation to a surgery versus non surgery trial in pleural infection including patient acceptability, clinician equipoise and potential challenges and barriers to recruitment

- To assess the feasibility of data collection and prioritise the important outcome data for a subsequent definitive study
- To gain experience in the design, set up and conduct of clinical trials in pleural disease.

Workstream 3

- To explore (using semi-structured interviews) the acceptability of the randomisation process and to identify relevant patient related outcome measures.
- To explore patient priorities in the treatment of pleural infection.
- Identify patient centred outcomes to inform the design of future clinical trials in pleural infection.
- To gain a better understanding of qualitative methodology and thematic analysis.

Workstream 4

- To assess the rate of bleeding complications of IET in a large international multicentre cohort.
- To document the consequences of fibrinolytic-induced pleural bleeding.
- To identify predictors of increased bleeding risk with IET in pleural infection

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## APPENDIX A1.1

Bedawi EO, Ur Rehman K, Sivakumar DP, et al. The impact of the COVID-19 pandemic on pleural infection incidence: a UK multicentre retrospective analysis. ERJ Open Res 2022; 8: 00206-2022 [DOI: 10.1183/23120541.00206-2022].

# The Impact of the COVID-19 Pandemic on Pleural Infection incidence: a UK multicentre retrospective analysis

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## Introduction

The fall in non-COVID-19 respiratory viruses, including seasonal influenza, during the pandemic is well reported (Angoulvant et al., 2021; Oster et al., 2020; Parry et al., 2020; Tan et al., 2020). It is thought to be a result of a combination of social distancing, lockdowns, improved hand hygiene and potentially virus-virus interactions and cross-protection impacting population dynamics. However, as vaccines weaken the transmission of SARS-CoV-2, clinicians remain vigilant for a potential resurgence of other respiratory pathogens and the implications of an ongoing rise in new SARS-CoV-2 variants.

A huge rise in pleural infection cases is well documented following the influenza pandemic in 1918 (Mozingo, 1918) and a recent epidemiological study from Arnold *et al* also found that for 9 of the 10 years studied, the highest annual point incidence of influenza nationally coincided with the highest admission rate for pleural infection (Arnold et al., 2021).

Pleural effusions have been noted in only a minority of severe COVID-19 cases (up to 5%) (Rathore et al., 2022). To date, no studies have examined the overall impact of the COVID-19 pandemic on adult pleural infection incidence. This study was therefore planned to assess the impact of the pandemic on incidence and profile of pleural infection.

## Methods

### *Participating centres*

A network of geographically diverse specialist pleural units across the United Kingdom (UK) actively screening pleural infection cases as part of recruitment to a prospective multicentre randomised controlled trial (RCT), the third Multicentre Intrapleural Sepsis Trial (MIST-3; ISRCTN18192121) conducted the study. These five centres were continuously screening for cases of pleural infection within their services and had dedicated pleural multidisciplinary team (MDT) meetings that captured and recorded all pleural infections across their services as standard practice.

***Data collection***

Two comparative periods were chosen as March 2020 – February 2021 (post-COVID) to represent the study cohort, against the same period pre-COVID (March 2019 – February 2020) as a control cohort. A retrospective review of screening logs and case notes was conducted.

***Outcomes***

The primary outcome measure was the difference in incidence (number of confirmed cases) of pleural infection admissions between the two time periods.

Secondary outcome measures included: comparison of patient demographics; interval between symptom onset and presentation; incidence of pleural infection during flu season; the effect of immunosuppression (patient receiving regular steroids, biologic agents, or active chemotherapy on admission); radiological evidence of pneumonic consolidation and/or COVID-19 infection (as per the reporting radiologist); microbiological profile.

As clinical outcomes would be skewed by involvement of the study cohort in the MIST-3 study, which involves early randomisation to one of three study intervention arms (standard care, intrapleural fibrinolytics, or an early surgical opinion) these were not evaluated.

In the study cohort, data on COVID-19 PCR positivity was also collected.

***Ethical approval***

As this was a retrospective analysis, patient consent and research ethics committee (REC) approval were not required.

***Eligibility criteria***

The inclusion criteria were adult patients (≥ 18 years) with a diagnosis of pleural infection based on standard, internationally agreed criteria (Davies et al., 2010) (identical to those used in large prospective RCTs) (Rahman et al., 2011). These were:

- a clinical history compatible with pleural infection
- a pleural collection that was either
    - purulent or
    - gram stain/culture positive or
    - acidic with a low pH <7.2 or

- low pleural fluid glucose (in the absence of an accurate pH measurement) or
- a septated pleural collection clinically considered most likely secondary to pleural infection

***Statistical analysis***

Statistical analyses were conducted using simple descriptive statistics. Continuous variables were reported as mean and standard deviation. Comparisons of proportions were conducted using the Pearson's Chi squared test ($p<0.05$). The analyses were conducted using SPSS (IBM, version 28)

## Results

***Primary outcome***

A total of 308 patients were included in the final analysis. In the same 1-year duration pre-COVID,184 new cases of pleural infection were identified across the five participating centres versus 124 new cases in the same 1-year period following the start of the pandemic. This equated to a decrease of 32.61% in admissions between the two years (figure 1).

***Secondary Outcomes***

Patient demographics [age and gender distribution, infection setting (hospital acquired vs community acquired), immunosuppression and median RAPID score] were similar in both groups with no statistically significant differences in the two time periods studied. The median interval between symptoms onset and hospital attendance was slightly longer in the post-COVID cohort (14 vs 10 days) but did not reach statistical significance ($p=0.16$). All patients had computed tomography (CT) scans and none of these reported evidence of co-existent COVID-19 pneumonia. Analysis of pleural infection cases diagnosed during the flu season (December, January, and February) showed 46/184 (25%) in the pre-COVID period, and 15/124 (12.1%) cases during those 3 months in the post COVID period. This difference was statistically significant [$\chi^2$ (1df, n=308) =7.765, p=0.005].

A greater proportion of pleural fluid purulence (49/124=39.5% vs 50/184=27.2%; p=0.04) and culture positive infections (48/124=38.7% vs 49/184=26.6%; p=0.03) were observed in the post COVID period. The species of microorganisms isolated on pleural fluid culture were similar in both cohorts.
SARS-CoV-2 PCR positivity was seen in 9/124 (7.25%) patients in the post-COVID cohort.

## Discussion

We present, to our knowledge, the first study examining the impact of COVID-19 on the incidence and profile of pleural infection. Epidemiological studies have suggested that the incidence has steadily increased year-on-year in the last decade (Bobbio et al., 2021; Farjah et al., 2007; Mummadi et al., 2021). In this representative sample of the UK population, covering 5 geographically diverse areas, our data demonstrate a 32.6% decrease in pleural infection in the year following the start of the COVID-19 pandemic. The higher proportion of purulent and microbiology positive infections in the post-COVID cohort may have been a result of poorer access to prior antibiotics in the community (Gray et al., 2020).
The low rate of co-existent COVID-19 PCR positivity (7.25%) and the absence of radiological evidence of COVID-19 pneumonia in any of the pleural infection cases, is in keeping with the literature, which suggests that empyema does not appear to be frequently associated with COVID-19 pneumonia (Rathore et al., 2022).
Compared to previous influenza pandemics, it is noteworthy that during the COVID-19 peaks, antibiotics were used sparingly to cover secondary bacterial infections, and this may have helped prevent empyema complications.
The potential role of public health measures in reducing pleural infection incidence is intriguing and one that has not been specifically explored in the existing literature. It is highly likely that decreased social mixing, isolation of older, more vulnerable patients with additional comorbidity, often at increased risk of pleural infection, as well as social distancing measures have had, in combination, a beneficial impact.
There were some limitations to this study. Being retrospective, it is bound by the validity and bias limitations of such a study design. However, the regular prospective screening procedures in place as part of recruitment to the MIST-3 and the prospective documentation of pleural infection cases through the weekly specialist MDTs at the participating centres are likely to have been more robust method of

capturing cases compared to hospital episode statistics or administrative databases. This data only captures patients admitted to hospital and therefore cannot exclude that a proportion of patients chose to be treated in the community to avoid capturing COVID-19 in hospital. The impact of vaccinations remains unclear. It also remains to be seen whether pleural infection incidence will return to pre-pandemic levels as enforcement of public health measures are relaxed.

## Conclusion

This study demonstrates a reduction in pleural infection incidence by almost a third following the start of the COVID-19 pandemic. Potential causes for lower rates of pleural infection may be secondary to reduced community transmission of viruses due to social distancing and use of personal protective equipment in both community and healthcare settings.

### Tables and figures

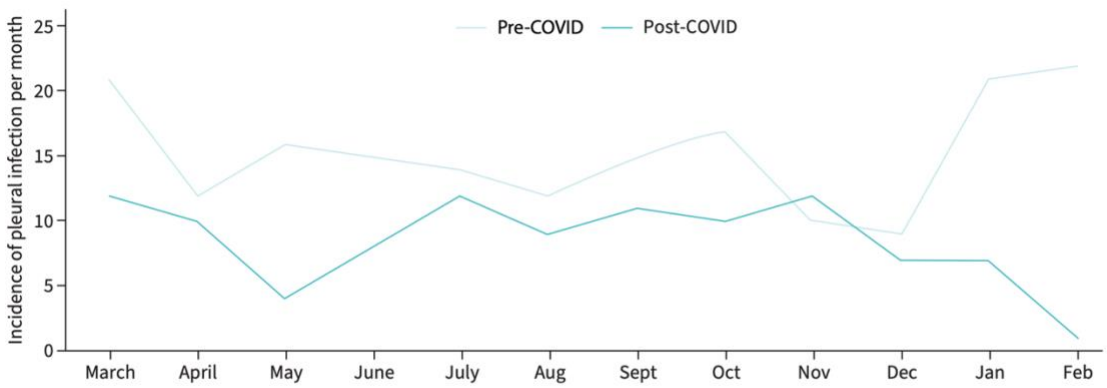


FIGURE 1 Incidence of pleural infection per month. Pre-COVID: March 2019 to February 2020. Post-COVID: March 2020 to February 2021.

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# CHAPTER 2

# Radiological and biological biomarkers as outcome predictors in pleural infection

This chapter includes a published study constituting an analysis of pleural fluid samples and sonographic septations data prospectively collected from the largest observational study of pleural infection in the literature – the Pleural Infection Longitudinal Outcome Study (PILOT).

Appendix A2.4 is a separate small study I conducted in the first year of my PhD looking at the ability of the change in opacification on the chest radiograph between day 1 and 7 of pleural infection treatment in predicting clinically important outcomes at 3 months. The radiographic data used in my analysis were prospectively collected as part of the MIST-2 randomised controlled trial. My findings were presented as a spoken abstract at the British Thoracic Society Winter Meeting in 2019 (Appendix A2.4a).

# The Biological Role of Pleural Fluid PAI-1 and Sonographic Septations in Pleural Infection: Analysis of a Prospectively Collected Clinical Outcome Study

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**Contributor statement**

EOB, NIK and NMR conceived and designed the study. JPC and EOB curated the PILOT database. EOB, NIK and YZ performed the laboratory processing and analyses. EOB and NMR analysed the data. MH, RA, RMM, AS and DNA contributed clinical data. EOB wrote the first draft of the manuscript. TD provided materials. RFM, TD and AMC provided expert knowledge. All authors reviewed and approved the final manuscript. EOB, NIK and NMR verified the underlying data and jointly act as guarantors.

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## 2.1 ABSTRACT


**Rationale**

Sonographic septations are assumed to be important clinical predictors of outcome in pleural infection but the evidence for this is sparse. The inflammatory and fibrinolysis-associated intrapleural pathway(s) leading to septation formation have not been studied in a large cohort of pleural fluid (PF) samples with confirmed pleural infection, matched with ultrasound and clinical outcome data.

**Objectives**

To assess the presence and severity of septations against baseline PF Plasminogen-Activator Inhibitor-1 (PAI-1) and other inflammatory and fibrinolysis-associated proteins as well as to correlate these with clinically important outcomes.

**Methods**

We analysed 214 pleural fluid samples from the PILOT study, a prospective observational pleural infection study, for inflammatory and fibrinolysis-associated proteins using the Luminex platform. Multivariate regression analyses were utilised to assess association of pleural biological markers with septation presence and severity (on ultrasound), and clinical outcomes.

**Results**

PF PAI-1 level was the only protein independently associated with septation presence ($p=<0.001$) and septation severity ($p=0.003$). PF PAI-1 levels were associated with increased length of stay (LOS) ($p=0.048$) and increased 12-month mortality ($p=0.003$). Sonographic septations alone had no relation to clinical outcomes.

**Conclusion**

In a large and well characterised cohort, this is the first study to associate pleural biological parameters with a validated sonographic septation outcome in pleural infection. PF PAI-1 is the first biomarker to demonstrate an independent association with mortality. While PF PAI-1 plays an integral role in driving septation formation, septations themselves are not associated with clinically important outcomes. These novel findings now require prospective validation.

## 2.2 INTRODUCTION

Fibrin is not present in the normal pleural space, yet disordered fibrin turnover and aberrant extravascular fibrin deposition are key components of pleural injury (Komissarov et al., 2018). Pleural injury is characterised by fibrin accumulation and a marked suppression of fibrinolysis resulting in the formation of fibrinous strands known as pleural septations, or loculations when they form closed networks that sequester inflammatory fluid and impair pleural drainage. Plasminogen-derived plasmin is the main mediator of fibrinolysis, however despite the presence of endogenous plasminogen in the injured pleural space, plasminogen activity (and thus fibrinolysis) is inhibited by significantly elevated levels of plasminogen activator inhibitor 1 (PAI-1) (Idell et al., 1991).

In pleural infection, significant variation has been observed in levels of endogenous pleural fluid (PF) PAI-1 in samples from participants recruited to the MIST-2 trial (Rahman et al., 2011). However, the degree of septation and loculation in these patients was not known as MIST-2 took place prior to the widespread use of bedside thoracic ultrasound (Komissarov et al., 2016; Rahman et al., 2011).

There is a paucity of evidence directly linking the presence of sonographic septations to clinically important outcomes. It has been suggested that the sonographic presence of pleural septation at diagnosis may be a prognostic indicator based on small retrospective studies (Chen et al., 2009, 2000) yet clinicians frequently use the presence of septations to alter treatment (specifically, larger chest tube insertion and/or upfront surgery or intrapleural therapy early in treatment). If septations and loculations are truly important predictors of clinical outcome, personalised therapy based on evaluation of the components of the fibrinolytic system in pleural fluids at baseline could be of clinical value, and PF PAI-1 or other established proteins in the inflammatory and fibrinolysis pathways are thus potentially important candidate biomarkers. Moreover, sonographic septations, which are easily detectable given the now commonplace use of thoracic ultrasound, may be an accurate radiological surrogate.

The aim of this study was to explore the inflammatory and fibrinolysis-associated intrapleural pathway(s) leading to formation of septations in the infected pleural space

by measuring a number of proteins from real life human samples, with key roles in the development and progression of pleural infection. Combined with matched ultrasound septation data and known clinical outcomes, the aim was to test the following hypotheses:

1. Septation formation is dependent on endogenous PF PAI-1 levels at baseline
2. PF PAI-1 is superior to conventional serum/pleural fluid biomarkers of pleural infection in its relationship with development of septations
3. PAI-1 and sonographic septation presence / severity is associated with clinically important outcomes

## 2.3 METHODS

The recently published Pleural Infection Longitudinal Outcome Study (PILOT) was an international multicentre prospective observational cohort study which enrolled adult patients with pleural infection (n=546; 29 sites). Participants were managed according to published guidelines (adapted for usual local practice). Details of the study inclusion and exclusion criteria are outlined in the PILOT manuscript (Corcoran et al., 2020) and Appendix A2.1. Baseline pleural fluid samples were collected from all patients who met the inclusion criteria from participating sites; Perth (processed locally) and select sites in the UK only to allow prompt receipt and processing by the central trial site (Oxford Respiratory Trials Unit, University of Oxford) and stored as per a trial specific procedure (TSP) (Appendix A2.2). Thus 243 samples were available for analysis, and baseline and clinical outcome data was available for all these patients. The PILOT study demonstrated that a clinical baseline score (RAPID) accurately predicted clinical outcome at 12 months.

### Pleural fluid analysis

Protein measurement assays were performed using a commercially available Luminex bead-based multianalyte profiling kit (Luminex® High Performance Assays, R&D Systems). Luminex assays were chosen over ELISA for the protein measurements due to increased precision, time efficiency and cost-effectiveness (Dupont et al., 2005). Absolute expression of total antigenic PAI-1, chemokine (C-C motif) ligand 2/monocyte chemoattractant protein-1/ (CCL2/MCP-1), urokinase type plasminogen

activator (uPA), D-dimer, interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α) were measured in the pleural fluid samples. These were selected based on existing knowledge of the procoagulant and inflammatory pathways involved in pleural infection. A spectrophotometer (NanoDrop™, ThermoFisher Scientific, UK) was used to measure the total protein expression in each sample. PF concentrations were normalised relative to total protein expression in pleural fluid. Only samples with a complete protein profile were used in the biomarker analysis. Further details of pleural fluid analysis are presented in Appendix A2.3.

**Clinical outcome data**

The primary endpoint of the PILOT study was all-cause mortality at 3 months with a data completion rate (DCR) of 542/546 (99.3%). Secondary endpoints included all-cause mortality at 12 months (DCR 542/546; 99.3%), length of hospital stay (LOS), and need for surgical drainage over 12 months (DCR 546/546; 100%). Use of combination intrapleural fibrinolytic and enzyme therapy (IET) was not a specific outcome of the PILOT study but as recorded on case report forms (CRFs), it was included in the analysis of this study (DCR 546/546; 100%).

**Ultrasound septation score**

An objective thoracic ultrasound septation score reflecting the sonographic extent of pleural fluid septation has been developed and validated, as described previously (Psallidas et al., 2017). This score categorises heterogeneously septated pleural effusions based on the maximum number of septations per image field of view into one of the following groups: non-septated; mildly septated (<2 septations per field); moderately septated (2-4 septations per field); or severely septated (>4 per field).

As part of the PILOT protocol, participants underwent ultrasound assessment prior to pleural intervention by a respiratory (or other) physician holding Royal College of Radiology Thoracic Ultrasound level 1(“Ultrasound training recommendations for medical and surgical specialties, Third edition, The Royal College of Radiologists” https://www.rcr.ac.uk/clinical-radiology/publications-and-standards) competency equivalent or above. Study case report forms (CRFs) documented septation score, with visual scales of ultrasound images included to guide the ultrasound operator in grading (Figure 2.1).

Baseline ultrasound data on the presence or absence of septations were available in 462/546 (84.6%) participants, with septation severity data in 434/462 (93.9%). The clinical population was divided and compared according to the septation status thus defined. The study flow diagram is shown in Figure 2.2

**Figure 2.1** – Objective septation scoring system scale - a. mildly septated effusion (<=2 septations); b. moderately septated effusion (2-4 septations); c. heavily septated effusion (>5 septations)

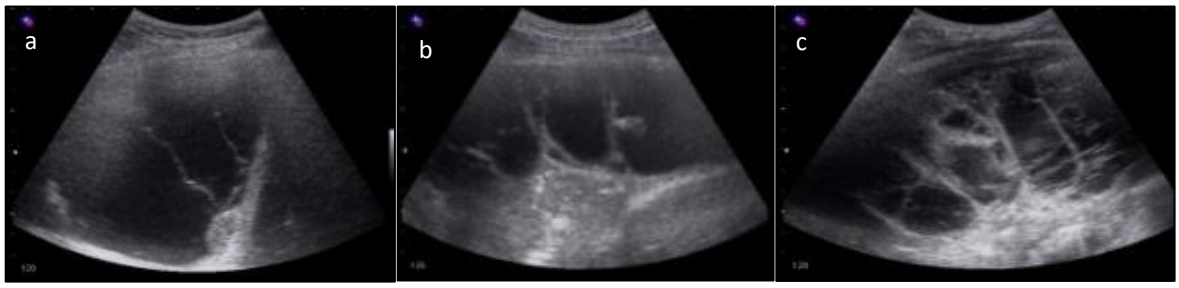
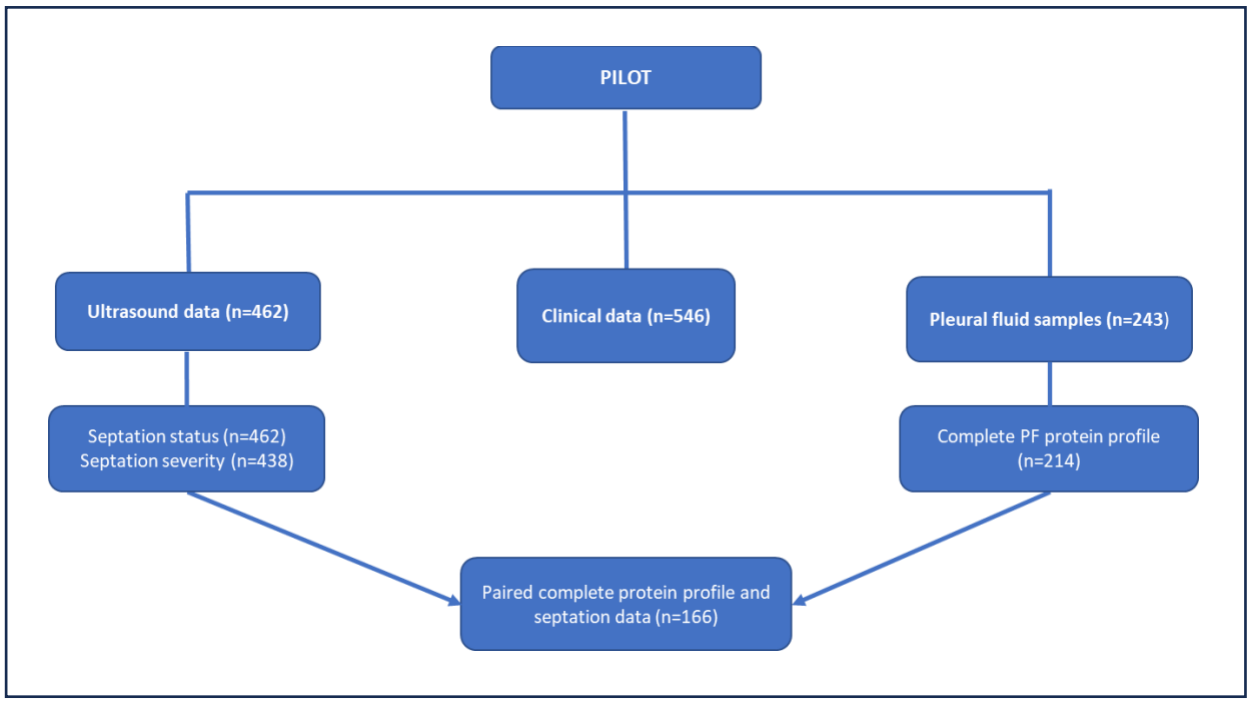



**Figure 2.2** – Study flow diagram




## Funding and ethical approval

The analysis of the PILOT samples was funded by Oxford NIHR Biomedical Research Centre and included in the PILOT ethics approval (Oxford B Research Ethics Committee Ref:13/SC/0204).

## Statistical analysis

Patient data are reported as the median/interquartile range (+/- range) for continuous variables. Chi squared statistics were used to compare differences in proportions between groups. Correlation between pleural fluid protein measurements, conventional biomarkers, septation presence and severity was assessed using Spearman's rank correlation coefficient (CC) with $p < 0.05$ used to define statistical significance. For outcome assessments, protein measurements and other biologically plausible conventional pleural fluid and serum biomarkers were analysed as independent variables in multiple (univariate) regressions with septation status as the dependent variable. Those with statistical significance $p < 0.2$ were exported into a stepwise multivariate regression model. When analysing the six biomarkers against each other, the p-value cut-off for statistical significance was $0.05/6 = 0.0083$.

For clinical outcomes, Chi squared statistics were used to determine the proportions of requirement for IET and surgery, 12-month readmission rates and mortality (3 and 12 months) between the different septation groups. LOS was analysed using Mann Whitney U and Kaplan Meier (KM). Linear regression was used to assess whether statistically significant biomarkers of septation status were associated with clinical outcomes. A Cox regression for survival analysis was conducted between septation and biomarker groups, as categorical variables. Statistical analysis was performed using SPSS 27.0 (IBM).

## 2.4 RESULTS

### Baseline demographics

Baseline demographic and infection characteristics were similar between septated and non septated groups (Table 2.1). Specifically, there were no differences in baseline RAPID category (Rahman et al., 2014) and chest tube size initially used. There was a higher proportion of macroscopically purulent fluid in the non-septated group (43.6% vs 21.7%; $\chi^2$ 1df 10.66; p=0.001) and a higher incidence of baseline tachycardia (HR >100bpm) in the septated group (36% vs 24%, $\chi^2$ 1df 4.57; p= 0.03)..

**Table 2.1.** Baseline demographics by septation status.

| | Not septated (n=94) | Septated (n=368) | p-value |
|---|---|---|---|
| Age, yr, median (IQR) | 69 (54-75) | 68 (53-77) | 0.65 |
| Male, n, (%) | 72 (76.6) | 256 (69.6) | 0.18 |
| Community acquired, n (%) | 86 (91.5) | 321 (87.2) | 0.18 |
| Poor dental hygiene, n (%) | 44 (46.8) | 168 (45.7) | 0.99 |
| Antibiotic use before diagnosis, n (%) | 56 (59.6) | 228 (62) | 0.52 |
| Fluid purulence, n (%) | 43 (45.7) | 79 (21.7) | 0.01 |
| Micro positive | 58 (61.7) | 223 (60.6) | 0.87 |
| Small bore drains (<=14F); n, (%) | 52 (55.3) | 191 (51.9) | 0.32 |
| Fever (T>37.8C) | 20 (21.7) | 77 (21.5) | 0.96 |
| Tachycardia (HR >100bpm) | 22 (24.2) | 129 (36) | 0.03 |
| **RAPID category** | | | |
| Low | 24 (25.5) | 111 (30.2) | 0.72 |
| Medium | 37 (39.4) | 139 (37.8) | |
| High | 20 (21.3) | 73 (19.8) | |
| **Comorbidities (%)** | | | |
| 0: n, (%) | 37 (39.4) | 145 (39.4) | 0.934 |
| 1 to 2: n, (%) | 45 (47.9) | 168 (45.7) | |
| 3 or more: n, (%) | 12 (12.8) | 51 (13.9) | |

Key: proportions were compared using $\chi^2$ statistics and medians were compared using independent K samples median test.

Assessment of the association between baseline variables and septation severity demonstrated no significant correlations (table 2.2). Complete baseline demographic data of the full PILOT study population can be found in the original PILOT paper (Corcoran et al., 2020)

**Table 2.2** - Baseline demographics based on septation severity

| | Not septated (n=94) | Mild (n=73) | Moderate (n=125) | Severe (n=142) | p-value |
|---|---|---|---|---|---|
| Age, yr, median (IQR) | 66 (52-78) | 67.5 (51-80) | 70 (53.5-77) | 66 (53-76) | 0.48 |
| Male, n, (%) | 72 (23.3) | 45 (14.6) | 95 (30.7) | 97 (31.4) | $\chi^2$ (3df) 6.57, p=0.09 |
| Community acquired, n (%) | 86 (22.6) | 62 (16.3) | 112 (29.4) | 121 (31.8) | $\chi^2$ (3df) 3.75, p=0.29 |
| Poor dental hygiene, n (%) | 47 (21.8) | 33 (15.3) | 63 (29.2) | 73 (33.8) | $\chi^2$ (3df) 0.37, p=0.95 |
| Antibiotic use before diagnosis, n (%) | 56 (20.7) | 48 (17.7) | 74 (27.3) | 93 (34.3) | $\chi^2$ (3df) 3.14, p=0.37 |
| **RAPID category** | | | | | |
| Low | 24 (18.9) | 22 (17.3) | 39 (30.7) | 42 (33.1) | $\chi^2$ (6df) 5.01, p=0.54 |
| Medium | 37 (23.1) | 29 (18.1) | 40 (25.0) | 54 (33.8) | |
| High | 20 (21.7) | 12 (13.0) | 34 (37.0) | 26 (28.3) | |
| **Co-morbidities (%)** | | | | | |
| 0 (n=167), % | 22.2 | 15.6 | 30.5 | 31.7 | $\chi^2$ (6df) 1.12, p=0.98 |
| 1 to 2 (n=202), % | 22.3 | 17.8 | 28.2 | 31.7 | |
| 3 or more (n=61), % | 21.9 | 17 | 28.8 | 32.3 | |

Key: proportions were compared using $\chi^2$ statistics and medians were compared using independent K samples median test.

## Incidence of septations in pleural infection

The incidence of sonographic septation at diagnosis overall was 368/462 (79.7%). Detailed breakdown of septation severity, based on recorded septation score, is presented in Table 2.3.

**Table 2.3.** The incidence of septations by septation score.

| Septation score | | n (434) | % |
|---|---|---|---|
| **0** | Not septated: 0 | 94 | 21.7 |
| **1** | Mild: 1 - 2 | 73 | 16.8 |
| **2** | Moderate: 3 - 4 | 125 | 28.8 |
| **3** | Severe: >5 | 142 | 32.7 |

## Correlations between serum / PF biomarkers and fibrinolysis-associated proteins

Complete PF protein profile data was available in 214/243 (88%) samples. There were no statistically significant associations between any PF fibrinolysis-associated proteins and RAPID score or number of comorbidities. PF uPA was correlated with all 3 commonly used PF indicators of cell death/activity; PF pH (CC -0.29; $p<0.001$), PF glucose (CC -0.45; $p<0.001$), and cell turnover; PF LDH (CC 0.39; $p<0.001$). PF IFN-$\gamma$ had a weak but statistically significant correlation with PF glucose (CC -0.27; $p=0.044$). PF PAI-1 had no correlation with PF pH, LDH or glucose.

With regards to conventional serum indicators of infection [serum C-reactive protein (CRP), peripheral blood white cell count (WCC) and platelets], modest correlations were seen between PF PAI-1 and CRP (CC 0.22; $p=0.007$), and PF CCL2/MCP-1 and WCC (CC -0.26; $p<0.001$).

## Septations and PF fibrinolysis-associated proteins

Paired complete protein profile and ultrasound data were available for 166 patients. Due to the smaller size of this analysis cohort compared to the overall PILOT population, baseline demographics were compared with the remaining PILOT population (as a control), to ensure this was a representative cohort. The groups were well matched, with the only statistically significant difference being an increased proportion of community acquired infections in the analysis groups (92.5% vs 86.9%, $\chi^2$ 1df 4.89; $p=0.03$) (Table 2.4).

**Table 2.4** – Comparison of patient demographic and baseline characteristics between the PILOT (control) dataset and the analysis (study) dataset

| | Control dataset | Study dataset | p-value |
|---|---|---|---|
| **Mean age +/- SD** | 64.85 +/-17 | 65.25 +/- 17 | 0.79 |
| **Male - no. (%)** | 237 (68.7%) | 148 (73.6%) | 0.19 |
| **Small bore tube <15F** | 165 (60.4%) | 115 (66.5%) | 0.2 |
| **Community acquired - no. (%)** | 298/343 (86.9%) | 186/200 (92.5%) | *0.03 |
| **Fluid purulence** | 93 (27.0%) | 54 (26.9%) | 0.98 |
| **Positive gram stain or culture** | 215 (62.7%) | 120 (60%) | 0.53 |
| **Pleural fluid pH** | | | |
| **Median** | 7.00 | 7.00 | 0.28 |
| **IQR** | 0.36 | 0.32 | |
| **Pleural fluid LDH** | | | |
| **Median** | 1953.5 | 1750 | 0.52 |
| **IQR** | 3973 | 3368 | |
| **RAPID score** | | | |
| **Median** | 3 | 3 | 0.16 |
| **IQR** | 2 | 2 | |
| **Abx use before diagnosis** | 197 (60.6%) | 129 (66.2%) | |
| **Poor dental hygiene** | 178 (54.4%) | 90 (46.9%) | |
| **WHO score - prior** | | | |
| **Median** | 0 | 0 | 0.45 |
| **IQR** | 1 | 1 | |
| **WHO score - current** | | | |
| **Median** | 2 | 2 | 0.8 |
| **IQR** | 1 | 2 | |
| **Serum CRP median (IQR)** | 200 (172) | 221 (185) | 0.46 |
| **Serum WCC median (IQR)** | 14.5 (9.64) | 13.7 (8.2) | 0.57 |
| **Serum platelets (IQR)** | 392 (216) | 403 (212) | 0.33 |

Of all assessed parameters, PF PAI-1 was the only protein to show a statistically significant correlation with septation status and severity (Table 2.5). Median values of PF proteins were compared by degree of septations (none, mild, moderate, heavy) (Table 2.6). Overall, concentrations of PF PAI-1 were significantly higher compared to other proteins. PF PAI-1 was the only parameter that independently discriminated septated and non-septated effusions (Figure 2.3). This finding was consistent across septation severity, with increased PAI-1 levels associated with worsening septation severity (Figure 2.4).

**Table 2.5.** Spearman's rank-order correlation coefficients (CC) between protein indices, septation status and septation severity.

| | Septation status | *p-value* | Septation severity | *p-value* |
|---|---|---|---|---|
| **PAI-1** | 0.36** | 0.009 | 0.29* | 0.016 |
| **CCL2/MCP-1** | 0.10 | 0.19 | 0.03 | 0.70 |
| **IFN-ɣ** | 0.01 | 0.86 | 0.05 | 0.51 |
| **uPA** | -0.1 | 0.21 | -0.08 | 0.33 |
| **D-dimer** | 0.04 | 0.64 | 0.02 | 0.83 |
| **TNF-α** | 0.08 | 0.28 | 0.09 | 0.28 |

Key: * p<0.05, ** p<0.01

**Table 2.6 -** Pleural fluid protein levels by septation severity.

| PF protein | Septation Score | | | | p-value |
|---|---|---|---|---|---|
| | **Nil (n=46)** | **Mild (n=28)** | **Moderate (n=39)** | **Severe (n=53)** | |
| **PAI-1,** ng/mL; median (IQR) | 725.2 (182-1480) | 1104.1 (513-1685) | 1464.9 (696-1893) | 1573.7 (1212-2111) | 0.003 |
| **MCP-1/CCL2**, ng/mL; median (IQR) | 0.59 (0.14-2.55) | 1.77 (0.54-8.93) | 3.83 (0.78-8.64) | 2.02 (0.56-5.03) | 0.16 |
| **IFN**-ɣ, ng/mL; median (IQR) | 0.02 (0.017-0.049) | 0.02 (0.019-0.039) | 0.02 (0.016-0.030) | 0.02 (0.017-0.032) | 0.79 |
| **uPA**, ng/mL; median (IQR) | 0.55 (0.19-2.05) | 0.34 (0.21-1.19) | 0.28 (0.18-0.57) | 0.35 (0.20-1.17) | 0.64 |
| **TNF**-α, ng/mL; median (IQR) | 0.05 (0.03-0.09) | 0.06 (0.03-0.11) | 0.06 (0.04-0.14) | 0.05 (0.04-0.09) | 0.59 |
| **D-dimer**, ng/mL; median (IQR) | 9.09 (6.21-14.98) | 9.83 (7.72-11.39) | 10.02 (8.55-13.96) | 10.90 (8.85-14.63) | 0.34 |

Key: The p-value represents the statistical significance in difference between the means of the four groups.

**Figure 2.3.** - Box and whisker plot of pleural fluid PAI-1 by septation status.

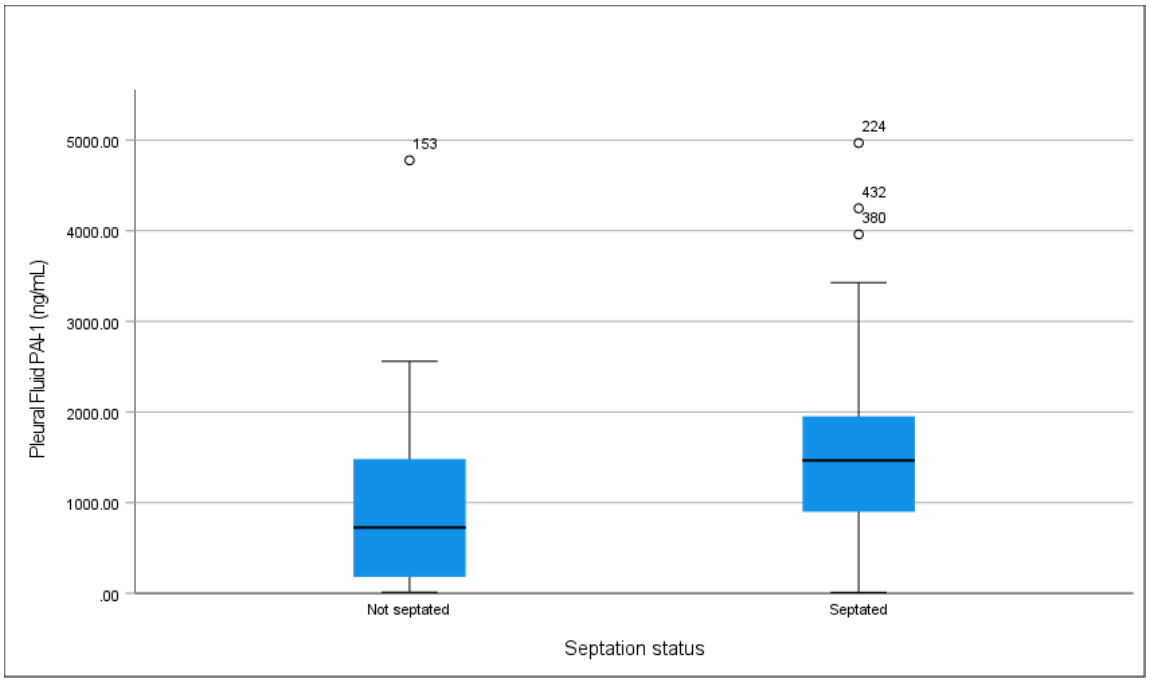
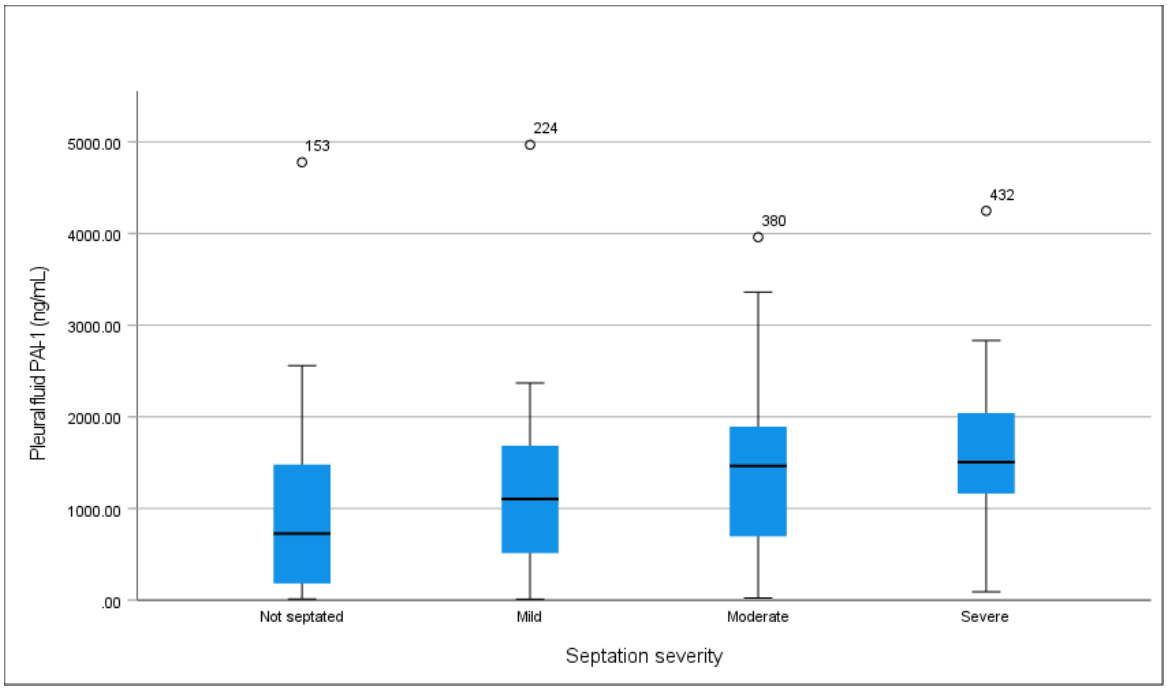



**Figure 2.4.** - Box and whisker plot of pleural fluid PAI-1 by septation severity*.




*The numbers over the boxes represent the study identifier for the outlier cases (1 case in each septation group)

### PF-PAI-1 as a biological correlate of septations

Conventional serum and pleural fluid biomarkers were analysed with pleural fluid biomarkers to assess for a relationship with septation status (Table 2.7). In the multivariate model, PF PAI-1 was the only biomarker independently associated with septation presence ($p<0.001$) (Table 2.8).

### The relationship of septations and PF-PAI-1 with clinical outcome

The presence of septations at baseline was independently associated with use of IET [19.6% vs 9.6%; p=0.023 (OR 2.30 95%CI 1.10-4.78)]. However, baseline septations were not associated with surgery, mortality, readmission or length of stay (Table 2.9). This was consistent when analysed for septation severity (Table 2.10). Using binary logistic regression and adjusting for use of IET, presence of septations had no relation to length of hospital stay (p=0.67), need for surgery at 3 months (p=0.25), mortality at 3 months (p=0.44) or 12 months (p=0.49) (Figure 2.5). There was no statistical difference in time-to-death between baseline presence or absence of septations (Figure 2.6).

PF-PAI-1 levels were not associated with use of IET (p=0.62) or surgery at 3 months (p=0.26). In a linear regression, higher PF-PAI-1 predicted longer length of stay; $t(1,214)=1.99$, p=0.048). In terms of mortality there was a trend towards, but not reaching, significance with death at 3 months (p=0.07). PAI-1 was converted into a categorical variable using the covariate mean (1974 ng/mL) to classify all cases into ‘PAI-1-low’ and ‘PAI-1 high’. In a KM survival analysis, the latter was associated with time-to-death at 12 months (Figure 3b). This result was consistent when repeated within the septated population alone.

**Table 2.7.** - Univariate regression analysis of serum and pleural fluid biomarkers.

| Biomarker | Non-septated (n=94) | Septated (n=368) | p-value (univariable analysis) |
|---|---|---|---|
| **Serum (conventional); median (IQR)** | | | |
| **WCC;** x$10^9$/L | 12.05 (9.4-18.6) | 14.70 (10.8-19.6) | **0.058*** |
| **CRP; mg/mL** | 198.2 (79.0-292.5) | 209.0 (132.9-300) | **0.15*** |
| **Platelets** | 378.0 (304-480) | 409.5 (298.5-527.5) | 0.13* |
| **Urea** | 4.8 (3.5-7.7) | 4.8 (3.6-7.6) | 0.21 |
| **Albumin** | 29 (24-33) | 28 (23-34) | 0.67 |
| | | | |
| **Pleural fluid (conventional); median (IQR)** | | | |
| **pH** | 7.02 (6.82-7.17) | 7.00 (6.80-7.16) | 0.97 |
| **LDH; IU/L** | 1869 (876-4681) | 2688 (1357-7331) | **0.12*** |
| **Glucose; mmol/L** | 1.0 (0.3-2.9) | 1.1 (0.3-3.3) | 0.30 |
| **Pleural fluid (proteins); median (IQR)** | | | |
| **PAI-1; ng/mL** | 725.2 (182.3-1480.6) | 1486.6 (908.8-1995.7) | **0.001**** |
| **MCP-1/CCL2; ng/mL** | 0.58 (0.14-2.55) | 2.37 (0.56-8.27) | 0.20* |
| **IFN-γ; ng/mL** | 0.023 (0.017-0.049) | 0.021 (0.017-0.036) | 0.86 |
| **uPA; ng/mL** | 0.55 (0.19-2.05) | 0.35 (0.19-0.91) | 0.24 |
| **D-dimer; ng/mL** | 9.09 (6.21-14.98) | 9.97 (8.57-13.23) | 0.65 |
| **TNF-α; ng/mL** | 0.05 (0.03-0.09) | 0.06 (0.04-0.11) | 0.38 |

Key: *included in the multivariate logistic regression model

**statistically significant on the multivariable model

**Table 2.8** - Multivariable logistic regression model of serum and PF biomarkers for septation status

| Factor | Regression coefficient | Std error | Z value (Wald) | p-value |
|---|---|---|---|---|
| **White cell count** | 0.009 | 0.015 | 0.341 | 0.559 |
| **Platelets** | 0.001 | 0.001 | 1.004 | 0.316 |
| **CRP** | 0.001 | 0.002 | 0.510 | 0.475 |
| **PF LDH** | 0.000 | 0.000 | 2.430 | 0.119 |
| **PF PAI-1** | 0.000 | 0.000 | 4.146 | 0.042 |
| **PF CCL2/MCP-1** | 0.000 | 0.000 | 0.735 | 0.391 |

**Table 2.9.** Clinical outcomes according to baseline presence or absence of septations.

| Outcome | Non-septated (n=94) | Septated (n=368) | p-value |
|---|---|---|---|
| **IET;** n (%) | 9 (9.6) | 72 (19.6) | $\chi^2$ (1df) 5.17, p=0.023 |
| **Surgery within 12 months;** n (%) | 20 (21.2) | 81 (22.0) | $\chi^2$ (1df) 0.01, p=0.92 |
| **Readmission within 12 months**; n (%) | 11 (11.7) | 59 (16) | $\chi^2$ (1df) 1.09, p=0.29 |
| **Length of stay (days);** median (IQR) | 14 (9-21) | 15 (10-22) | Mann Whitney p=0.31 |
| **3 month mortality*;** n (%) | 6 (6.4) | 40 (10.9) | $\chi^2$ (1df) 1.68, p=0.19 |
| **1 year mortality;** n (%) | 10 (10.7) | 69 (18.7) | $\chi^2$ (1df) 3.13, p=0.07 |

Key: *Primary outcome of the PILOT study.

**Table 2.10.** Clinical outcomes according to baseline septation score.

| Outcome | Septation score (n=434) | | | | p-value |
|---|---|---|---|---|---|
| | Non-septated (n=94) | Mild (n=72) | Moderate (n=125) | Severe (n=143) | |
| **IET; n (%)** | 9 (9.6) | 11 (15.1) | 31 (24.8%) | 26 (18.3%) | Ordinal $\chi^2$ (1df) 4.02; p= 0.045 |
| **Surgery within 12 months; n (%)** | 20 (21.2) | 14 (19.2) | 21 (16.8) | 37 (26.1) | Ordinal $\chi^2$ (1df) 3.63; p= 0.41 |
| **Readmission within 12 months; n (%)** | 11 (11.7) | 16 (21.9) | 19 (15.2) | 21 (14.8) | Ordinal $\chi^2$ (1df) 3.61, p=0.31 |
| **Length of stay (days); median (IQR)** | 14 (9-21) | 13 | 15 | 15 | Mann Whitney p=0.73 |
| **3 month mortality; n (%)** | 6 (6.4) | 10 (13.9) | 13 (10.4) | 15 (10.5) | Ordinal $\chi^2$ (1df) 1.26, p=0.74 |
| **1 year mortality;** n (%) | 10 (10.7) | 9 (12.5) | 27 (21.6) | 23 (16.1) | Ordinal $\chi^2$ (1df) 1.19, p=0.27 |

Key: p values represent results of ordinal $\chi^2$ test (linear by linear association)

**Figure 2.5. -** Kaplan-Meier plot for time-to-discharge (days)

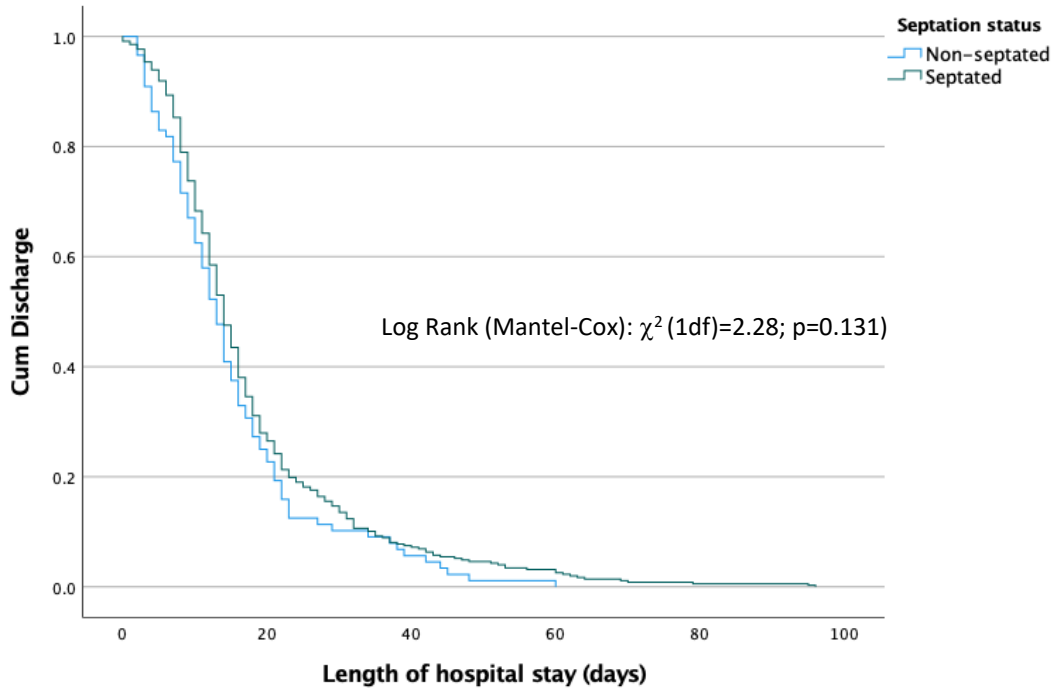
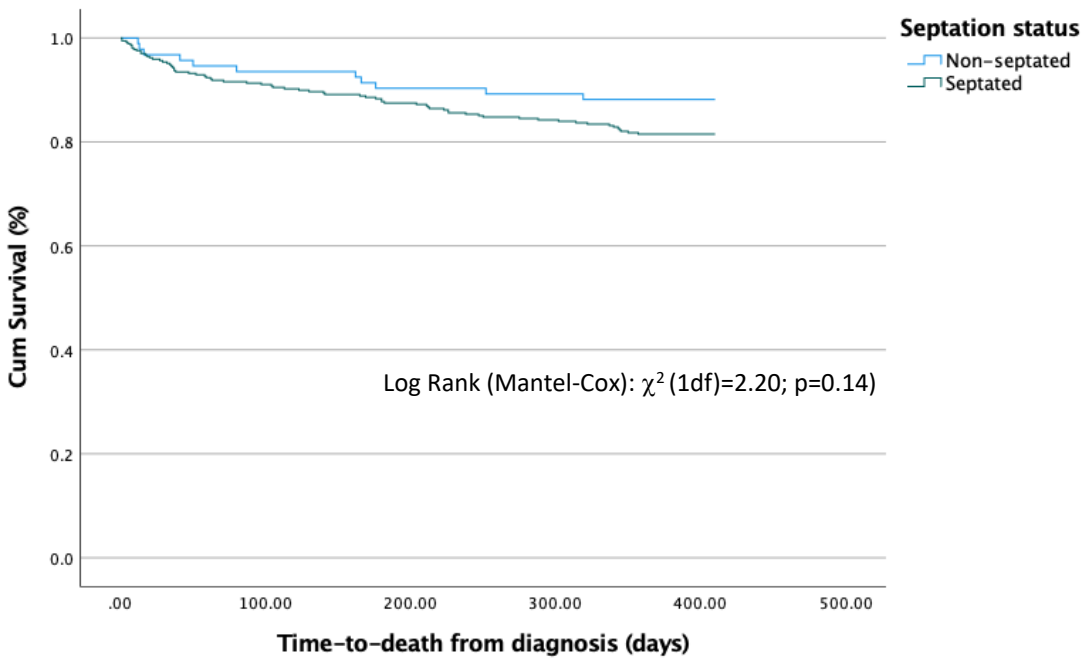


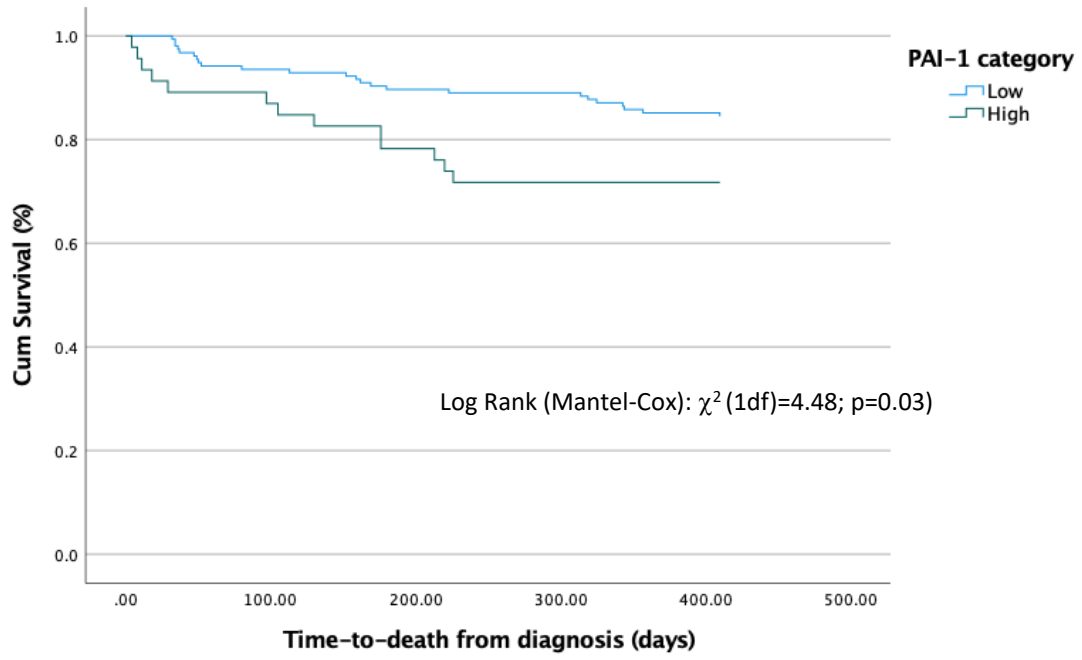

**Figure 2.6.** - Kaplan Meier survival curves presenting one-year mortality for septated and non septated case




| Days | 0 | 100 | 200 | 300 | 400 |
|---|---|---|---|---|---|
| **Non-septated** | 94 | 88 | 86 | 84 | 83 |
| **Septated** | 368 | 335 | 322 | 310 | 298 |

**Numbers at risk**

**Figure 2.7.** Kaplan Meier survival curves presenting one-year mortality PAI-1 high and PAI-1 low cases (Multivariate Cox regression p value)




| Days | 0 | 100 | 200 | 300 | 400 |
|---|---|---|---|---|---|
| **PAI-1 low** | 164 | 153 | 146 | 145 | 138 |
| **PAI-1 high** | 50 | 43 | 38 | 34 | 34 |

**Numbers at risk**

## Macroscopic pleural fluid purulence

Given the increased representation of frank purulence in the non-septated cohort (45% vs 21%) (Table 2.1), further analysis was performed to explore the composition of inflammatory and fibrinolysis-associated proteins in fluid samples comprising frank pus versus non-purulent macroscopic appearances. The only significant difference in patient characteristics between the two groups was a significantly greater proportion of poor dental hygiene in the purulent group (60.3% vs 44.3%, $\chi^2$ 1df 11.1; p=0.009) (Table 2.11).

**Table 2.11** - Baseline patient characteristics of patients with pus

| Patient characteristics | Pus (n=151) | Non-pus (n=386) | p-value |
|---|---|---|---|
| Age, yr, median (IQR) | 59 (43 – 70) | 64 (47 – 72) | 0.11 |
| Male, n, (%) | 103 (68.2) | 247 (64.0) | 0.65 |
| Community acquired, n (%) | 136 (90.7) | 340 (88.3) | 0.44 |
| Poor dental hygiene, n (%) | 91 (60.3) | 171 (44.3) | 0.009 |
| Baseline pyrexia, n (%) | 25 (17.0) | 86 (22.8) | 0.14 |
| Baseline tachycardia | 48 (32.7) | 130 (34.7) | 0.66 |

The frequency of pleural infection diagnoses based on the aspiration of frank pus in the PILOT study overall was 151/537 (28.1%) (Figure 2.8) and in 65/214 (30.3%) ($\chi^2$ 1df 0.38; p=0.54) of the pleural fluid samples with complete protein profile data used in this study. The pleural fluid protein compositions were completely different with purulent samples containing significantly higher median levels of IFN-ɣ, TNF-α and uPA with significantly lower levels of PAI-1, MCP-1/CCL2, and D-dimer (Table 2.13). The PF proteins were entered into a stepwise multinomial regression model and low PAI-1 and high uPA were the only proteins independently associated with the presence of purulent fluid (Table 2.14).

**Figure 2.8** – Macroscopic pleural fluid appearances as reported in the PILOT study

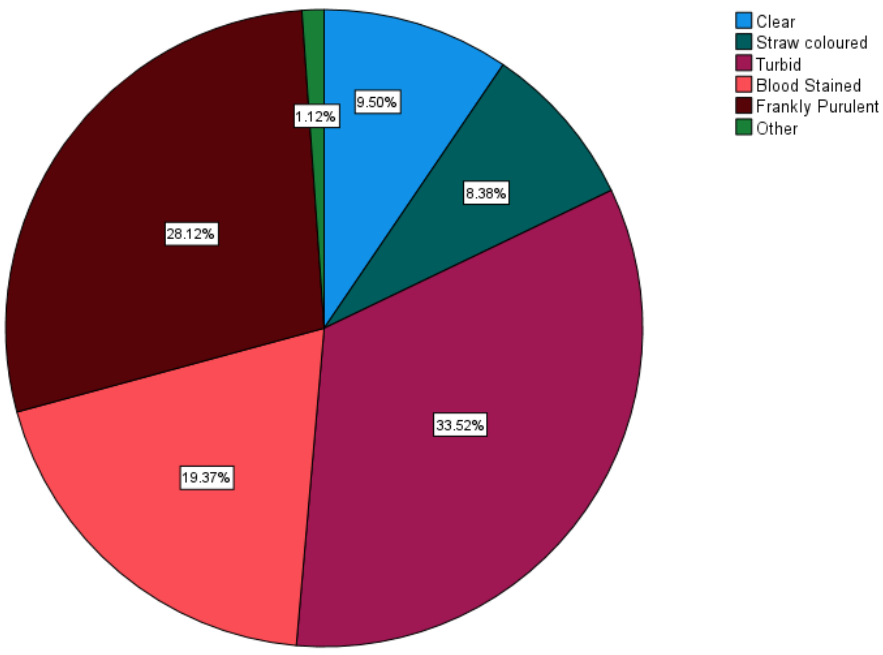


**Table 2.12** – Conventional serum and pleural fluid parameters in the pus and non-pus populations

| Conventional parameters | Grand Median | Pus (n=146) | Non-pus (n=370) | P value |
|---|---|---|---|---|
| WCC; $x10^9$/L | 14.2 | 15.9 (13.2 – 21.4) | 13.5 (10.4 – 18.7) | 0.130 |
| CRP; mg/ml | 205.6 | 226.0 (154.6 – 311.5) | 189.9 (126.0 – 295.0) | 0.55 |
| Platelets; $x10^9$/L | 396.0 | 480.0 (372.0 – 515.0) | 389.0 (310.0 – 496.0) | 0.048* |
| Albumin; g/dL | 28.00 | 25.0 (20.0 – 29.5) | 28.0 (23.0 – 34.0) | 0.029* |
| Urea; mmol/L | 4.85 | 5.0 (3.5 – 8.4) | 4.8 (3.6 – 7.2) | 0.56 |
| **Pleural fluid** | | | | |
| LDH; IU/L | 2034.0 | 5300.0 (2294.5 – 19630.0) | 1430.0 (823.0 – 2570.0) | <0.001* |
| Glucose; mmol/L | 2.0 | 0.8 (0.3 – 2.4) | 2.4 (0.6 – 4.2) | 0.004* |

*statistically significant at p<0.05

**Table 2.13.** Pleural fluid protein levels based on presence/absence of frank purulence

| PF Protein | Grand Median | Purulent (n=65) | Non-purulent (n=149) | p-value |
|---|---|---|---|---|
| **PAI-1,** ng/mL; median (IQR) | 1330.28 | 565.29 (133.43 – 1329.29) | 1559.80 (904.51 – 2019.49) | <0.001 |
| **MCP-1/CCL2, n**g/mL; median (IQR) | 2.05 | 0.86 (0.08 – 3.21) | 4.48 (1.31 – 10.10) | 0.034 |
| **IFN-γ**, ng/mL; median (IQR) | 0.022 | 0.027 (0.016 – 0.043) | 0.021 (0.018 – 0.034) | 0.009 |
| **D-dimer, n**g/mL; median (IQR) | 9.85 | 9.07 (4.10 – 11.35) | 10.14 (8.69 – 14.73) | 0.001 |
| **TNF-α**, ng/mL; median (IQR) | 0.058 | 0.078 (35.5 – 140.8) | 0.052 (41.2 – 113.8) | 0.012 |
| **uPA; n**g/mL; median (IQR) | 0.39 | 1.06 (0.41 – 2.24) | 0.27 (0.16 – 0.46) | <0.001 |

Key: proportions were compared using $\chi^2$ statistics and medians were compared using independent K samples median test.

Comparing conventional pleural infection serum and pleural parameters of cell death turnover, patients with frank purulence had a higher serum platelet count, lower serum albumin concentration and a 3.5-fold higher median pleural fluid LDH (Table 2.15).

**Table 2.14** - Multivariable logistic regression model of PF proteins for frank pus

| Factor | Regression coefficient | Std error | Z value (Wald) | p-value |
|---|---|---|---|---|
| PF PAI-1 | 0.000 | 0.000 | 16.83 | <0.001 |
| PF CCL.2/MCP-1 | 0.000 | 0.000 | 2.97 | 0.085 |
| PF IFN.gamma | 0.001 | 0.001 | 1.74 | 0.19 |
| PF uPA | 0.001 | 0.000 | 20.79 | <0.001 |
| PF TNF.alpha | 0.000 | 0.000 | 2.35 | 0.13 |
| PF D.dimer | 0.000 | 0.000 | 0.59 | 0.44 |

## 2.5 DISCUSSION

Using human biological samples from the largest prospective observational pleural infection cohort to date in the world literature, this study confirms the findings of studies using animal models and smaller retrospective clinical studies in demonstrating that levels of endogenous PF PAI-1 in pleural infection are considerably elevated intrapleurally (Idell et al., 2017; Tucker et al., 2016; Zentina et al., 2019). These data infer that despite multiple factors being associated with a general increased level of endogenous PF PAI-1 (Eren et al., 2014; Morrow et al., 2021; Ploplis, 2011), the differences in concentrations of PF PAI-1 and the presence or severity of septations in this study population were independent of any pre-existing patient factors such as age or co-morbidity.

Endogenous PF PAI-1 appears to play an integral role in the biological development and progression of septations. PF PAI-1 had a stronger association with septations than any other conventional serum, blood, or pleural fluid parameter as well as the inflammation and fibrinolysis-associated proteins measured in this study. Of particular interest was the finding that pro-inflammatory cytokines (CCL2/MCP-1, IFN-$\gamma$ and TNF-$\alpha$) measured from patients with active pleural infection were not associated with septation severity. This observation is intriguing and may suggest that once pleural injury induces suppression of the fibrinolytic system, inflammation has a lesser role than PAI-1 in driving septation progression. This may explain why septation severity was not associated with differences in fever or tachycardia or markers of pleural and systemic inflammation (PF LDH, serum CRP, blood WCC).

This large prospective cohort is the first to show that approximately 4 in 5 patients with pleural infection will present with some degree of sonographic septation, this incidence having been previously unknown. One in three will have a severely septated appearances. Detection of septations is often used by clinicians as a decision-making parameter, or a tool to select treatment, based on assumptions such as reduced likelihood of successful pleural drainage. However, these assumptions are challenged by the presented evidence; there was no increased need for more invasive intervention compared to standard treatment with an ultrasound-guided optimally placed chest tube with regular saline flushes. Whether subjects with sustained

elevations in PF PAI-1 are more predisposed to florid septation and failed drainage was not examined in this study.

Two retrospective studies (Chen et al., 2009, 2000) have previously suggested septations are associated with poorer clinical outcomes and need for more invasive intervention. However, due to sample size and methodology, these data are likely to be flawed. The data from our study demonstrate an increased septation severity is associated with a greater use of IET and surgical referral at 3 months. However, neither septation state nor severity at baseline, in isolation, were associated with a need for surgery at 3 months, longer length of hospital stay, or likelihood of readmission at 12 months. This being the case, it is not possible to exclude the possibility that septation detection results in different behaviour by clinicians, as demonstrated by their independent relationship with IET use in the multivariate model.

In most cases, the diagnosis of pleural infection is straightforward based on well-established conventional blood, serum and PF biomarkers, and initial management of pleural infection is focused on early chest tube drainage and prompt antibiotics. In a cohort of 93 patients with parapneumonic effusions, Arnold and colleagues demonstrated that high PF soluble urokinase plasminogen activator receptor (suPAR) predicted pleural fluid pH and subsequent chest tube insertion (Arnold et al., 2020). This represented a step forward in our understanding of the biology of pleural infection progression. Albeit in a smaller cohort, pleural fluid suPAR concentrations were higher in patients with loculated collections (graded as absent/present), which likely represented two ends of the septation spectrum. In the present study, using a pre hoc definition and a validated method for quantification of septation severity, we have now demonstrated PF PAI-1 to be an accurate biological correlate of a radiological outcome across the spectrum of septation development.

Importantly, this is the first study to demonstrate that pleural biology relates to clinically important outcomes in pleural infection. PF PAI-1 was independently associated with length of stay and mortality, a finding thus far not demonstrated by suPAR (Arnold et al., 2020). A recent study by Hoshino et al examining sepsis biomarkers and coagulation/fibrinolysis markers on ICU admission found serum PAI-1 to be the only independently predictor of 28-day mortality in sepsis patients (Hoshino et al., 2017). Schmitt et al found that acute fibrinolysis shutdown, judged by raised serum PAI-1

levels, occurred early in sepsis and was associated with increased morbidity and mortality in septic shock (Schmitt et al., 2019). The underlying pathomechanisms and specific temporal kinetics of PAI-1 in the pleural space are yet to be fully understood but it is plausible that this process is exaggerated, or occurs more rapidly, within the confines of the pleural space prior to significant systemic compromise reflecting the increased mortality associated with this condition.

Septations are an attractive radiological biomarker, particularly with bedside ultrasound becoming routinely used in clinical practice by respiratory physicians. However, despite data from both this study and that by Arnold et al demonstrating the relationship between biomarkers of fibrinolysis inhibition such as PF-PAI-1 and PF suPAR with the development of septations, this large prospective cohort to our knowledge provides the strongest evidence that septations do not, in isolation, bear relation to clinical outcomes. We therefore hypothesize that septations are likely an epiphenomenon in the progression of pleural sepsis, and this may be the reason that lone fibrinolytic therapy (in the absence of DNase) does not result in improved clinical outcomes in randomised trials of adult pleural infection (Rahman et al., 2011).

Treatment with IET is based on activation of endogenous plasminogen providing sustained fibrinolytic activity that degrades intrapleural fibrin. Several factors may be associated with treatment outcome including the rate of intrapleural inactivation of a fibrinolytic, levels of endogenous plasminogen, a higher level of active PAI-1 and extracellular DNA, and potentially the formation of biofilms (Zhang et al., 2020). These may collectively, or synergistically contribute to poor outcomes in pleural infection (Thomas et al., 2020).

The analysis of purulent pleural fluid samples adds important insight into pathogenesis. It has not yet been fully explained why some patients present with unilocular purulent collections, while other develop more complex, septated effusions. In this study, purulent collections were shown to contain higher levels of pro-inflammatory cytokines, cell death and turnover as demonstrated by the higher levels of IFN-gamma, TNF-alpha and PF LDH with relatively suppressed intrapleural levels of fibrinolysis inhibition (PAI-1). These data suggest that lesser inhibition of plasminogen activator activity associated with reduced pleural fluid levels of PAI-1 may thereby favour intrapleural purulence. The inhibition of fibrinolysis favours intrapleural

organization with septation, but whether it reduces intrapleural inflammation remains unclear (Tucker et al., 2016). Therefore, is a surge in fibrinolysis inhibition an intrinsic host defence mechanism to reduce inflammation and sepsis? The significance of cross-talk between bacteria and an inflammatory pleural environment remains unclear. One may infer that the invading pathogen(s) plays a significant role judging by the increased proportion of patients with poor dental hygiene who appear to develop purulent collections, and a detailed analysis of microbiology and its association with septation is now required.

This study has some limitations. PAI-1 testing is complex. Total PAI-1 antigen assays measure the sum of active PAI-1, tPA/PAI-1, and latent PAI-1 ("Laboratory Techniques in Fibrinolysis Testing," 2019). PAI-1 levels, but not its activity were measured in this study. Whether PAI-1 is cleaved in pleural fluid by proteases or if a proportion of PAI-1 reverts to its latent form remains unknown. As pleural infection is a one-off event where sampling occurs at baseline followed by urgent drainage being clinically required, intermittent drainage and repeated sampling (e.g. via an indwelling pleural catheter) was not feasible (or ethical) to assess for diurnal variability in PAI-1 or measure how levels progressed with treatment. To the best of our knowledge, no other studies have addressed these limitations and they should be prioritised in future studies. Ideally, rapid centrifugation followed by immediate storage of the cell-free fluids at -80C is required to reliably perform these analyses. However, in a large scale, multicentre study such as PILOT, this was not feasible. The sample collection and processing protocol applied is standardised within our group to ensure samples are sent promptly, received and processed centrally in a timely and uniform fashion and has been used with success in other studies for proteomics analyses (Psallidas et al., 2018). The validated septation score method allowed assessment of septation severity but is not immune to a degree of inter-operator variability (as septation appearance can differ depending on angle of the probe against the rib space) but operators were asked to specifically use the maximal degree of septation to quantify severity, which should have minimised this. Furthermore, it should be clear that final clinical outcome was not knowable at the time the ultrasound images were taken and scored by clinicians.

The size of the analysis cohort paired with complete biological samples and ultrasound data was smaller than the PILOT population as a whole but nonetheless we have

demonstrated that this was a representative cohort based on similar patient demographics and baseline characteristics (table 2.4). Despite this, the current study still represents the largest analysis using human samples associating baseline parameters of inflammation and fibrinolysis in pleural fluid with radiological and predefined clinical outcomes. Secondly, as the PILOT study did not collect blood samples, measurement of serum PAI-1 was not possible and a correlation of these levels with PF PAI-1 may have enabled a more complete understanding of its role in the pathogenesis of pleural infection. The most commonly used plasminogen activator (tPA) is rapidly inactivated by PAI-1 in the pleural space. We are here unable to assess whether PAI-1 levels were associated with tPA treatment failure due to the small number of patients in the PILOT study who received IET, as the majority of trial recruitment occurred prior to IET becoming commonplace. Nonetheless our data suggest that PAI-1 has some influence on the outcome of pleural injury and may dampen the ability of fibrinolytics to activate plasminogen.

## 2.6 CONCLUSION

In summary, this is the first study to associate pleural biological parameters with a validated sonographic septation outcome as well as clinically important outcomes. Within a large cohort of patients with confirmed pleural infection, increased levels of endogenous PF-PAI-1 was associated with more severe sonographic septation, longer hospital stay and reduced survival at 12 months. Plasminogen activation suppression with downstream suppression of local fibrinolysis appears to have a more dominant role compared to the pro-inflammatory state in driving septation development and progression. Increasing severity of septations was associated with a higher rate of clinician-driven intervention with IET and surgery, but were not independently associated with clinical outcomes. These signals require prospective validation before the utility of PF-PAI-1 in pleural infection prognostication and management can be fully elucidated.

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# APPENDIX A2.1

## PILOT study inclusion criteria

Patients were included if they had a clinical presentation consistent with pleural infection and any of the following criteria:

1. Pleural fluid that was macroscopically purulent; or

2. Pleural fluid that was positive on culture for bacterial infection; or

3. Pleural fluid that demonstrated bacteria on Gram staining; or

4. pleural fluid with a pH ≤7.2 (measured by blood gas analyser) or low glucose level (≤3 mmol·L−1 or ≤55 mg·dL−1) in a patient with clinical evidence of infection; or

5. contrast-enhanced computed tomography (CT) evidence of pleural infection (consolidation of underlying lung with enhancing pleural collection) in a patient with clinical evidence of infection, alongside exclusion of other sources of infection.

Evidence of infection was assessed by the recruiting physician on the basis of fever, an elevated peripheral blood white-cell count, or elevated serum inflammatory markers such as C-reactive protein (CRP).

Study exclusion criteria were as follows:

1. age <18 years;

2. no pleural fluid available for analysis;

3. previous pneumonectomy on the side of pleural infection; and

4. expected survival of <3 months due to co-morbid disease, as judged by the recruiting physician.

## REFERENCE

Corcoran, J.P., Psallidas, I., Gerry, S., et al 2020. Prospective validation of the RAPID clinical risk prediction score in adult patients with pleural infection: the PILOT study. Eur Respir J. https://doi.org/10.1183/13993003.00130-2020

# APPENDIX A2.2

**Trial Specific Procedure for the PILOT study: Pleural fluid Sample Collection, processing and storage (UK sites only)**

## Introduction and scope

The purpose of the Trial Specific Procedure is to describe the procedures relating to pleural fluid sample collection, processing, and storage for the purpose of the PILOT study. It applies to researchers taking part in the PILOT study and performing the above procedures as per the protocol and the delegation log.

## Procedure

*Sample Collection*

All patients who have consented to study sample collection for the PILOT study should have these additional pleural fluid samples taken on the day of enrolment.

The unprocessed trial samples must be labelled with the patient trial number (generated at patient enrolment) and the time and date of collection. Staff collecting samples should complete the relevant sample form; either Form PILOT 01.02-A (Oxford site), Form PILOT 01.02-B (all other UK sites) provided with the study materials. All sample collection tubes should also be labelled using the pre-printed sheet of labels provided with the study materials.

*Baseline study pleural fluid samples:*

1x StarLab 15.0mL tube (blue screw top, DNase/RNase/DNA/RNA free)

## Sending samples

**For UK sites**; unprocessed trial samples taken at sites should be sent as soon as possible in the sample boxes provided to: PILOT study, Oxford Respiratory Trials Unit, Churchill Hospital, Headington, Oxford. OX3 7LE.

Study samples may be stored in a fridge (4ºC) until sending, if necessary.
To avoid potential spillages in the post, please make sure the lids are firmly screwed onto the plastic containers. Please use as many plastic containers and posting boxes as you need when sending samples.

## Sample processing

- The 15.0mL StarLab (or equivalent) pleural fluid tube is centrifuged at 800g for 10 minutes. This should simply be divided into 2.0mL sterile aliquots (up to 6 per patient) and transferred into 2.0mL microcentrifuge tubes (Fisher Scientific, DNase/RNase/DNA/RNA free) for freezing and storage.

**Sample storage** *(Oxford only)*

- Create subject and sample set on Sapphire and print sample labels.
- Freeze aliquots at -80 ºC
- Record number of aliquots stored on PILOT freezer log.
- Check samples into appropriate freezer trays on Sapphire system

# APPENDIX A2.3

## Expansion of lab methods on PAI-1 analysis

Protein measurement assays were performed using a commercially available Luminex kit for Serpin E1/PAI-1 (Luminex high performance assay, R&D) as our analyte of interest. We chose to use Luminex assays over ELISA for the protein measurements due to increased precision, time efficiency and cost-effectiveness (dupont et al., 2005). These will allow us to measure the absolute expression of PAI-1 in the pleural fluid samples (in pictogram/ml). We then used a spectrophotometer (NanoDrop$^{TM}$ ) to measure the total protein expression in each sample . As the pleural fluid samples have varying levels of protein enrichment, we then calculated the relative PAI-1 expression of each sample by dividing the absolute PAI-1 by the total protein concentrations.

### Principles of the Luminex assay

Luminex kits are designed for use with dual laser, flow-based sorting and detection analysers, which, in my case, was the Bioplex$^{TM}$ 200 system in the Nuffield Department of Medicine laboratories at the University of Oxford. The kits are based on analyte-specific antibodies that are pre-coated onto colour-coded microparticles, known as ‘beads’. The beads, standards and samples are pipetted into wells of a 96 well plate. During an incubation period, the immobilised antibodies then bind the analytes of interest. After washing away any unbound substances, a biotinylated antibody cocktail specific to the analyte of interest is added to each well. Following a further wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE), which binds to the biotinylated detection antibodies, is added to each well. A final wash removes unbound Streptavidin-PE and the microparticles are resuspended in buffer and read using the Luminex analyser. One laser is microparticle-specific and determines which analyte is being detected. The other laser determines the magnitude of the phycoerthrin-derived signal, which is in direct proportion to the amount of analyte bound.

**Laboratory methods**

A 2-step dilution of the analyte was conducted as per the manufacturer recommendations to achieve a 1:200 ratio of pleural fluid to diluent. 1.5ml centrifuge (Eppendorf) tubes were labelled with the sample numbers. 10microlitre of processed pleural fluid sample (collected from the PILOT study) was added to 90microlitre diluent in each tube (first set). This process was then repeated by adding 10microlitres of the diluted sample to 90microlitre of diluent (second set).

The first column of a 96-well Luminex plate was reserved to calculate the standard (or calibration) curve to allow proper calculation of these known protein concentrations using the serial dilution method (Table A2.3.1). Diluent was added in two wells to be used as the blank samples. The standard curve was then used to calculate the PAI-1 protein levels for the PILOT samples as these are indirect protein measurements dependent on the fluorescence of the beads. A separate standard curve was calculated for each of the plates but the various proteins were measured at the same time ('cocktail') as each protein would have different fluorescence. 50 ul of diluted pleural fluid were added to each well with duplicates for each sample. 500 ul of premixed microparticle cocktail (containing the beads) was mixed with 5ml of diluent. 50 ul per well of this mixture was then added to the plate. The plate was covered with adhesive foil and incubated at room temperature on a standard plate shaker at 800rpm for 2 hours.

Following the first incubation a magnet was used to keep the magnetic beads in the plate while the fluid was discarded. The plate was washed three times with wash buffer as per protocol. For each wash 100 ul of wash buffer was added to each well using a multi-channel pipette and the plate was incubated for two minutes and then the buffer was discarded. The diluted biotin-antibody cocktail was prepared by adding 500 ul of biotin-antibody cocktail to a vial containing 5ml of Diluent RD2-1 (included in the kit). 50microlitres per well of biotin antibody micro-cocktail was added and the plate was incubated at room temperature on the microplate shaker (800rpm) for a further hour.

**Analysis**

Each well was matched with original trial sample number. The blank wells were used to subtract the background fluorescence from all the measurements. The standard curve was calculated from the wells in the first column using a polynomial regression. A polynomial regression was recommended by the manufacturer and provides a better fit for the data.

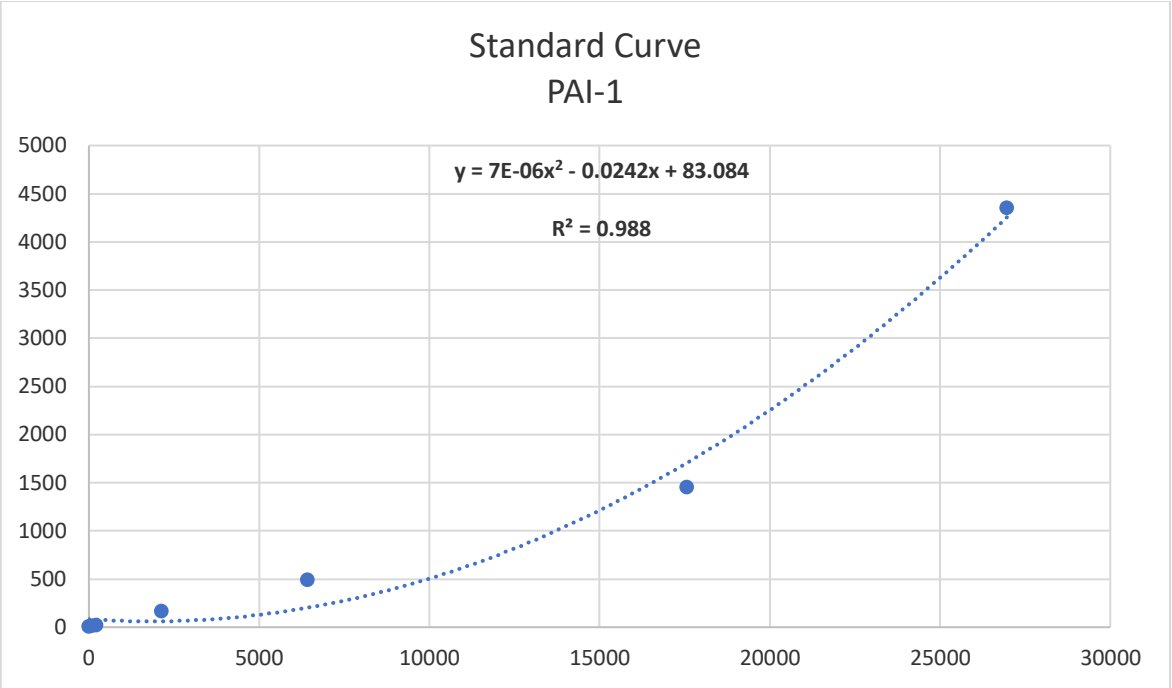
The polynomial regression was used to create a function (standard curve) to calculate the protein measurements; $y=7E\text{-}06x^2 – 0.0242x + 83.084$ with y = concentration of protein (pg/ml) and x= intensity of fluorescence (Figure A2.3.1). The $R^2$ (0.98) suggests that the standard curve can be used to calculate the protein concentration in the samples with a high level of accuracy. Each measurement was then multiplied by 200 to account for the dilution (Figure A2.3.2).

**Table A2.3.1 - Samples used to calculate the standard curve**

| Measured FI | FI-Blank | Value (pg/mL) |
|---|---|---|
| 41 | 0 | 0 |
| 116.5 | 75.5 | 5.967078 |
| 266 | 225 | 17.90123 |
| 2180 | 2139 | 161.1111 |
| 6462.5 | 6421.5 | 483.3333 |
| 17599 | 17558 | 1450 |
| 27016 | 26975 | 4350 |

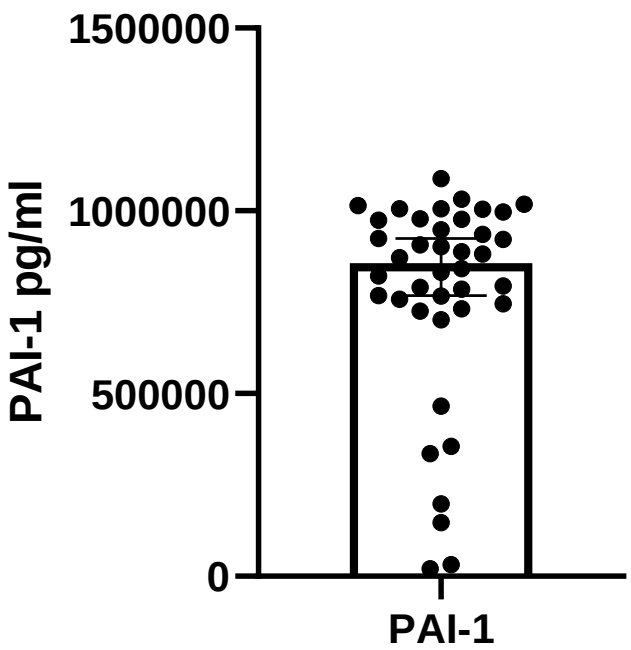
FI = Fluorescence intensity readout (arbitrary units)
FI - Blank = FI – background fluorescence (from the empty wells containing diluent only)
Value = concentration of protein (pg/mL)

**Figure A2.3.1 – Graph displaying the standard curve used to calculate the PAI-1 concentrations**




Y axis – Fluorescence intensity (arbitrary units)
X axis – PAI-1 concentration (pg/mL)

**Figure A2.3.2 – Column scatter plot of the PAI-1 levels from the first 40 samples**



### Measurement of the fluorescence intensity

The fluorescence intensity was measured using a Bio-Plex 200 ® System (Bio RAD™) (Figure A2.3.3). Bio-Plex assays are bead-based assays that can be performed in a mixed array (multiplexed). The machine combines two lasers, fluidics, and real-time digital signal processing to distinguish up to 100 different sets of color-coded polystyrene beads, each bearing a different assay.

**Figure A2.3.3 - Bio-Plex® 200 System used to measure the fluorescence**

# APPENDIX A2.4

## The chest radiograph as an outcome predictor in pleural infection

### Introduction

Potential outcome biomarkers such as PAI-1 and suPAR are not currently validated and such assays are not readily available in clinical practise. In contrast, the chest radiograph is the most common investigation undertaken in daily practice for pleural infection diagnosis, and has important clinical value in diagnosis of a number of other diseases. Chest radiographs are safe (minimal radiation), low in cost, very accessible in most healthcare settings, and are often the first investigation to lead to a suspicion of pleural infection. They are therefore very well placed as a baseline measure and, for the aforementioned reasons, serial radiographs can also be helpful in monitoring treatment impact. They are regularly used in guiding treatment decision-making in pleural infection, particularly as other modalities such as thoracic ultrasound (TUS), which may be more sensitive, are operator-dependent and may not be available in all healthcare scenarios (for example, low and middle income countries). To be used reliably to monitor treatment progress, TUS depends on the same competent operator being present on a daily basis throughout the patient's treatment, particularly as documentation is often inconsistent. A chest radiograph is easy to interpret and enables standardised comparisons.

For the reasons above, it was considered as the main outcome measure for the MIST-2 study (Rahman et al., 2011). Incorporating a digital measurement analysis strategy for the chest radiograph (explained further below) allowed it to be a more objective outcome measurement, increasing the power of the study as a continuous outcome (pleural opacity measured as a number from 0-100).

MIST-2 was a blinded, 2-by-2 factorial trial in which 210 patients with pleural infection were randomly assigned to receive one of four study treatments for 3 days: double placebo, intrapleural t-PA and DNase, t-PA and placebo, or DNase and placebo. The primary endpoint was the change in pleural opacity, measured as the percentage of the ipsilateral hemithorax occupied by effusion on the chest radiograph on day 7 as compared with day 1. Secondary outcomes included relative change in chest radiograph opacification, referral for surgery, duration of hospital stay and death from

any cause at 3 months. Clinicians had access to the chest radiograph for decision-making and there were standard procedures for referral to surgery. Local investigators recorded the reasons for referring patients for surgical treatment, which were subject to an independent, blinded review to identify reasons for surgery before data analysis.

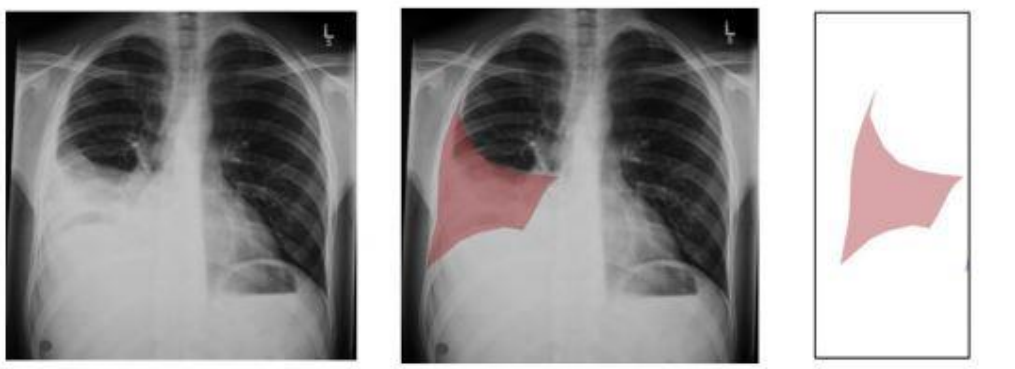
In a separate primary endpoint validation study, the optimal chest radiograph analysis strategy was defined through 10 randomly selected patients who had undergone both thoracic computed tomography (CT) and contemporaneous postero-anterior chest radiographs before and seven days after pleural fluid drainage. All CT and chest radiograph assessments were conducted blind of each other. The area of pleural opacity on the chest radiograph was measured digitally by two separate assessors (see next section). Linear regression analysis modelling of the CT measured volume of pleural fluid change using this digital chest radiograph measurement strategy ($r^2=0.71$, $F=19.1$, $p=0.002$) showed that 71% of the variability of the CT measured volume change was explained by this quantification strategy.

**Chest radiograph measurement strategy in MIST-2**

Chest radiographs were saved as a digital image (JPEG format). The digital image of the chest radiograph was opened in Paint Shop Pro version 11.0 (Corel, California, USA) and the area occupied by the pleural collection and the hemithorax manually drawn around using the “pen tool”, forming a polyhedron exactly matching the area of interest. The chest radiograph image was then removed and the polyhedron area saved as a separate JPEG file (Figure 1a and 1b). The same process was then repeated for all areas of interest on the chest radiograph. The area of each polyhedron (effusion and hemithorax) was measured using a Java image processing programme developed by the National Institute of Health, USA (ImageJ – website http://rsb.info.nih.gov/ij/), which is able to accurately measure areas occupied by polyhedrons in absolute pixel terms. This permitted calculation of the percentage hemithorax area occupied by effusion (Figure 1c). The process of calculation of the hemithorax area and pleural collection area was repeated for each chest radiograph (i.e. separately calculated hemithorax and pleural opacification areas for day 1 and day 7 chest radiographs).

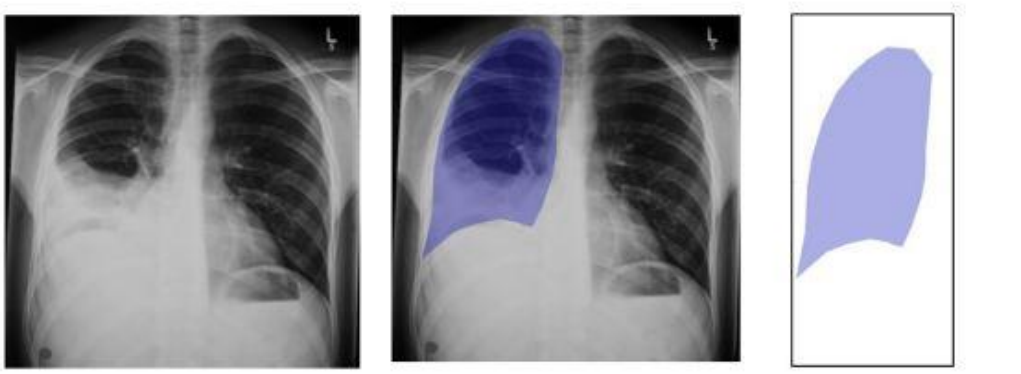
**Figure 1: Assessment of percentage hemithorax area occupied by effusion**

**Figure 1a – Digital measurement strategy of effusion area**



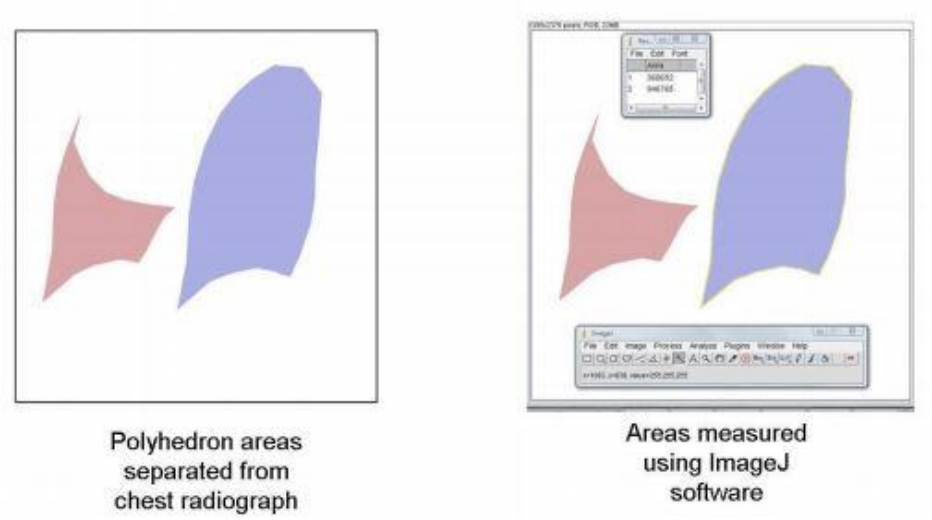
Original chest radiograph | Effusion area measured | Area of effusion alone preserved for analysis

**Figure 1b – Digital measurement strategy of hemithorax area**



Original chest radiograph | Hemithorax area measured | Area of hemithorax alone preserved for analysis

**Figure 1c – Area measurement using "ImageJ" software**



Polyhedron areas separated from chest radiograph | Areas measured using ImageJ software

**Study Rationale**

The results of MIST-2 showed that the primary endpoint was positive with statistical significance (p=0.005) for combination therapy with tPA and DNase versus double placebo, tPA alone or DNase alone. Although the effect size was small based on absolute values (8%), the relative reduction in the infected pleural collection was approximately doubled when compared to t-PA alone (2%) or DNase alone (4.5%) (Rahman et al., 2011). Over the last 12 years since the publication of these results, approximately 800 patients have been reported in the literature who have gone on to have their pleural infection safely and successfully treated with this intrapleural enzyme therapy. This included a large cohort of 107 unselected patients from centres in three countries, of whom 92.3% were safely and successfully treated without need for surgical intervention (Piccolo et al., 2014). However, the fact that the combination arm of the MIST-2 RCT had only 52 patients has meant that tPA/DNase treatment has struggled to be incorporated into national guidelines and become standard practice, with concerns about efficacy and bleeding risk yet to be fully alleviated (see Chapter 5). Some members of the chest and pleural physician community have also been sceptical about the use of a radiographic outcome measure as a surrogate for clinical efficacy. The aspect of a radiographic outcome as a true surrogate for clinical outcome has not been directly addressed.

The aim of this study was to establish whether or not a radiographic outcome measure (such as improvement in chest radiograph) could indeed be used as a surrogate marker for treatment success and predict clinically important patient outcomes using the image data available from the entire MIST-2 dataset (n=210 participants enrolled). The outcomes chosen were based on those important to clinicians and patients and were here deliberately considered as clinically meaningful (i.e. not just radiographic) and were:

a) length of stay in hospital
b) need for surgery within 3 months of diagnosis
c) death at 3 months.

## METHODS

Retrospective analyses were conducted using the prospectively collected data from the MIST-2 database. Four separate analyses were conducted. The database was screened for completeness and the potential impact of missing data was deemed to be negligible for the purpose of this analysis. Therefore no imputations for missing values were performed, but only complete cases were analysed.

Linear regression was used to model the change in CXR opacity (using the measurements obtained by the two assessors in the MIST-2 study) with the number of days in hospital as a continuous variable. Logistic regression models were used to investigate association of CXR opacity change and need for surgery and death at 3 months (yes/no), as dependent variables (categorical). The independent variable was absolute change in chest radiograph opacity (MIST-2 primary endpoint), chosen as statistically more powerful (reported in exact measurement) than relative change (reported as a percentage). This was corrected for day 1 radiograph appearance to account for baseline variability. Finally, a fourth analysis was conducted for relative change (a secondary endpoint in MIST-2) modelled using logistic regression against surgery or death at 3 months, as a combined outcome. This composite outcome was chosen to increase statistical efficiency by increasing the number of events (i.e. surgery or death) as these are both considered clinically important negative outcomes in pleural infection.

Relative change was explored as a more clinically relevant measure in daily practice. To illustrate this point, if the pleural opacity had been digitally measure as occupying 40% of the hemithorax at baseline and then measured as occupying 20% of the hemithorax after treatment, this equates to a 20% absolute reduction but a 50% relative improvement. This amount of clearance is more akin to what clinicians assess when comparing the two films in the clinical setting.

The results were reported using p statistics to measure significance with a pre-set cut of $<0.05$ for statistical significance. To understand how much variation in the dependent variable can be explained by the model, $R^2$ was used for linear regression and Nagelkerke $R^2$ was used for logistic regression, as per American Psychological Association (APA) recommendations. Nagelkerke $R^2$ is a measure of goodness of fit in logistic regression analysis. It ranges from 0 to 1, with values closer to 1 indicating

a better fit of the model. However, unlike in linear regression analysis, where $R^2$ can be interpreted as the proportion of variance explained by the model, Nagelkerke $R^2$ cannot be interpreted as easily. A common rule of thumb to interpret it, is a value of 0.2 or less indicates a weak relationship between the predictors and the outcome. A value of 0.2 to 0.4 indicates a moderate relationship. A value of 0.4 or higher indicates a strong relationship.

IBM SPSS® version 25 was used for all analyses.

## RESULTS

### Analysis 1: Length of hospital stay (linear regression)

Data was available for 190/210 patients (90.5% data completion) and these were included in the analysis. The mean length of stay was 13 days (min 6.95, max 29.3; SD 3.995). A linear regression analysis was conducted to assess the association of length of stay in hospital based and change in radiograph opacification from day 1 to day 7, corrected for day 1 chest radiograph opacification (Table 1). Analysis demonstrated a significant association ($F(2,182) = 10.5$, $p<0.001$, with an $R^2$ of 0.103.

**Table 1 – Difference in chest radiograph change between participants who received and those who did not receive surgery**

| Absolute change (day 1 to day 7) | n | Mean | SD |
|---|---|---|---|
| **Non-Surgery** | 159 | -20.5 | 22.3 |
| **Surgery** | 31 | -16.1 | 18.8 |

### Analysis 2: Surgery at 3 months (logistic regression)

A binomial logistic regression was used to assess how well need for surgery at 3 months (dependent variable) was predicted from absolute change in chest radiograph opacification (independent variable), corrected for baseline (day 1) chest radiograph opacification. In total, 190 patients were included in the analysis (98.4% data completion rate). The dependent variable (surgery at 3 months) was measured on a

dichotomous scale (1=yes, 0=no). The independent variable was the absolute change in CXR opacification, digitally measured and reported as a number from 0 to 100.

The logistic regression model demonstrated absolute change in CXR was highly associated with outcome ($\chi^2$ (2df) = 18.6, $p$<0.005), with the model, correcting for baseline chest radiograph opacity (day 1), explaining 16% (Nagelkerke $R^2$) of the variance in need for surgery and correctly classifying 84.2% of cases.

### Analysis 3: Mortality at 3 months (logistic regression)

In total, 189 patients were included in the analysis (92.6% data completion). No association between mortality and CXR change was demonstrated ($\chi^2$ (2df) = 4.05, $p$=0.13 (Table 2).

**Table 2 – Logistic regression model for relationship between absolute change, surgery and mortality**

| Absolute change (day 1 to day 7) | n | $\chi^2$ | df | p-value | Nagelkerke $R^2$ |
|---|---|---|---|---|---|
| **Surgery at 3 months** | 190 | 18.6 | 2 | <0.05 | 0.158 |
| **Mortality at 3 months** | 189 | 4.05 | 2 | 0.13 | 0.052 |

### Analysis 4: Combined outcome (surgery/death at 3 months)

A binomial logistic regression analysis was conducted to assess the association of a combined clinical outcome (death or surgery at 3 months, as used in the MIST1 randomised study (Maskell et al., 2005) (dependent variable) with relative change in chest radiograph opacification (independent variable). In total, 188 patients were included in the analysis (97.4% data completion rate). The dependent variable (combined outcome) was measured on a dichotomous scale (1=yes, 0=no). The independent variable was the relative change in CXR opacification, digitally measured and reported as a percentage. This approached, but did not reach statistical significance ($\chi^2$ (1df) = 2.97, $p$=0.08), with the model explaining 24% (Nagelkerke $R^2$)

of the variance in need for surgery or death occurring at 3 months, and correctly classifying 77% of cases (Table 3).

**Table 3 – Logistic regression model for relationship between relative change and combined outcome**

| Relative change (day 1 to day 7) | n | $\chi^2$ | df | p-value | Nagelkerke $R^2$ |
|---|---|---|---|---|---|
| **Combined outcome (death/surgery)** | 188 | 2.97 | 1 | 0.08 | 0.24 |

## DISCUSSION

These analyses show that change in chest radiograph opacification is associated with length of stay and need for surgery, although is not significant in predicting mortality. There were no predefined surgical referral (or 'medical treatment failure') criteria in MIST-2. Local investigators recorded the reasons for referring patients for surgical treatment, which were later subject to an independent, blinded review to identify reasons for surgery before data analysis. This review found that all referrals were due to clinical evidence of worsening infection. Patients who needed referral to surgery had an almost 25% less improvement in their chest radiograph (relative change) between day 1 and day 7 (Table 1). Despite the use of a validated digital measurement tool, chest radiograph data has its limitations and is not perfect. It must be acknowledged that the $R^2$ values are poor (<=0.2) but clearly the variation in length of stay and need for surgery cannot be explained by change in chest radiograph alone. In the clinical setting, these are often confounded by a number of factors including treatment received, age, baseline fitness, co-morbidities and local clinical practice. The data is also limited by sample size and number of events. Despite being the second largest cohort (after the MIST-1 study) of randomised, prospectively collected pleural infection patients, the surgical and mortality event rate at 3 months was just 16/209 (7.6%) and 31/209 (14.8%) respectively. With such modelling methodology, the power of analysis is dependent on the event rate.

The positive association of chest radiograph with surgery may also be explained by the fact that in the clinical setting, a lack of chest radiograph improvement may be one

of the factors dictating the decision to proceed to surgery (in combination with a non-resolving inflammatory markers or persistent pyrexia) that would then lead to more detailed cross sectional imaging using CT. However, need for surgery cannot predict length of stay and, if anything, the literature advocating surgery in empyema would suggest a reduced length of stay (although obviously this data is frequently prone to selection bias of younger, fit patients) (Redden et al., 2017). Therefore poor chest radiograph response appears to be associated with negative clinical outcomes, and clearance of infected material as measured by chest radiograph, is clearly an important surrogate marker of response, and may be considered in deciding treatment strategies.

The pleural infection guidelines (Davies et al., 2010), valid at the time of publication of the MIST-2 (Rahman et al., 2011) recommended assessing for medical treatment failure at day 5-7 and hence chest radiographs were appropriately done on day 1 and day 7. However, further radiological or ultrasound surrogates with a stronger association that could be performed earlier in the course of treatment would be of great value to the treatment pathway and are urgently needed.

**APPENDIX 2.4A ORAL ABSTRACT PRESENTED AT BRITISH THORACIC SOCIETY WINTER MEETING 2019**

# Does the appearance of the radiograph matter in pleural infection?

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## Introduction

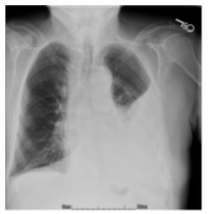
- The chest radiograph is used in clinical practice to guide decision-making in the treatment of pleural infection.
- This outcome was used as the primary endpoint in the second Multicentre Intrapleural Sepsis Trial (MIST-2)
- It was defined as change in area of pleural opacity, measured as percentage of the ipsilateral hemithorax occupied by effusion, between day 1 (D1) and day 7 (D7).
- The value of this radiographic outcome measure as a surrogate for predicting clinically important outcomes e.g. in time in hospital (LOS), need for surgery at 3 months, and mortality at 3 months, has not been directly addressed.

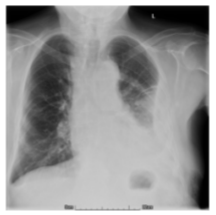
## Methods

- Retrospective analyses of the prospectively collected MIST-2 database were conducted (n=210)
- Regression analyses were modelled with number of days in hospital (linear), and surgery or death at 3 months, both individually and as a combined outcome (yes/no; logistic), as dependent variables.
- The independent variables were *absolute change* in chest radiograph opacity (MIST-2 primary endpoint), and *relative change*, which is more clinically applicable in daily practice (MIST-2 secondary endpoint)
- Each of the analyses was corrected for day 1 radiograph appearance to account for baseline variability
- SPSS (v25) was used for all analyses

## Results

- Both absolute and relative change in chest radiograph opacity between D1 and D7 correlated with hospital LOS and either surgery OR death at 3 months (combined outcome) ($p<0.01$)
- Analysing components of combined outcome individually;
  - both absolute and relative change were associated with need for surgery at 3 months ($p<0.01$ and $p<0.02$ respectively)
  - absolute change in chest radiograph was borderline significant in predicting death at 3 months ($p=0.089$) and relative change was not predictive ($p=0.16$)





**Mortality at 3 months**

| | | B | S.E. | Wald | df | Sig. |
|---|---|---|---|---|---|---|
| Step 1[a] | Day 1 to day 7 change | .025 | .015 | 2.884 | 1 | .089 |
| | day1hemithoraxoccupied | .026 | .014 | 3.694 | 1 | .055 |
| | Constant | -3.258 | .645 | 25.550 | 1 | .000 |

**Surgery at 3 months**

| | | B | S.E. | Wald | df | Sig. |
|---|---|---|---|---|---|---|
| Step 1[a] | Day 1 to day 7 change | .037 | .012 | 9.912 | 1 | .002 |
| | day1hemithoraxoccupied | .045 | .012 | 15.241 | 1 | .000 |
| | Constant | -3.033 | .512 | 35.066 | 1 | .000 |

**Length of hospital stay** ANOVA[a]

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 639.651 | 1 | 639.651 | 4.217 | .041[b] |
| | Residual | 27756.349 | 183 | 151.674 | | |
| | Total | 28396.000 | 184 | | | |
| 2 | Regression | 2937.210 | 2 | 1468.605 | 10.499 | .000[c] |
| | Residual | 25458.790 | 182 | 139.883 | | |
| | Total | 28396.000 | 184 | | | |

a. Dependent Variable: time_in_hosp

b. Predictors: (Constant), Day 1 to day 7 change

c. Predictors: (Constant), Day 1 to day 7 change, day1hemithoraxoccupied

## Conclusions

- The change in chest radiograph during the course of treatment of pleural infection is a robust and clinically important surrogate endpoint which appears to predict meaningful outcomes
- Although surgery may be decided solely on the basis of the radiograph (which may explain this result), change in radiograph appearance predicts other important outcomes (LOS).
- This data supports the clinical utility of the chest radiograph in assessing treatment response in pleural infection, and suggests it is a robust research outcome measure

REFERENCE: [1]Rahman NM, M.N., West A et al., Intrapleural use of tissue plasminogen activator and DNase in pleural infection. N Engl J Med, 2011. **365**(6): p. 518

# CHAPTER 3

## Early Video Assisted Thoracoscopic Surgery (VATS) or Intrapleural Enzyme Therapy (IET) in Pleural Infection – a feasibility randomized controlled trial

## (The third Multicentre Intrapleural Sepsis Trial - MIST-3)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**Conflicts of Interest:**

The authors have no conflict of interest.

**Contributor statement:**

EOB, DS, EPB and NMR conceived and designed the study. EOB, KGB, DDF, JPC, RP, AW, TS, RMM, LGL, DNA, RA, RB, VG, MH, DM, AS, JMW, NAM DS, AK, JGE, EI, AM, EC, LO, MP, EPB recruited patients and collected data. MCJ, SD, EOB and NMR analysed data and facilitated its interpretation. EH and MD carried out trial and data management duties. EOB, EH, GB, JH, MS, SD, RFM, EPB and NMR provided trial oversight. EOB and NMR drafted the manuscript. The final draft of manuscript and supplementary material were reviewed and approved by all authors. MCJ, SD, EOB and NMR accessed and verified the trial data. EOB and NMR had full access to all data in this study and had final responsibility for the decision to submit for publication.

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## 3.1 ABSTRACT

Rationale

Assessing the early use of video-assisted thoracoscopic surgery (VATS) or intrapleural enzyme therapy (IET) in pleural infection requires a phase III randomized controlled trial (RCT).

Objectives

To establish the feasibility of randomization in a surgery versus non-surgery trial as well as the key outcome measures which are important to identify relevant patient-centred outcomes in a subsequent RCT.

Methods

The MIST-3 (third Multicentre Intrapleural Sepsis Trial) (ISRCTN registry no. 18192121) was a prospective multicentre RCT involving 8 UK centres combining on-site and off-site surgical services. The study enrolled all-comers with a confirmed diagnosis of pleural infection and randomized those with ongoing pleural sepsis after an initial period (up to 24 hours) of standard care to one of 3 treatment arms - continue standard care, early IET, or **surgical opinion with regards to early VATS**. The primary outcome was feasibility based on >50% eligible patients being successfully randomized, >95% of randomized participants retained to discharge and >80% of randomized participants retained to 2 weeks follow up. The analysis was performed as per intention to treat.

Main Results

Of 97 eligible patients 60 (62%) were randomized, with 100% retained to discharge and 84% retained to 2 weeks. Baseline demographic, clinical and microbiological characteristics of the patients were similar across groups. Median time-to-intervention (TTI) was 1.0 and 3.5 days in IET and surgery, respectively (p=0.02). Despite the difference in TTI, LOS (randomization to discharge) was similar in both intervention arms (7 days) compared to standard care (10 days) (p=0.70). There were no significant inter-group differences in 2-month readmission and further intervention, although the study was not adequately powered for this outcome. Compared to VATS, IET demonstrated a larger improvement in mean EQ-5D-5L health utility index from

baseline (0.35) to 2 months (0.83) (p=0.023). One serious advert event was reported in the VATS arm.

Conclusion

This is the first multicentre RCT of early IET vs early surgery in pleural infection. Despite the logistical challenges posed by the COVID-19 pandemic, the study met its predefined feasibility criteria, demonstrated potential shortening of LOS with early surgery, and signals toward earlier resolution of pain and a shortened recovery with IET. The study findings suggest that a definitive phase 3 study is feasible but highlights important considerations and significant modifications to the design that would be required to adequately assess optimal initial management in pleural infection.

Word count: 338

Key words: pleural empyema, video-assisted thoracic surgery, intrapleural, pleural effusion, randomized controlled trial

## 3.2 INTRODUCTION

Pleural infection affects an estimated 80,000 patients annually in the United States (US) and United Kingdom (UK) combined (Idell et al., 2017). The incidence has steadily increased (Arnold et al., 2021; Bobbio et al., 2021; Mummadi et al., 2021), and clinical outcomes remain poor with 30-day and 1-year mortality rates of 10% and 20% respectively (Cargill et al., 2019; Corcoran et al., 2020).

The largest international multicentre prospective observational study to date (PILOT (Corcoran et al., 2020)) demonstrated standard medical therapy with chest tube and antibiotics fails in 33.5% of cases(Corcoran et al., 2020). Such patients are treated with one or both of two established treatment modalities – surgical intervention or combination intrapleural enzyme therapy (IET) with tissue plasminogen activator (tPA) and deoxyribonuclease (DNase).

Minimally invasive surgical techniques using video assisted thoracoscopic surgery (VATS) have potentially widened the population suitable for surgical intervention. However, large case series (Farjah et al., 2007; Marks et al., 2012) demonstrate patients undergoing surgery are consistently younger and have fewer comorbidities than unselected populations (Maskell et al., 2005; Rahman et al., 2011). There are potentially significant numbers of patients in whom the mortality from uncontrolled pleural sepsis may outweigh the risks of surgery / general anaesthetic. Delays in surgical intervention are a predictor of conversion of thoracoscopic to open surgery (Lardinois et al., 2005; Stefani et al., 2013); it is therefore plausible that earlier surgical intervention may be beneficial. However, there remains no strong data to support the use of early surgery to improve key clinical outcomes. Two small randomized controlled trials (RCTs) of chest tube drainage versus surgery have demonstrated reduced length of hospital stay with initial surgical treatment(Bilgin et al., 2006; Wait et al., 1997). However, these studies contained methodological issues including absence of standardized decision-making criteria and have not altered practice.

Since publication of the MIST-2 study, the use of IET has revolutionized medical management. Although MIST-2 was limited by a small number of patients in the IET arm (n=52) and a primary outcome of radiographic clearance, multiple case series (Kheir et al., 2018; Majid et al., 2016; McClune et al., 2016; Piccolo et al., 2014; Popowicz et al., 2017) comprising >600 patients have supported a reduced need for

surgery and length of stay. A multicentre retrospective study of 1850 patients treated with IET confirmed a low rate of bleeding complications (4.2%) and no major adverse events (Akulian et al., 2022).

Thus, both surgery and IET appear to be effective interventions and early introduction in treatment may improve outcomes. Direct comparison of early VATS and IET requires a phase 3 RCT; no such study has been conducted to date. The MIST-3 study was designed to assess the feasibility of early randomization to a surgical versus non-surgical (IET) intervention, and to specifically address the selection bias of previous studies (Bilgin et al., 2006; Wait et al., 1997). The study aimed to randomize all participants enrolled, regardless of fitness for surgery, and sought to establish key outcome measures relevant to a subsequent definitive randomized controlled trial. Information was collected on feasibility of recruitment, participant acceptability and the ability to collect outcome data.

## 3.3 METHODS

### Trial Design and participants

MIST-3 was an open label, multicentre 3-arm randomized controlled feasibility trial undertaken in 8 UK centres in the United Kingdom combining on-site and off-site* thoracic surgical services as follows:

- Oxford – John Radcliffe Hospital
- Bristol – Bristol Royal Infirmary and Southmead Hospitals*
- Sheffield – Northern General Hospital
- Glasgow – Queen Elizabeth University Hospital and Golden Jubilee National*
- London – Guy's Hospital and St Thomas's Hospital*
- Plymouth – Derriford Hospital
- Leicester – Glenfield Hospital
- Blackpool – Blackpool Victoria Hospital

All eligible patients were included on screening logs and reasons for inclusion / exclusion / randomization recorded. The trial was registered on ISCRTN (number

18192121) and received ethical approval by the Cambridge East Research Ethics Committee (19/EE/0174). The central sponsor institution was the University of Oxford.

Eligibility criteria were:

1. Clinical presentation compatible with pleural infection
2. Pleural collection with a chest drain in-situ
3. Pleural fluid on sampling that was macroscopically purulent, positive on Gram staining or culture for bacterial infection, or pleural fluid pH < 7.2 (measured by blood-gas analyser) as per previous studies (Maskell et al., 2005; Rahman et al., 2011) and international guidelines (Bedawi et al., 2022b; Roberts et al., 2023).
4. Evidence of residual collection/ongoing sepsis, including the presence of fever and elevated serum levels of inflammatory markers such as C-reactive protein or an elevated peripheral blood white-cell count, as assessed by the recruiting physician.
5. Willing to give written informed consent.

Exclusion criteria were an age < 18 years; previous treatment with intrapleural fibrinolytics, DNase, or both for empyema; known sensitivity to DNase or t-PA; coincidental stroke; major haemorrhage or major trauma; major surgery in the previous 5 days; previous pneumonectomy on the infected side; pregnancy or lactation. Patients with an expected survival of less than 3 months, owing to a pathologic condition other than that responsible for the pleural abnormalities were also excluded.

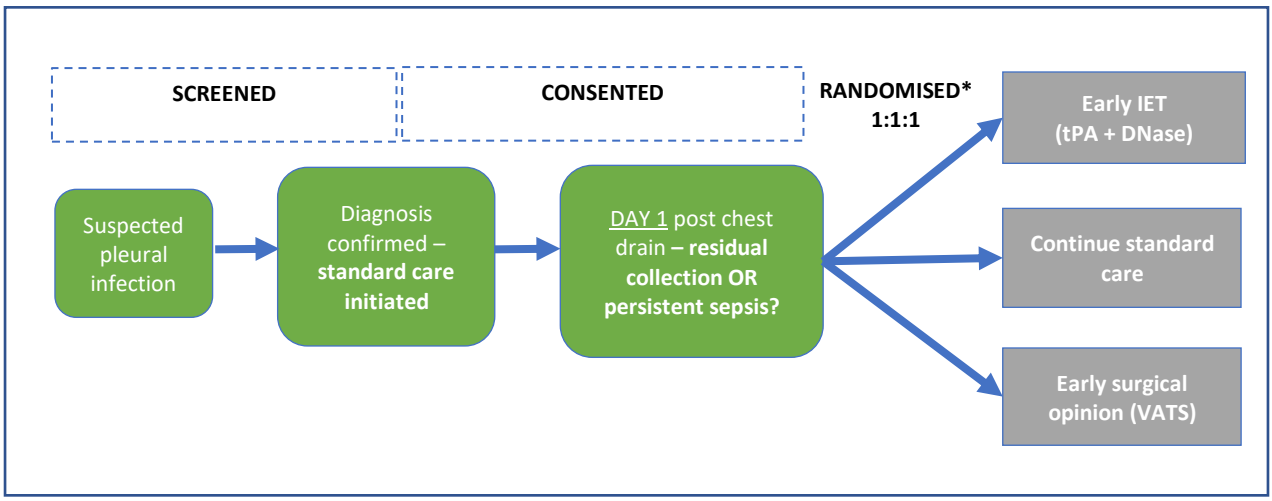
Patients who met eligibility criteria were screened and enrolled once diagnosis was confirmed. The date of chest tube insertion was considered trial day 0. To exclude cases where initial intervention resulted in complete pleural drainage, a run-in period of standard care (antibiotics and chest tube drainage) of up to 24 hours occurred post drain insertion. If a significant residual collection remained, the patient was eligible for randomization (confirmed by local PI) based on one or more predefined criteria of ‘medical treatment failure’ prior to randomization (trial day 1) (Figure 3.1).

**Medical Treatment Failure**

An objective criteria list was applied at the 24-hour timepoint after initial drainage. Criteria for medical treatment failure were defined as

1. The presence of a residual and clinically significant pleural collection, as judged by the local PI, based on current radiology (chest radiograph, ultrasound and/or CT); and
2. At least one of the following:
    a. Clinical evidence of ongoing sepsis (persistent fever, tachycardia, and hypotension)
    b. Serum C-reactive protein (CRP) that fails to fall by more >= 50% peak admission value
    c. Lack of significant response in the peripheral blood white cell count (WCC) as judged by the local investigator.

**Figure 3.1 – Trial Design**




*Minimization for site and RAPID category

## Randomization

Eligible participants were randomized via an online randomization system (Sortition®) on day 1 on a 1:1:1 basis to continue standard care, early IET intervention or referral for early VATS. Randomization was stratified by centre and baseline RAPID risk score (Corcoran et al., 2020; Rahman et al., 2014) (Figure 3.1).

## Interventions

Complete details of intervention and treatment in each randomization arm are outlined below. In brief, patients randomized to standard care were managed as per current BTS treatment guidelines (Roberts et al., 2023). Patients assigned to IET underwent treatment with intrapleural tPA (10mg bd) and DNase (5mg bd) through the chest tube (maximum 6 doses over 72 hours) (Rahman et al., 2011). Treatment was started as soon as possible after randomization. Sites were able to reduce dosages of tPA on an individual case basis at the discretion of the local PI (Popowicz et al., 2022, 2017).

Patients assigned to surgery underwent surgical assessment by a local thoracic surgeon and if suitable, underwent surgery in accordance with the trial surgical standard operating procedure (see appendix). The decision to proceed to surgery was at the discretion of the local surgical team.

### *Standard Care*

Participants assigned standard care were managed as per current (2010) BTS treatment guidelines (Davies et al., 2010) (BTS 2022 guidelines out for public consultation at time of reporting). Participants were admitted to hospital and started on broad spectrum antibiotics as per local guidelines. A chest tube (minimum 12F) was inserted using image guidance and local anaesthetic, and the participant was monitored with radiology, blood, and clinical parameters for treatment failure. This was assessed at 3-5 days post chest drain insertion and according to objective decision-making criteria as defined above.

### *IET*

Patients assigned IET underwent treatment with intrapleural tPA (10mg bd) and DNase (5mgbd) through the chest tube inserted during usual care, administered as per the MIST-2 trial protocol (12 hourly over 72 hours) (Rahman et al., 2011). Treatment was started as soon as possible after randomisation as per local administration protocols. Based on studies demonstrating safety and feasibility of concurrent administration (i.e., DNase and tPA in one intrapleural administration, followed by 1 hour of clamping, then repeating the procedure 12 hourly), this was the schedule used in MIST-3, to ease pragmatic delivery of the protocol. Sites were not

permitted to exceed the recommended doses but were able to reduce doses on an individual case basis at the discretion of the local PI, and doses were recorded on the CRFs.

*Surgery*

Patients assigned to the surgical arm underwent immediate referral post randomisation to local surgical services for assessment by local thoracic surgeon. If suitable, VATS was conducted in accordance with the trial surgical standard operating procedure (see supplementary materials). The decision on requirement for and safety of performing VATS was at the discretion of the receiving surgeon. Variation in timing of surgery, surgical bed, and operation theatre availability (time from randomisation to incision), and the proportion of participants considered 'fit' for surgery (i.e., the number of patients who underwent a surgical procedure) were collected on the CRFs.

*Compliance and crossover*

Non-compliance with protocol treatment in each of the 3 arms was defined as follows:

- Standard care: participants received IET or surgery during hospital stay.
- IET: treatment was abandoned for a reason other than the clinician deemed the treatment had been completed successfully and no further doses required.
- VATS: participants who did not receive surgery (aim for a surgical evaluation within 48 hours of randomization)

For safety reasons, crossover was permitted if a different intervention was deemed clinically necessary (at PI discretion) and the trial intervention could not be achieved within 48 hours of randomization.

**Outcomes**

The primary outcome was assessment of feasibility of randomizing participants to the 3 arms of the study using recruitment rate, retention rate and the proportion of participants screened who consented to be randomized, according to pre-defined criteria:

- >50% of eligible patients successfully randomised.
- >95% of randomised participants retained to discharge.
- >80% of randomised participants retained to 2 weeks follow up.

Secondary outcomes included length of hospital stay, frequency of readmission, requirement for reintervention, visual analogue scores of pain and quality of life. Further details are included in the full trial protocol (see appendix A3.5).

**Data Collection**

Baseline clinical data were collected at enrolment. Full details including inpatient study interventions are outlined below. Length of stay was calculated from date of randomization to date of patient being medically fit for discharge. Deaths occurring before discharge were excluded from the analysis. Follow-up data was collected at 2 weeks and 2 months with an optional 6 month follow up to allow for monitoring of late effects of treatment in each arm.

Patient distress and anxiety was collected using the Hospital Anxiety and Depression Scale (HADS). **H**ealth utility scores using EQ-5D-5L measurements were assessed and International Physical Activity Questionnaire (IPAQ in MET minutes) checked from raw values at baseline, at 2 weeks, 2 months and 6 months follow up. Pain scores were measured using the 100mm visual analogue scale (VAS) (Hawker et al., 2011). Further details on the utilized tools and data collection are described below.

*Baseline and Inpatient*

Baseline clinical data at enrolment included participant demographics and comorbidities, recent blood test and radiology results (within the previous week), details of symptoms and treatment received for current pleural infection episode until the date of enrolment, previous intrapleural treatment or thoracic surgery, vital signs, and patient weight.

Details of compliance including the type of surgery (VATS, open) and time to surgery (from randomisation to point of surgical intervention) in the surgical arm were recorded. In the IET arm, the proportion initiating treatment, completing treatment,

administered dose reductions, and missed doses were recorded. Reasons for non-compliance in each of the intervention arms were also recorded.

*Hospital Anxiety and Depression Scale*

HADS is a self-report rating scale of 14 items on a 4-point Likert scale (range 0-3). The range for HADS score is: 0-7 normal, 8-10 mild, 11-15 moderate, 16-21 severe. HADS was measured at a single time point at day 2-3 post randomisation. The total score is the sum of 14 items with each subscale of anxiety and depression the sum of 7 items, with higher scores indicating worse symptoms of anxiety and/or depression. The HADS scores were computed using the values corresponded to answers to each question (14 in total) by referring to the HADS scale. HADS depression and anxiety scores were separately computed before adding them up into the total score. If the participant did not answer one of the questions, the HADs score was treated as missing.

*Pain scores*

VAS booklets were completed by participants once a day for a maximum of 7 days following chest tube insertion (or discharge if earlier than 7 days). Inpatient pain was measured by taking the average of the daily pain scores reported. Pain scores at home were measured weekly until 2 months post discharge and were calculated by taking the average over the weekly pain scores reported during this 8-week period.

**Statistical analysis**

As this was a feasibility study, a formal sample size was not calculated. A total of 75 patients were planned to be randomized (25 in each arm) over 18 months from 6 centres, based on recruitment to an observational study in pleural infection (PILOT), which recruited 20 participants per month in 20 centres (Corcoran et al., 2020).

To assess feasibility, the proportion of eligible participants was compared to the total number of patients screened, and the proportion of participants who consented to randomization was compared to the total number eligible. The proportion of patients who became ineligible due to good initial treatment response to standard care (thus

not meeting the criteria for medical treatment failure at 24 hours post chest tube insertion), and the recruitment and retention rates to discharge and 2-weeks were measured.

Baseline comparability of the two intervention groups in terms of minimization factors and baseline characteristics are described as proportions for categorical variables, and as mean and standard deviation (SD) or median and interquartile range (IQR) for continuous variables depending on the distribution. The number of withdrawals, losses to follow-up, deaths and details of treatment received were summarized by treatment group. Further admissions and interventions were summarized alongside safety events.

All patient reported and clinical outcomes were analysed on an intention-to-treat basis. Treatment difference and 95% Confidence intervals are reported throughout. Hospital length of stay (LOS) was summarized using a Kaplan Meier plot with deaths censored. Hospital LOS was defined as date of randomization to date of discharge. A mixed effects model adjusting for treatment, RAPID category, size of chest tube inserted and baseline values as fixed effects and recruiting centre as a random effect was fitted for continuous outcomes available at multiple time points.

Mean HADS, EQ-5D utility index, EQ-5D 100mm VAS and pain scores were compared between groups using one way analysis of variance (ANOVA) with post-hoc comparisons using the Tukey HSD performed for statistically significant differences ($p<0.05$)

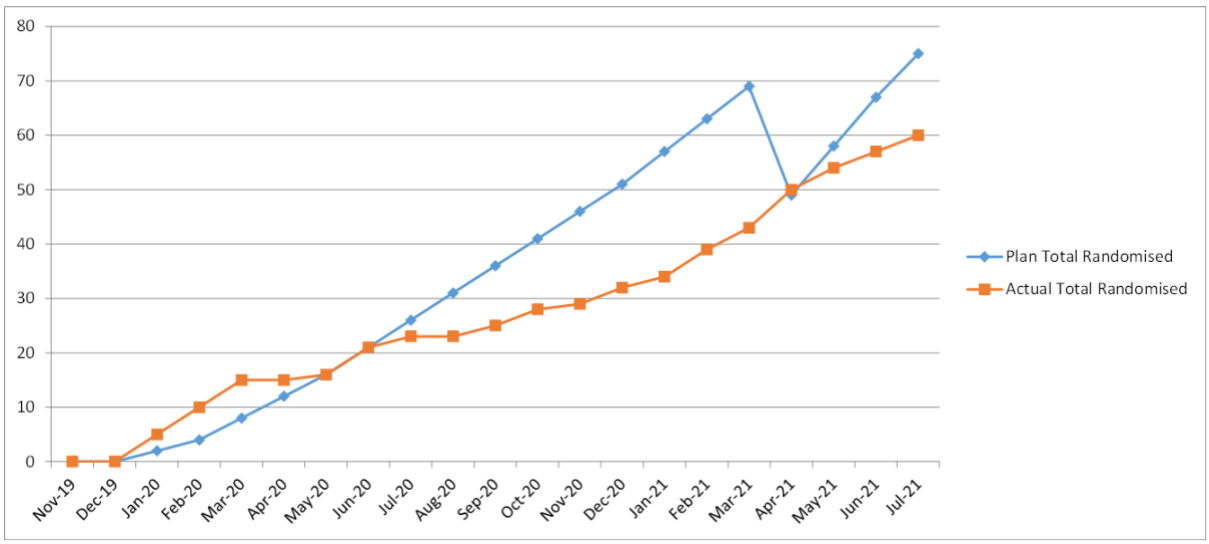
### Impact of COVID-19

The COVID-19 pandemic presented significant challenges. The first wave in the UK began in March 2020, 4 months after trial recruitment began (November 2019). The main impact was to trial recruitment rates, which, having been ahead of target, dropped substantially (Figure 3.2). Based on a separate analysis of screening data, pleural infection rates in the UK dropped by approximately one third during the pandemic (Bedawi et al., 2022a) with hospital admissions and research efforts predominantly COVID-19 related. Performing timely surgery and intervention in the context of COVID-19 became challenging as infection and prevention control (IPC) measures became more restrictive and theatre capacity was reduced.

### COVID-19 Mitigation strategies

No deviations from the planned enrolment and randomisation procedures were made to mitigate against the effect of the pandemic. Some study assessments (such as follow up visits, which patients were sometimes reluctant to attend during the pandemic) were made optional to streamline the trial pathway, to reduce the data collection burden on sites and focus on essential data required to meet study outcomes. A further 2 sites were added bringing the final number of recruiting sites to 8. The original planned follow-up time (6 months) was shortened to 2 months to facilitate a 4-month recruitment extension. Sites were asked to specifically record any protocol deviations strictly related to COVID-19 restrictions. All trial modifications were agreed by the trial steering committee (TSC) prior to implementation and protocol amendments submitted for ethics and sponsor approval accordingly. No interim data analyses were pre-planned or performed to inform the trial modifications.

**Figure 3.2 – MIST-3 Trial recruitment.**



## 3.4 RESULTS

### Recruitment and feasibility

Between 1 Nov 2019 and 30th July 2021, 8 centres representing a geographical spread across the UK with a combination of on-site and off-site access to thoracic surgery services submitted screening logs for 178 patients. Of those screened, 110 patients met initial eligibility criteria; 13/110 (11.8%; 95%CI 0.06-0.19) patients had a good response to initial treatment and were excluded from randomization. A total of 60 participants from the remaining 97 eligible were randomized (61.9%, 21 ongoing standard care, 19 early IET therapy and 20 early surgical referral). All randomized participants were included in the analysis. The flow of participants through the study from screening to follow-up and availability of data is shown in Figure 3.3.

Baseline demographic, clinical and microbiological characteristics were similar across all three groups (Table 3.1).

### Data completion

Participant retention rate to hospital discharge was 100%. Two week and two month follow up completion rates were 84.5% and 87.5% respectively (Table 3.2). Considering the first wave of the COVID-19 pandemic occurred 4 months into trial recruitment, the 6 month follow up was made optional and completion rates were removed from the analysis. There were two participant withdrawals, one from the standard care arm (patient choice) and one from the IET arm (lost to follow up). Both withdrawals occurred after discharge.

HADS was conducted during the inpatient phase 1-2 days post randomization and completion rate was 93% (20/21 (standard care), 18/19 (IET) and 18/20 (VATS)). At 2-week follow-up, completion rates of IPAQ and EQ-5D-5L questionnaires were 79% and 81% respectively. Completion rate of both IPAQ and EQ-5D-5L at 2 months and 6 months was 84% and 71% respectively. Data completeness for at each follow up timepoint is shown in appendix A3.1. Analysis was performed for the ITT population using available cases with unacceptable values set to missing.

**Figure 3.3 – CONSORT diagram from screened to analysis.**

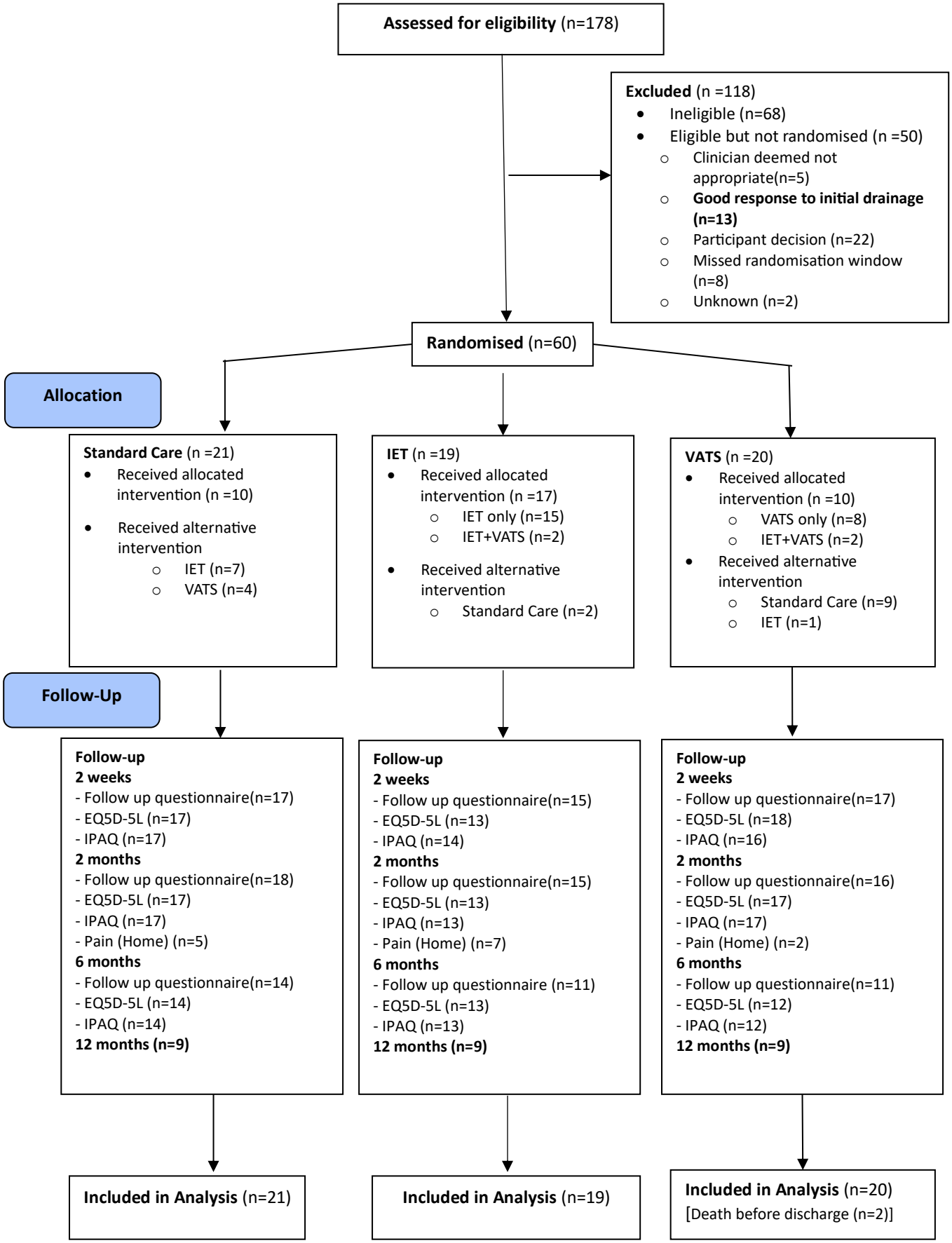


**Table 3.1 – Baseline characteristics according to treatment groups**

| Characteristics | Standard Care (n=21) | IET (n=19) | VATS (n=20) |
|---|---|---|---|
| **Age, years** - median (IQR) | 58 (51 – 72) | 66 (56 – 71) | 66 (59 – 74) |
| **Male sex** | 13 (61.9%) | 14 (73.7%) | 11 (55.0%) |
| **RAPID score** | | | |
| 0 to 2 (Low) | 9 (42.9%) | 9 (47.4%) | 9 (45.0%) |
| 3 to 4 (Moderate) | 8 (38.1%) | 7 (36.8%) | 7 (35.0%) |
| 5 to 7 (High) | 4 (19.0%) | 3 (15.8%) | 4 (20.0%) |
| **Comorbidities** | | | |
| Respiratory disease | 8 (38.1%) | 4 (21.1%) | 6 (30%) |
| Gastro-intestinal | 7 (33.3%) | 7 (77.8%) | 8 (40%) |
| Renal | 2 (9.5%) | 2 (10.5%) | 2 (10%) |
| Cardiac | 8 (38.1%) | 10 (52.6%) | 10 (50%) |
| **Pleural fluid characteristics** | | | |
| Purulence | 10 (47.6%) | 11 (57.9%) | 7 (35%) |
| Micro positive | 6 (28.6%) | 4 (21.1%) | 5 (25%) |
| **Pleural fluid pH** | | | |
| Median | 6.98 | 6.90 | 7.03 |
| IQR | (6.89 - 7.17) | (6.74 – 7.03) | (6.80 – 7.25) |
| **Pleural fluid LDH (IU/L)** | | | |
| Median | 1160 | 1650 | 1660 |
| IQR | (172 – 2160) | (820 – 4360) | (600 – 3000) |
| **Chest tube size** | | | |
| 12F | 17 (81.0%) | 14 (73.7%) | 18 (90.0%) |
| 16F | 1 (4.8%) | 0 (%) | 0 (%) |
| 18F | 3 (14.3%) | 4 (21.1%) | 2 (10.0%) |
| Other | 0 (%) | 1 (5.3%) | 0 (%) |

## Treatment compliance and crossover

Overall treatment compliance was 10/21 (47.6%) in standard care, 14/19 (73.6%) for IET and 10/20 (50%) for VATS (Table 3.3).

All non-compliance in the standard care arm was due to clinician concern that the patient required further intervention. Seven patients received IET and 4 received VATS. Five crossovers occurred in the standard care arm within 48 hours (4 to IET and 1 to VATS) and were therefore classed as protocol deviations (Table 3.3).

**Table 3.2 - Feasibility outcomes**

| Screened | | |
|---|---|---|
| **178** | | |
| **Eligible** | **Not Eligible due to being a quick responder*** | **Quick responder rate** |
| **110** | 13 | 12% (95%CI 6% - 19%) |
| **Actual Eligible** | **Randomised** | **Randomization Rate‡** |
| **97** | 60 | 62% (95%CI 54% - 66%) |
| **Survivors to discharge** | **Completed hospital discharge** | **Retention to discharge+** |
| **58** | 58 | 100% (95%CI 100% - 100%) |
| **Survivors to 2-weeks** | **Completed week 2 follow-up** | **Retention to 2-weeks†** |
| **58** | 49 | 84% (95%CI 73% - 93%) |

* Quick responders are those screened, not eligible as they did not have a residual collection on day 1 or CRP dropped by more than one half
‡ Randomised out of actual number eligible
+ Number who completed hospital discharge/number of survivors
† Number who completed 2 week/number of survivors to 2 weeks post discharge

**Table 3.3 – Compliance with study intervention**

| | Standard Care (n=21) | IET (n=19) | VATS (n=20) |
|---|---|---|---|
| **Received as randomised*** | | | |
| Yes | 10 (47.6%) | 12 (63.2%) | 10 (50.0%) |
| No | 11 (52.4%) | 7 (36.8%) | 10 (50.0%) |
| **Treatment(s) received during hospital stay** | | | |
| No VATS or IET | 10 (47.6%) | 2 (10.5%) | 9 (45.0%) |
| IET | 7 (33.3%) | 15 (78.9%) | 1 (5.0%) |
| VATS | 4 (19.0%) | 0 (0%) | 8 (40.0%) |
| IET +VATS | 0 (0%) | 2 (10.5%) | 2 (10.0%) |

Reasons for non-compliance in the IET arm included patient intolerance due to pain (n=3), clinician-assessed bleeding risk (n=1) and concern about subdiaphragmatic communication (n=1) (table 3.4). The mean number of IET doses received by participants randomized to the IET arm was 4.8 (SD 1.4). Of the 59 doses of tPA

administered in the study, 40/59 (68%) were 10mg and 19/59 (32%) were 5mg. Full details of IET dosing are presented in table 3.5.

All patients in the VATS arm had a documented surgical evaluation within 48 hours of randomization. Analysing reasons for non-compliance in the VATS arm (table 3.4), 7/10 were deemed not to require surgery (clinical improvement) and did not receive further intervention to discharge. In 2/10 patients, the anaesthetic risk was deemed too great, and these patients did not receive another intervention before discharge. One patient could not have VATS due to lack of surgical capacity and crossed over to IET.

Treatment compliance in the surgical arm was compared between recruitment sites with immediate access to surgeon 'on-site' and 'off-site' centres, and this was not different (Fisher Exact test=0.39; 1df; p=0.53). No patients who received VATS required conversion to thoracotomy.

Two patients in the IET arm went on to receive VATS during hospital admission, due to IET failure. Two patients in the VATS arm received IET while awaiting surgery. These were not classed as non-compliers.

**Table 3.4 – Reasons for non-compliance with intervention**

| | Standard Care (n=21) | IET (n=19) | VATS (n=20) |
|---|---|---|---|
| Patient intolerance | X | 3 (15.8%) | 0 (0%) |
| Complications | X | 0 (0%) | 0 (0%) |
| Unavailability of staff to administer | X | 0 (0%) | 0 (0%) |
| Clinician choice | X | 4 (21.1%) | 0 (0%) |
| Operator access | X | 0 (0%) | 0 (0%) |
| Theatre access | X | 0 (0%) | 0 (0%) |
| Anaesthetic risk deemed too high | X | 0 (0%) | 2 (10.0%) |
| Clinician choice (improving/no longer required) | X | 0 (0%) | 7 (35.0%) |
| Surgical capacity | X | 0 (0%) | 1 (5.0%) |
| Not Applicable | 21 (100%) | 12 (63.2%) | 10 (50.0%) |

### Time to intervention

The median time to intervention was 1 day in the IET arm (IQR 0-1) and 3.5 days for VATS (IQR 1.2-4.0) (Mann Whitney U test p=0.02). In the IET arm, 6 patients commenced IET within 24 hours of randomization and only 1 patient took >48 hours to initiate therapy (table 3.5). In the VATS arm, 5/10 (50%) underwent an operation within 3 days of randomization and 8/10 (80%) within 5 days of randomization (table 3.6).

**Table 3.5 – Details of intervention – IET**

| | Standard Care (n=21) | IET (n=19) | VATS (n=20) |
|---|---|---|---|
| **IET** | | | |
| N, Mean (SD) | 7, 1.7 (1.4) | 14, 0.6 (0.6) | 3, 2.0 (1.0) |
| Median (IQR) | 1.0 (1.0 - 2.5) | 1.0 (0.0 - 1.0) | 2.0 (1.5 - 2.5) |
| Range | 0,4 | 0,2 | 1,3 |
| **Number of total IET doses received (max=6)** | | | |
| 1 | 1 (14.3%) | 0 (0%) | 2 (66.7%) |
| 2 | 2 (28.6%) | 1 (5.9%) | 0 (0%) |
| 3 | 1 (14.3%) | 2 (11.8%) | 0 (0%) |
| 4 | 1 (14.3%) | 3 (17.6%) | 1 (33.3%) |
| 5 | 1 (14.3%) | 1 (5.9%) | 0 (0%) |
| 6 | 1 (14.3%) | 7 (41.2%) | 0 (0%) |
| Missing | 0 (0%) | 3 (17.6%) | 0 (0%) |
| Mean (SD) | 3.3 (1.8) | 4.8 (1.4) | 2.0 (1.7) |
| **Time to first dose of IET**** | | | |
| Within 24 hours | 1 (14.3%) | 6 (35.3%) | 0 (0%) |
| 24-48 hours | 3 (42.9%) | 7 (41.2%) | 1 (33.3%) |
| > 48 hours | 3 (42.9%) | 1 (5.9%) | 2 (66.7%) |
| Missing | 0 (0%) | 3 (17.6%) | 0 (0%) |

**Table 3.6 – Details of intervention – VATS**

| | Standard Care (N=21) | IET (N=19) | VATS (n=20) |
|---|---|---|---|
| Time to intervention in days | | | |
| **VATS Surgery** | | | |
| N, Mean (SD) | 4, 7.2 (6.7) | 2, 10.5 (6.4) | 10, 5.8 (9.4) |
| Median (IQR) | 5.0 (4.2, 8.0) | 10.5 (8.2, 12.8) | 3.5 (1.2, 4.0) |
| Range | 2,17 | 6,15 | 1,32 |
| **VATS** | **4 (19.0%)** | **2 (10.5%)** | **10 (50.0%)** |
| Operation within 3 days of randomisation (N, %) | 1 (25.0%) | 0 (0%) | 5 (50.0%) |
| Operation within 5 days of randomisation (N, %) | 3 (75.0%) | 0 (0%) | 8 (80.0%) |

## Length of stay

Overall median length of stay across the study population was 9 days (IQR 6 -15). Median length of stay according to RAPID category (low, moderate, high) was 6, 8.5 and 13 days respectively (p=0.032). Median length of stay was 10 days (IQR 7-13) in the standard care arm, 7 days (IQR 5.5-10) in the IET arm and 7 days (IQR 5.5-10.5) in the VATS arm (p=0.70). Further analysis showed no intergroup differences between individual arms (table 3.7).

**Table 3.7 – Length of stay comparison between intervention arms (p-value)**

| | p-value |
|---|---|
| Standard care vs IET | 0.52 |
| Standard care vs VATS | 0.43 |
| IET vs VATS | 0.62 |

## Further intervention post discharge

Further pleural infection related admissions and intervention post discharge were analysed. These were similar across the 3 treatment groups: 5/21 (23.8%) for standard care, 5/19 (26.3%) for IET and 6/20 (30%) for VATS ($c^2$ 2df=0.20, p=0.90). Details of intervention are presented in Table 3.8.

**Table 3.8 – Further admission and surgery**

| | Standard Care (n=21) | IET (n=19) | VATS (n=20) |
|---|---|---|---|
| **Further hospital admission** | 5 (23.8%) | 5 (26.3%) | 6 (30.0%) |
| **Further intervention** | | | |
| Surgery | 1 (4.8%) | 0 (%) | 1 (5.0%) |
| Chest drain | 0 (%) | 1 (5.3%) | 1 (5.0%) |
| Other | 1 (4.8%) | 1 (5.3%) | 0 (%) |

## Mortality

In the ITT analysis, overall mortality at 12 months was 10% (6/60). Mortality showed a trend toward being higher in the VATS arm (4/20; 20%) compared to in the standard care arm (1/21, 4.8%) and the IET arm (1/19, 5.3%) ($c^2$ 2df=3.33, p=0.19). Two deaths occurred before discharge (2/60; 3.3%), both of which were in the VATS arm (table 3.9). Deaths were analysed per protocol due to the potential implications for the primary feasibility outcome. Deaths occurring in the IET, and standard care arm had both received treatment as randomized. In the VATS arm, only one patient who died had received VATS (due to post-operative haemorrhage), with the remaining 3 not receiving any intervention beyond standard care, none of whom succumbed directly to untreated pleural sepsis (table 3.10).

**Table 3.9 – All-cause mortality between treatment groups**

| | Standard Care (N=21) | IET (N=19) | VATS (N=20) |
|---|---|---|---|
| **Mortality** | 1 (4.8%) | 1 (5.3%) | 4 (20.0%) |
| **Mortality before discharge** | 0 (%) | 0 (%) | 2 (10.0%) |
| **Days on trial** *median (IQR)* | 59 (59, 59) | 174 (174, 174) | 58 (20, 115) |

**Table 3.10 – Treatment details for participants who died during the trial.**

| | Treatment allocated | Treatment received | Days on trial | Follow-up point at the time of death | **Final cause of death** |
|---|---|---|---|---|---|
| **1** | VATS | No VATS or IET | 23 days | before discharge | Aspiration Pneumonia |
| **2** | Standard Care | No VATS or IET | 59 days | two months | Septic shock secondary to community acquired pneumonia on a background of acute myeloid leukaemia and chronic kidney disease |
| **3** | IET | IET | 174 days | six months | Natural death in nursing home |
| **4** | VATS | No VATS or IET | 92 days | two months | Metastatic lung cancer |
| **5** | VATS | No VATS or IET | 185 days | twelve months | Subdural hematoma |
| **6** | VATS | VATS | 10 days | before discharge | Large retroperitoneal hematoma secondary to hepatic artery rupture* |

*Reported SAE

### Health quality of life and physical activity

Mean HADS score across the entire study at day 1-2 post randomization was 16.7 (SD 9.3, 95% CI 14.2-19.2), with no differences between groups (table 3.11).

EQ-5D utility index scores range from 0 (equivalent to death) to 1 (representing perfect health). The IET arm showed the greatest improvement in mean EQ-5D utility index score from baseline to 2 months (0.35 to 0.83) compared to standard care (0.48 to 0.62) and VATS (0.38 to 0.59). Comparing the difference in the change in EQ-5D utility index scores between IET and VATS, this was statistically significant in favour of IET (p=0.023] (table 3.12).

The IET arm showed the greatest improvement in mean EQ-5D 100mm VAS for patient perception of overall health from baseline to 2 months (45.1 to 79.5) compared to standard care (49.9 to 63.6) and VATS (54.2 to 72.0). VATS did not show a benefit compared to standard care (p=0.24) but there was a difference favouring IET comparing to standard care (p=0.027) (table 3.12).

Inpatient mean pain scores were high across all interventions. This was highest in the IET arm (mean 36.4 SD 19.0), followed by standard care (mean 32.8, SD 16.8) and VATS arm (mean 29.2, SD 14.5) (p=0.89). At 2 months post discharge, mean pain scores were reduced in all groups; IET was associated with the lowest score (4.9; SD 2.1), followed by standard care (19.4, SD 8.2) and then VATS (22.2, SD 9.5) (table 3.12). The difference between groups did not meet statistical significance (p=0.08).

**Table 3.11 - Hospital Anxiety and Depression Scale (HADS), (N, Mean (SD))**

| | Standard Care (N=21) | IET (N=19) | VATS surgery (N=20) |
|---|---|---|---|
| Anxiety | 20, 8.3 (5.8) | 19, 7.6 (5.4) | 18, 7.9 (4.1) |
| Depression | 20, 8.5 (5.8) | 18, 8.7 (6.0) | 18, 9.6 (4.9) |
| HADS overall score | 20, 16.8 (9.9) | 18, 15.9 (10.3) | 18, 17.5 (8.0) |

**Table 3.12 – EQ-5D and pain scores**

| | **Standard care (n=21)** | **IET (n=19)** | **VATS (n=20)** |
|---|---|---|---|
| | mean (SD) | mean (SD) | mean (SD) |
| **EQ-5D utility index*†** | | | |
| Baseline | 0.485 (0.181) | 0.351 (0.157) | 0.382 (0.159) |
| 2 weeks | 0.629 (0.232) | 0.704 (0.223) | 0.591 (0.276) |
| 2 months | 0.616 (0.389) | 0.833 (0.126) | 0.587 (0.354) |
| **EQ-5D VAS*‡ (Patient perception of overall health)** | | | |
| Baseline | 49.9 (20.7) | 45.1 (16.2) | 54.2 (23.8) |
| 2 weeks | 59.5 (29.1) | 67.7 (14.2) | 66.2 (17.0) |
| 2 months | 63.6 (25.8) | 79.5 (16.0) | 72.0 (19.2) |
| | | | |
| **Pain score post tube insertion** | 32.8 (16.8) | 36.4 (19.0) | 29.2 (14.5) |
| **Pain score in 2 months post discharge** | 19.4 (8.2) | 4.9 (2.1) | 22.2 (9.5) |

‡EQ5D VAS scores range from 0 (worst possible health) to 100 (best possible health)

† EQ-5D utility scores range from 0 to 1 with 1 representing perfect health and 0 equivalent to death. Participants who died prior to an EQ5d measurement time point utility scores were imputed as 0.

### Per protocol analysis

Due to the variable compliance with the trial protocol, a per protocol analysis of the main outcomes was performed (n=35; standard care=10, IET=15, VATS=10). The median LOS was 9.5 (IQR 4-16) in the standard care arm, 7 days (IQR 5-12.5) in the IET arm and 9.5 (IQR 7-17) in the VATS arm (ANOVA between groups; p=0.47). Requirement for readmission and/or further intervention at 6 months follow up was highest in the standard care arm at 5/10 (50%), similar in IET (7/15; 47%) but lowest in those who received upfront VATS at 2/10 (20%) [$c^2$ 2df; p=0.246] (table 3.13). EQ-5D utility index, EQ-5D 100mm VAS and pain scores maintained similar intergroup differences between baseline and 2 months, in favour of IET but these were not statistically significant.

**Table 3.13 – Per protocol analysis of hospital readmission and/or reintervention**

| | 2w FU | 2m FU | 6m FU | TOTAL |
|---|---|---|---|---|
| **Standard care** | 2/10 | 2/10 | 1/10 | 5/10 = 50% |
| **IET** | 2/15 | 3/15 | 2/15 | 7/15 = 47% |
| **VATS** | 1/10 | 1/10 | 0/10 | 2/10 = 20% |

**Table 3.14 – Per protocol analysis of EQ-5D utility index, EQ-5D 100mm VAS and Pain scores**

| | Standard care (n=10) | IET (n=15) | VATS (n=10) |
|---|---|---|---|
| | mean (SD) | mean (SD) | mean (SD) |
| **EQ-5D utility index** | | | |
| Baseline | 0.55 | 0.40 | 0.39 |
| 2 months | 0.74 | 0.84 | 0.65 |
| **EQ-5D VAS*‡ (Patient perception of overall health)** | | | |
| Baseline | 49.9 (20.7) | 45.1 (16.2) | 54.2 (23.8) |
| 2 weeks | 59.5 (29.1) | 67.7 (14.2) | 66.2 (17.0) |
| 2 months | 63.6 (25.8) | 79.5 (16.0) | 72.0 (19.2) |
| | | | |
| **Pain score post tube insertion** | 32.8 (16.8) | 36.4 (19.0) | 29.2 (14.5) |
| **Pain score in 2 months post discharge** | 19.4 (8.2) | 4.9 (2.1) | 22.2 (9.5) |

### Safety and adverse events

One serious adverse event occurred in a VATS patient who received surgery and died 10 days post randomization. The patient developed acute kidney injury post-operatively with a drop in haemoglobin and was found to have a large retroperitoneal hematoma secondary to hepatic artery rupture.

Most non-serious adverse events were in the IET arm, and the most common event was pain (Table 3.15).

**Table 3.15 – Adverse events according to treatment received**

| | Standard Care (n=21) | IET (n=19) | VATS (n=20) |
|---|---|---|---|
| **Number of participants with probably or possibly related AEs*** | 1 (4.8%) | 3 (15.8%) | 0 (0%) |
| Pain | 0 | 2 | 0 |
| Acute Kidney Injury (AKI) | 1 | 0 | 0 |
| Dizziness/presyncope | 0 | 1 | 0 |
| Other | 0 | 1 | 0 |
| **Number of participants with unrelated AEs*** | 6 (28.6%) | 7 (36.8%) | 5 (25.0%) |
| Pain | 1 | 4 | 1 |
| GI upset (nausea/vomiting) | 2 | 3 | 0 |
| Transient delirium or confusion | 1 | 0 | 1 |
| Acute Kidney Injury | 0 | 0 | 2 |
| Swelling/erythema around drain site | 0 | 0 | 1 |
| Other | 6 | 5 | 3 |
| **Number of participants with serious AEs*** | 1 (4.8%) | 0 (0%) | 1 (5.0%) |
| Acute Kidney Injury | 1 | 0 | 0 |
| Hepatic artery rupture | 0 | 0 | 1 |
| *Number of participants reported at least one AE* | | | |

## 3.5 DISCUSSION

The treatment paradigm in pleural infection is based on expert consensus and has remained largely unchanged in the last two decades. The addition of combination intrapleural fibrinolytic and enzyme therapy has been a major advance. However, modern surgical techniques have meant that VATS has become a more accessible treatment for a significantly larger proportion of patients. Despite these developments, the two treatments have not been compared in a prospective multicentre study resulting in variability in clinical practice and guideline recommendations (Bedawi et al., 2022b; Chaddha et al., 2021; Roberts et al., 2023). Outcomes in pleural infection remain unacceptably poor and new treatment approaches are urgently needed.

In this study, the first head-to-head randomized study of IET versus surgery early in treatment, we demonstrated that patients presenting with pleural infection are amenable to early escalation to more ‘aggressive’ therapies. After an initial period of standard care (chest tube drainage and antibiotics), using a protocolized definition of treatment failure, and despite a concurrent COVID-19 pandemic, 62% of eligible patients were successfully randomized to early IET or early surgical evaluation. Of the eligible patients who were not randomized, only 23% were due to a direct participant refusal; hence in general there is equipoise and acceptance amongst clinicians and patients for participating in a surgery versus non-surgery trial in pleural infection.

While early crossover (within 48 hours of randomization) was permissible if deemed ‘clinically necessary’, the high proportion of early crossover in participants randomized to standard care likely represents a general trend towards early escalation amongst clinicians, suggesting ‘standard care’ has evolved ahead of guideline-driven practice. The degree of crossover provides credence for the exclusion of a standard care arm in future trials with head-to-head randomization to IET and surgery alone. Furthermore, the observation that these patients were not heavily skewed towards one intervention (four received surgery, seven received IET) suggests reasonable equipoise amongst clinicians. The run-in period of initial treatment of antibiotics and chest tube remains justified to exclude quick responders that may not require further intervention, which occurred in approximately 13% of participants. This is an important finding and will inform future sample size calculations in a definitive trial.

In terms of the 'active' interventions, treatment compliance was notably lower in the surgical arm (50%) compared to the IET arm (79%). The most common reason for patients not undergoing surgery was the risk/benefit balance of VATS no longer being in favour of proceeding with surgery by the time an operation was feasible. This should be considered when planning future phase 3 studies in which there is a large difference in delivering the trial intervention between two arms. Nonetheless, while most eligible patients agreed to be randomly allocated to surgery, and that anaesthetic risk only precluded a minority of participants, a minimum fitness criterion may potentially optimize compliance in a future phase 3 study whilst maintaining the strength of MIST-3 in avoiding the selection bias of previous surgical RCTs (Bilgin et al., 2006; Wait et al., 1997), and where patients in their 7$^{th}$ and 8$^{th}$ decade were successfully treated surgically. With increasing experience, expertise and safety demonstrated with VATS as a treatment modality, we strongly advocate that such patients are included in future trials given their increasing representation in pleural infection cohorts (Arnold et al., 2022).

Adverse events related to treatment arm were minimal throughout the study (overall adverse events 4/60; 6.7%). The most common AE was pain in the IET arm, which is well documented (20,29,30). It is noteworthy that despite increased pain during administration, the reduction in mean pain score at 2 months in the IET arm was clinically significant (MCID=16mm) (Dahlberg et al., 2020), which when combined with the significantly favourable EQ-5D changes at 2 months compared to baseline, suggest there are potentially important treatment effects in favour of IET that require further evaluation in a definitive phase 3 study.

A notable finding among the secondary outcomes was length of hospital stay. Despite median time to intervention varying significantly between IET and VATS (1 day vs 3.5 days), it is of added value that these were similar in a recent United States pilot single-centre RCT (Wilshire et al., 2023), reflecting generalizability across both healthcare systems. The median length of hospital stay was the same (7 days). Whilst MIST-3 was not powered to assess this outcome, this finding suggests intervention at the earlier stages of the condition may be beneficial when compared to the observed overall median LOS of 10-14 days in large studies (Corcoran et al., 2020; Rahman et al., 2011), and that an adequately powered RCT is needed to address this question.

Patient reported outcome measures (PROMs) were a key focus in this study as these have not been specifically evaluated in pleural infection. The HADS score is a simple and effective measure of psychological and emotional distress (Djukanovic et al., 2017). The study was not sufficiently powered to detect intergroup differences but brings to light the extent of psychological impact of pleural infection.

In terms of the other qualitative secondary outcomes, EQ-5D-5L and IPAQ questionnaires at 2 weeks and 2 months of follow-up had high completion rates. The inpatient and home EQ-VAS pain scores questionnaire had a lower completion rate, especially for participants randomized to early surgery. The challenges of collecting accurate patient reported outcome data are expected in acutely unwell patients and were likely compounded by intra- and inter-hospital patient transfers throughout their treatment journey, particularly in the surgical arm. Interestingly, EQ-VAS data have been shown to have a predictable and consistent relationship with the EQ-5D profile (Feng et al., 2014) and it may be that sole use of the EQ-5D-5L in future studies reduces questionnaire burden and represents sufficient comparison between treatment arms, particularly as the latter combines physical activity, pain, and anxiety/depression metrics within it.

The impact of the COVID-19 pandemic on the MIST-3 trial cannot be understated. The trial was recruiting well ahead of target until March 2020 when the first wave of the pandemic struck UK hospitals. Hospitalizations became predominantly COVID-19 related and a substantial reduction in non-COVID-19 related admissions was observed across the western world (Birkmeyer et al., 2020; Bodilsen et al., 2021; Kapsner et al., 2020). Data from our own centres in a related study estimated that the incidence of pleural infection hospitalizations was reduced by approximately one third (Bedawi et al., 2022a). The reasons for this are uncertain but likely attributed to the combined effect of shielding and isolation of vulnerable populations, reduced social mixing, widespread use of personal protective equipment and liberal use of antibiotics to prevent secondary bacterial infections in patients with viral illness.

Previous surgical RCTs have been confounded by absence of blinding, highly selected study population and no objective decision criteria (Bilgin et al., 2006; Wait et al., 1997). The deliberate inclusion of all-comers in MIST-3 saw patients being referred for surgical evaluation who are normally denied this intervention in clinical practice due to

risk of adverse surgical outcome. Despite overall small numbers, we note that patients in their 7th and 8th decades of life were successfully treated surgically, with anaesthetic risk precluding only a minority. With increasing experience, expertise and safety demonstrated with VATS as a treatment modality, and based on the feasibility findings in this study, we strongly advocate that these patients are included in future trials given their increasing representation in pleural infection cohorts (Arnold et al., 2022).

To our knowledge, MIST-3 is the first prospective multicentre study to successfully randomly assign patients to IET versus surgery in pleural infection. The results provide evidence for feasibility and acceptability but arguably the study's main success has been in identifying key aspects of study design and methodology that will inform future protocols for a trial of this kind. Strengths of the study include clear and standardized pleural infection diagnostic criteria and protocolized definition of medical treatment failure prior to randomization. Although the study is small and not powered for any treatment differences between groups, early intervention in general appeared to show a significant benefit in terms of length of stay compared with standard care, and this finding adds credence to the need for a larger definitive study. Despite ITT analysis being the gold standard as it gives an unbiased estimate of treatment effect, its interpretation becomes difficult when a significant proportion do not receive the intervention as randomized, as occurred in the surgical arm. We fully accept that a much larger study with longer follow-up would be needed to provide reliable evidence on mortality, quality of life improvement and long-term survival between IET and surgery. Based on the results of the MIST-3 study, a further definitive phase 3 study is being developed that does not include a standard care arm.

## 3.6 CONCLUSION

This is the first multicentre RCT of early intrapleural enzyme therapy vs early surgery in pleural infection, demonstrating feasibility of recruitment, potential shortening of length of stay with VATS, but signals toward earlier resolution of pain and return to usual function with IET. The study findings suggest that with some modification to the trial design, a definitive phase 3 study is feasible and required to assess optimal initial management in pleural infection. Planning for this is underway.

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# APPENDIX A3.1

### Table A3.1 - CRF completeness at 2 weeks, 2 months, and 6 months

| Completeness of CRF | Standard Care (n=21) | IET (N=19) | VATS (n=20) | Expected (n)‡ | Completed (n) | Completion Rate (%) * |
|---|---|---|---|---|---|---|
| **2-week CRFs†** | | | | | | |
| Follow-up | 17 (81.0%) | 15 (78.9%) | 17 (85.0%) | 58 | 49 | 84.5% |
| IPAQ | 17 (81.0%) | 13 (68.4%) | 16 (80.0%) | 58 | 46 | 79.3% |
| EQ-5D-5L | 17 (81.0%) | 14 (73.7%) | 16 (80.0%) | 58 | 47 | 81.0% |
| **2-month CRFs †** | | | | | | |
| Follow-up | 18 (85.7%) | 15 (78.9%) | 16 (80.0%) | 56 | 49 | 87.5% |
| IPAQ | 17 (81.0%) | 13 (68.4%) | 17 (85.0%) | 56 | 47 | 83.9% |
| EQ-5D-5L | 17 (81.0%) | 13 (68.4%) | 17 (85.0%) | 56 | 47 | 83.9% |
| **6-month CRFs †$** | | | | | | |
| Follow-up | 14 (66.7%) | 11 (57.9%) | 11 (55.0%) | 55 | 36 | 65.5% |
| IPAQ | 14 (66.7%) | 13 (68.4%) | 12 (60.0%) | 55 | 39 | 70.9% |
| EQ-5D-5L | 14 (66.7%) | 13 (68.4%) | 12 (60.0%) | 55 | 39 | 70.9% |

**Calculated as number completed divided by actual people that were alive up to that time point*

*†These are excluding those face-to-face follow-up data that could not be extracted during the pandemic*

*‡Number of survivors up to this time point*

*$ The follow-up visits at 6 months is optional.*

# APPENDIX A3.2

## Standard Operating Procedure (SOP) for participants assigned to Video Assisted Thoracoscopic Surgery (VATS) in the MIST-3 trial

| | |
|---|---|
| **Version Number:** | V1.02 |
| **Date Finalised:** | 18Jul2019 |
| **Name of Author:** | Eihab Bedawi<br>Dionisios Stavroulias<br>John Edwards<br>Najib Rahman |
| **Name of Reviewer:** | Emma Hedley<br>Joy Wiles |
| | |

### 1. Scope

This SOP is written to outline the use of VATS and to standardise the non-surgical management of the surgical arm patients unfit for surgery in the MIST3 randomised study.

### 2. Individuals and Centres

For quality assurance purposes, centres and individuals who undertake VATS procedures for this study must demonstrate significant experience (at least 50 procedures performed as first operator). All operations are to be performed by specialist thoracic consultant surgeons or cardiothoracic surgical trainees under appropriate supervision.

### 3. Use of VATS

Patients in the MIST3 study may be treated with a VATS procedure if randomised to this treatment arm. Those recruited will be adult patients with pleural infection, requiring hospital admission for antibiotics and chest tube drainage. Those randomised to the VATS arm of the study will receive an early surgical review; if surgery is not deemed to be appropriate (following surgical and anaesthetic evaluation), then patients will be managed according to best practice (see section 6) and according to the opinion of the local surgeon. Therefore, this SOP applies to any patient randomised to the surgical arm of the MIST3 study.

## 4. VATS procedure

Patients randomised to the VATS arm, deemed eligible for surgery (with no significant medical comorbidity precluding an operation), will be managed according to BTS guidelines whilst awaiting an operation. Those patients who meet the following criteria will be offered an operation:

- Persisting sepsis despite adequate chest tube drainage.
- Residual pleural collection despite adequate chest tube drainage.

A VATS procedure will be first line management for those undergoing surgery. This will be performed under general anaesthetic, with lung isolation achieved (method of lung isolation as per anaesthetist's experience). Either 1, 2 or 3 port VATS will be performed depending on surgeon preference and experience. The technical details of port placement and instrumentation for surgery will not be covered in this SOP and will vary according to unit and surgeon. With each approach all loculations must be broken down, full drainage of the empyema achieved, and decortication performed as required to achieve complete re-expansion of the underlying lung (under direct vision on-table). One 28F drain (minimum – larger and more drains can be used according to surgical preference) should be left at the end of the procedure, with surgeon discretion as to whether a second drain is required.

## 5. Complications

Should complete re-expansion of the lung not be achieved using a thoracoscopic approach, then on-table conversion to posterolateral thoracotomy may be performed. This should ideally be a muscle sparing approach, aiming to minimise patient morbidity. Intra-operative bleeding not controlled thoracoscopically may also require a thoracotomy. Other technical reasons encountered by the operating surgeon requiring conversion to open procedure must also be documented. Empyema surgery is associated with a well-documented list of complications. Post-operative complications include, but are not limited to mortality, wound infection, prolonged air leak, repeat operation, blood transfusion, respiratory failure, and tracheostomy.

## 6. Non-surgical management

For those patients who are deemed not fit for an operation under general anaesthetic, they will continue to receive best standard care. Antibiotics will be continued as guided

by microbiology. Chest drain size may be increased to a 28Fr (minimum). If there are further residual collections, if amenable, image guided drainage may be attempted. Rib resection and drainage under local anaesthetic and sedation may be considered as a last resort procedure. For the purposes of the MIST-3 trial, no intrapleural agents of any kind are permitted (including saline irrigation).

### 7. Post-operative period

A chest radiograph will be performed routinely on all post-operative patients on day 0, day 1 and post drain removal. All other chest radiographs taken will be based on clinical need.

Patients will be prescribed venous thromboembolism prophylaxis (according to local guidelines) and supported nutritionally as required. Analgesia will be administered according to local post-operative guidelines and escalated as per the WHO pain ladder. Drains will be connected to negative pressure suction, at -2.5kPa, where possible, portable suction units will be utilised. Drain output will be monitored as per local practice, and a 24hrly figure of fluid and air drainage recorded. Antibiotic therapy will be continued as guided by the microbiology results where positive cultures have been obtained, and in accordance with local microbiology best practice guidelines. Drains will be removed when the volume of fluid output is minimal and following the cessation of any air leak in the preceding twenty-four-hour period.

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## APPENDIX A3.3

## Strategies to optimise MIST-3 study recruitment

In a systematic review of 45 randomised controlled trials, Treweek et al described six principal categories of intervention adopted for trial recruitment: trial design, obtaining consent, approaching participants, financial incentives, training for recruitment and trial coordination (Treweek et al., 2013). This section will outline how some of these factors were considered in the design and amendment of the trial protocol, set up of the study, as well as additional steps taken to overcome challenges and maximise recruitment since the trial began.

The key focus throughout the process of designing the trial was aligning the protocol with standard clinical practice as much as possible to minimise burden on participants (as well as recruiting sites), which has been recommended as a strategy of improving recruitment (Shelby-James et al., 2012). Follow up visits and interventions were aligned with clinical follow up visits, including completion of CRF's, questionnaires and conduct of interviews.

In the MIST-3 protocol, potential participants can be approached for consent prior to confirmation of diagnosis but only randomised once they meet the inclusion criteria (diagnosis confirmed and failed initial period of chest tube drainage). The main reasons for this are to allow as long as possible for patients to consider enrolment, as well as the fact that, in practice, often chest tube insertion is performed at the time of pleural fluid sampling as part of the same procedure, with a short pause during which a pleural fluid sample is run through a blood gas analyser to check the pH in the absence of pus. My experience of recruiting the first few participants locally, has given me some valuable insight that may allow reconsideration of this strategy if, as the trial progresses, the screening logs show that eligible patients are declining to take part. Early in their illness, patients with pleural infection are often significantly unwell with pleuritic chest pain, fatigue from a catabolic state, difficulty breathing and pyrexia. They may also have significant anxiety about the nature of their condition, which may not yet be explained or which they may not have had time/been able to fully understand. They may also be apprehensive about forthcoming invasive procedures, involving mention of needles and a tube inserted into the chest. Adding to this psychological burden by approaching them at this stage to discuss a trial relating to

surgery and clot busting drugs, in case of “treatment failure”, may understandably not be welcomed. My impression is that waiting until after patients have had the opportunity to receive 2 or 3 doses of antibiotics, have their chest tube inserted, will allow for a much more engaged discussion of the trial. This has currently not been formally changed on the protocol, but a minor amendment may be considered in the future if required, after discussion with the TMG. The qualitative data from the participant interviews will undoubtedly offer us a greater understanding of the recruitment experience for these patients.

The systematic review (Treweek et al., 2013) found conflicting results for the effect of additional education for recruiters on study recruitment, but demonstrated little effect on recruitment for centres receiving on-site initiation visits versus none. However, in view of the small number of sites, the involvement of both medical and surgical teams, the above-average complexity of the trial entry process (with the intention of capturing data from those who consent to randomisation and interview, randomisation, or interview alone as well as those who refuse to consent to either, but agree to follow up), I selected the face-to-face approach for the site initiation visits (SIVs). These meetings were hugely beneficial, allowing the opportunity for engaged discussions and clarification of any queries regarding the trial procedures. There were some common themes that emerged from these discussions, the most dominant pertaining to the surgical arm, including the physician-surgeon interactions following randomisation, arrangements for an early surgical review, care of the participants randomised to surgery but not fit for an operation (e.g., whether they would be under the care of the physician or the surgeon and which ward they would be managed on). As some of these aspects were anticipated (early randomisation to surgery being the novel aspect of the trial), I had set a pre-requisite that each site initiation visit include representation from both the surgical and medical sides. In the one site where this was not possible, the surgical sub-PI dialled in to the SIV. This allowed opportunity for the surgical TSP to be discussed including options for patients not fit for surgery, and local arrangements to be put in place for participants randomised to surgery, from initial contact to delivery of intervention.

Personalised site feedback on recruitment has previously been reported to be an effective intervention in reducing time to meet recruitment targets in one RCT, although not statistically significant (Monaghan et al., 2007). As well as collective email updates on a fortnightly basis to the PIs at all sites together, individual site emails

will be sent to sites who do not appear to be engaging with recruitment (judged by monthly review of screening logs) offering recruitment tips, response to any queries in a gentle, non-critical manner. These sites will be discussed separately at TMG/TSC meetings, should they continue to make inadequate progress.

Additionally, MIST-3 is being managed centrally by the Oxford Respiratory Trials Unit, with a trials manager assigned to co-ordinate site set-up and TSC/TMG meetings as well as answering queries from individual recruiting sites and assisting with the monthly newsletter production. Although not reaching statistical significance, it has been reported that trials with Clinical Trials Unit (CTU) input appeared more likely to achieve successful recruitment (65% versus 48% for trials without CTU input (Sully et al., 2013).

Other strategies I adopted in MIST-3 to maximise successful recruitment include designing a MIST-3 poster advertising the study that each site can modify with local contact details and display around their site (Appendix A3.3a), a suggested MIST-3 script (Appendix A3.3b) on how to explain the trial to potential participants as well as a colour trial entry flow chart as an aide memoire for the trial procedures (appendix A3.3c). Regular contact with ward teams and remote monitoring of the ward lists on EPR also helped ensure that potentially eligible participants were not missed.

The remainder of my MD studies will focus on the identification and evaluation of biomarkers (biochemical and radiological) that may enable early risk stratification in terms of outcomes such as requirement for surgery and length of hospital stay. Such biomarkers, if identified, could be evaluated prospectively in future trials.

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**APPENDIX A3.3a – Site Recruitment poster**

ORTU
Improving Healthcare Through Research

# MIST-3

UNIVERSITY OF OXFORD

## Early Surgery (VATS) vs Fibrinolytics in Empyema – a randomised trial

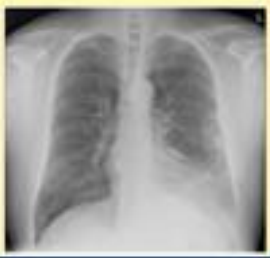
## RECRUITING NOW!!!


**UNILATERAL PLEURAL EFFUSION?**
**SIGNS/SYMPTOMS OF INFECTION?**
**EMPYEMA?!**

**Please contact the pleural team URGENTLY on:**

_____

_____



**APPENDIX A3.3b – Study recruitment script**

# The third Multi-Centre Intra-Pleural Sepsis Trial (MIST-3):

# Early Video Assisted Thoracoscopic Surgery (VATS) or Intrapleural Enzyme Therapy (IET) in Pleural Infection – feasibility, randomised trial

## RECRUITMENT SCRIPT

Approach patient day after chest drain insertion once a clinical decision has been made that there has been a suboptimal response to chest tube drainage. At this stage, patients will hopefully be feeling a little bit better and in a better position to discuss taking part in a trial.

**REMEMBER TO CHECK INCLUSION AND EXCLUSION CRITERIA PRIOR TO APPROACHING PATIENT**

**Always begin by ensuring patient understands their diagnosis.**

*"Hello Mr/Mrs X, my name is X, and I am one of the (role)*
*I'm sorry to see you are unwell in hospital. You are being treated for a condition called pleural infection – which means you have developed an infected collection or type of 'abscess' in the chest on the outside of the lung. You are receiving antibiotics and you have had a chest tube inserted yesterday with salt water flushes to try to drain this out. From the response to chest tube drainage so far you are likely to require further treatment to help us resolve this completely. There are 2 treatment options that we would normally use in this situation. The first involves injecting some 'clot busting' medications directly into the collection through your chest tube to help break it up and make it easier to drain. The other option would be to ask our surgical colleagues to perform a more 'direct' drainage by making a small incision in the chest wall the size of a keyhole to insert a camera and an instrument into the chest and drain the collection under direct vision and a short general anaesthetic. These 2 treatments have never been compared directly so we do not know which of these treatments is better. Normally we would wait another 2 or 3 days to consider this but there is good evidence in the medical literature that delays to treating this condition are associated with worse outcomes and longer periods of recovery.*
*On that basis, we are currently running a research study to see if earlier treatment can help resolve your illness and help you recover more quickly. The way we do this is we ask a computer to assign you to one of the treatments I have mentioned. The possible outcomes are that we continue to treat you in the same way for another day or so and*

*then make a decision as to what to do next in the usual way, known as 'standard care'. A 'standard care' outcome does not withhold any of the other treatment options from you. The other outcomes would be we proceed straight to a surgical drainage or to use the clot busting medications through your chest tube. What I would like to emphasise is that these treatments are both established treatments used in clinical practice and are not 'research' treatments in themselves. Any potential risks or side effects would be discussed with you before the treatment is administered to you. The rest of your care will continue in the same way, and you will not need any additional tests or follow up visits specifically for the trial. All we would do is monitor your progress a bit more closely than usual, mostly from your medical records, and once you are discharged and feeling better, provided you have given us permission to do so, we may contact you to have a short chat about your treatment experience.*
*If this is something that would potentially interest you, I can offer you a short one-sided patient information sheet to have a look at now before you decide along with a detailed one to read at your leisure. How does that sound? It is completely voluntary, and I am happy to answer any questions you may have about the study.*

**If positive** – offer PIS (highlighting summary PIS on the first 2 pages)

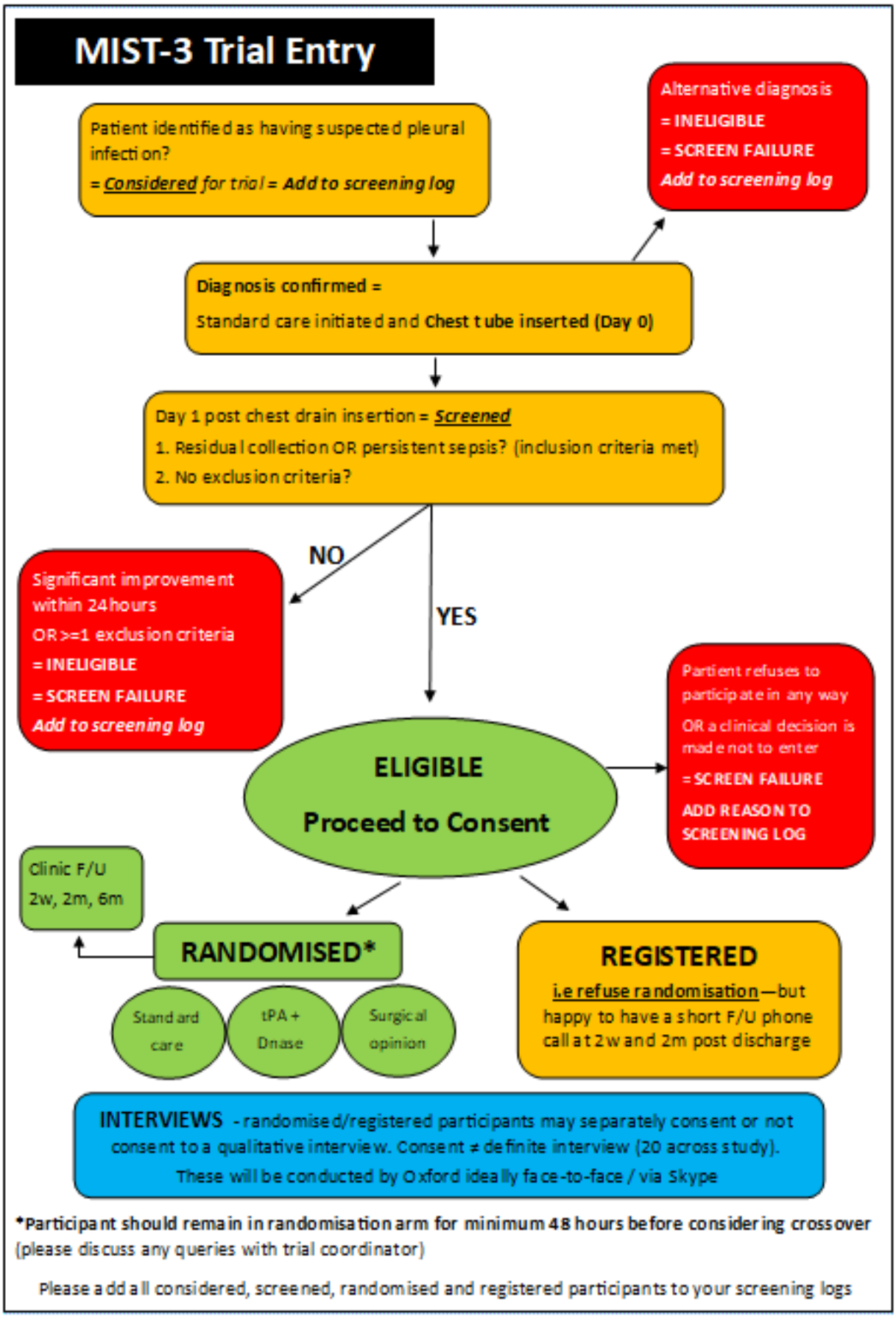
**If not keen:**

*"No worries at all, thank you for taking the time to discuss this. We/the team will continue to treat you in the usual way and will consider how to proceed in the next day or 2. Once you have recovered and are back home, it would be extremely valuable for the study team to understand your reasons for not wanting to take part in the study and the concerns you had. Would you be happy for a member of the research team to contact you to discuss this at a time convenient to yourself?"*

**Remember to offer consent for interview only regardless of consent to randomisation.**

**If not interested in interview or randomisation, no need to complete consent form but please complete screening log.**

**APPENDIX A3.3c – MIST-3 Trial Entry Flow chart**



## APPENDIX A3.4

## MIST-3 FULL TRIAL PROTOCOL

**Trial Title:** Early Video Assisted Thoracoscopic Surgery (VATS) or Intrapleural Enzyme Therapy (IET) in Pleural Infection - a feasibility randomised trial.

**Internal Reference Number / Short title:** MIST 3
**Ethics Ref:** 19/EE/0174
IRAS ID: 255746
**Date and Version No**: 14Apr2021_V10.0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**Sponsor:** University of Oxford

**Funder:** National Institute of Health Research, Grant PB-PG-0416-20020

1. **LAY SUMMARY**

Pleural infection is a serious complication of pneumonia where infected fluid collects around the lung in a large abscess. It can affect anyone and occurs in 40 patients every day in the UK. Treatment requires antibiotics and drainage of fluid using a chest tube inserted with local anaesthetic between the ribs, and admission to hospital for 2 weeks.

When these treatments fail, patients either die (about 20% of cases) or are referred for major surgery (a further 20%). Surgery is important when initial treatment fails but has several side effects and is not an option for elderly and sick patients, where the death rate is 40%.

A new treatment (called Intrapleural Enzyme Therapy or IET) can be given through the chest tube early in treatment, which improves drainage and reduces the need for surgery and the time spent in hospital. Keyhole surgery is also now available to drain infected fluid (Video Assisted Thoracoscopic Surgery or VATS), and some people believe that this should occur early in treatment to prevent death and long hospital admissions, but this has not been proven. Early treatment with either IET or VATS may therefore improve care, but we do not know the long-term effects (e.g., restriction in breathing) or impact on quality of life.

In this study, we will consult with patients to understand what factors are important to them when treating this disease. This will help us to understand what should be measured in a study to best improve care. We will conduct a study where patients are randomised (assigned by computer) to usual treatment (chest tube and antibiotics), early VATS or early IET. We will measure whether it is acceptable to patients to be randomised in this way and whether a larger study in the future is important and possible.

## 2. **SYNOPSIS**

| | | |
|---|---|---|
| Trial Title | Early Video Assisted Thoracoscopic Surgery (VATS) or Intrapleural Enzyme Therapy (IET) in Pleural Infection - a feasibility randomised trial. | |
| Internal ref. no. (or short title) | MIST 3 | |
| Clinical Phase | Feasibility | |
| Trial Design | Randomised trial | |
| Trial Participants | Adults with pleural infection requiring admission to hospital for antibiotics and chest tube drainage. Defined as:<br>1) A clinical presentation compatible with pleural infection<br>2) A pleural collection with a chest drain in situ<br>3) Has pleural fluid requiring drainage which is either:<br>• purulent **or**<br>• gram stain positive **or**<br>• culture positive **or**<br>• acidic with a pH <7.2 **or**<br>• low pleural fluid glucose (<2mmol / L) in the absence of accurate pH measurement<br>4) Residual collection/ongoing sepsis after 24h standard care<br>5) Willing and able to give written informed consent | |
| Planned Sample Size | Total 75 randomised (25 in each arm); however more participants will be required to be screened to fulfil the randomised requirement | |
| Treatment duration | Whilst as an inpatient for pleural infection (from 48 hours to 7 days post treatment, whilst an inpatient only) | |
| Follow up duration | 2 months (optional follow up at 6 months). Any participants randomised post 1st June 2021 will only receive the 2 month follow up visit. | |
| Planned Trial Period | 24 months | |
| | Objectives | Outcome Measures |
| Primary | To assess the feasibility of randomising 75 participants with pleural infection to standard care, early VATS or early IET. | Recruitment rate, retention rate and the proportion of participants screened, who consented to be randomised and who consented to be interviewed. |
| Secondary | 1. Explore the risks/benefits from a participant perspective of a referral to standard care, VATS or IET treatment strategy<br><br>2. Understand the acceptability of randomisation in a surgery versus non-surgery trial.<br><br>3. Establish feasibility of collecting accurate long-term (6 month,) outcomes in randomised participants including mortality, hospital stay, readmissions, lung function (optional), further surgery, functional ability, | 1. Conduct structured interviews with a proportion of randomised participants and carers (Oxford recruiting site only).<br><br>2. Proportion of participants who accepted/did not accept to be randomised. Conduct structured interviews with a proportion of participants to collect information about their concerns and reasons for accepting/not accepting randomisation.<br><br>3. Review completeness of data collected up to 6 months from randomisation, regarding mortality, length of hospital stay (time from starting intervention until discharge), number of hospital readmissions, completion of lung function tests (FEV1/FVC) |

| | | |
|---|---|---|
| | participant reported outcomes and quality of life.<br><br>4. Assess feasibility of trial interventions<br><br>5. Establish treatment costs including standard care, intrapleural drugs, surgery, initial and subsequent hospitalisation, outpatient, A&E and primary care contacts.<br><br>6. Assess which outcomes of pleural infection are most important to the participants.<br><br>7. Proportionate adverse events for the intervention arms | (optional), proportion of participants requiring further surgery. Assess the number of qualitative assessments completed such as functional assessments, questionnaires, and visual analogue scores. Collect data on quality of life.<br><br>4. Record type of surgery (VATS, thoracotomy) and time to surgery (from randomisation to surgery point of surgical intervention) in the surgical arm and details of compliance (proportion initiating treatment/completing treatment/requiring dose reductions/missed doses) in each interventional arm along with the reasons for non-completion.<br><br>5. Costs of surgery will be assessed using a micro-costing study evaluating staff time, theatre time and consumables. Other healthcare resource use will be obtained from participants' trial records; hospital records; and participant self-report through questionnaires. Resource use will be costed using appropriate unit costs.<br><br>6. Perform structured qualitative interviews with a proportion of participants who have had pleural infection to collect information on their priorities of care.<br><br>7. Record agreed adverse events |
| Investigational Medicinal Product(s) and interventions | 1. Recombinant human deoxyribonuclease (DNase)And Recombinant human tissue plasminogen activator (tPA, Alteplase)<br><br>2. Video Assisted Thoracoscopic Surgery<br><br>3. Chest drain insertion, broad spectrum antibiotics and Intrapleural saline flushes (standard care) | |
| Formulation, Dose, Route of Administration | DNase 5mg BD (diluted in 30mls sterile water) intrapleural<br>Alteplase 10mg BD (diluted in 30mls sterile water) intrapleural | |

***Some of the study assessments and visits have been made optional to streamline the trial pathway, following the slow recruitment due to COVID-19. This will reduce the data collection burden on sites and focus on the essential data required to meet the study outcomes. The maximum follow up time has been shortened to 2 months to facilitate a 4 month recruitment extension (April – July 2021) due to COVID-19. The 6 month follow up visit is now optional. Those participants randomised after 1st June 2021 will only be required to have a 2 month follow up visit in keeping with the trial timelines.**

## 3. BACKGROUND AND RATIONALE

### Introduction

Infection of the pleural space is common and the incidence is increasing in both adult (1, 2) and paediatric (3) populations. There are currently around 80,000 cases per year in the US and UK (estimated 15,000 new cases per year in the UK). These infections carry a significant health burden; over 35% are fatal or require thoracic surgery (4); 26% of such participants require a hospital admission lasting more than a month (4); the associated estimated cost of care is around £5900 per participant (internal audit data).

### Current clinical care

Standard treatment for pleural infection, advocated in guidelines from all major respiratory specialist societies (5, 6), is a combination of appropriate antibiotics and drainage of infected pleural fluid/pus with a chest tube. More complex surgical drainage techniques (e.g. video assisted thoracoscopic surgical pleural drainage, open thoracotomy with decortication, or rib resection and open drainage (5, 6)) is advocated in participants with a “poor likely response to medical therapy”, or a poor response to initial treatment. Definitive surgical treatment in selected participants with pleural infection is essential. Pleural infection is a progressive disease with pleural fibrosis developing with time (7); this can prevent effective drainage with the least invasive surgical techniques (VATS), and precipitates the need for open thoracotomy which is associated with higher adverse event rates (see below). Early surgery may be appropriate as the infection is debilitating, and there are progressively increasing anaesthetic and perioperative risk. Previous studies demonstrate that around 60% of participants will respond favourably to medical treatment (4), therefore participants are generally treated with a combination of antibiotics and chest tube drainage initially, with referral for surgical intervention in those who have evidence of ongoing sepsis syndrome despite these treatment measures. This pathway is based on expert opinion rather than empirical evidence and an adequately powered, randomised controlled trial is needed to establish the optimal treatment pathway.

### Surgery for pleural infection

The timely use of surgical drainage techniques has been a cornerstone of the treatment of pleural infection for many years (8), and is accepted to be sometimes life-saving. Such treatment is not based on large, randomised trials, but large cohort studies such as a recent analysis of 4,424 cases of adult pleural infection suggest

effective surgical drainage is associated with improved outcome (1). This data is collected from US 'billing' records, and is affected by reporting and selection bias, but it and other small surgical series (9-13), strongly support the importance of surgery that is advocated by standard treatment guidelines (5, 6).

Some authors have advocated surgery as immediate treatment for all participants with pleural infection (13-15), although two moderate sized clinical trials in children showed no clinical benefit and greater cost from this more radical approach (16, 17). Early surgery in adults has been advocated in pleural infection (18) on the basis of two randomised studies which compared standard care (antibiotics and chest tube, plus fibrinolytics in one study) to early VATS (19, 20). Both demonstrate earlier hospital discharge and lower mortality with VATS but are underpowered and methodologically flawed (unclear criteria for medical failure, lack of objective decision-making criteria, no blinding).

The disadvantages of surgical drainage are substantial and preclude its use in all participants. Surgical thoracic procedures carry associated anaesthetic/perioperative risks (21) (operative mortality ~2%, major complication rate ~8% in reported VATs series), and thoracotomy also causes substantial post-operative pain. 61% of participants experience some pain at one year after surgery and 3-5% describe this as severe (22). 66% of participants require analgesia at six months and 38% of participants still have pain 3 years after surgery, falling to 30% at 4 years (23). Video assisted thoracoscopic (VATS) drainage significantly improves on this adverse event rate. However, 4% of participants still experience significant pain at 2 years (24), and a proportion (reported variously from 8 to 59% (25-27)) of VATS procedures require conversion to open thoracotomy at the time of surgery, with the attendant increase in morbidity.

Despite the likely benefit of surgery in selected participants, there is evidence to suggest that older participants with more co-morbidity receive less access to this treatment (perhaps because of concerns about anaesthetic / perioperative risk). We have collated surgical empyema case series from the UK (13, 28-31) and US (1, 10, 32-37) (including a very large recent cohort (1)), and demonstrated that the typical age of participants in these series is 49.5 years in the UK and 52.6 years in the USA. This is significantly below the median age of 61 years seen in an unselected and well documented UK sample of 454 participants (unpublished data) (4). Within this sample, those who received surgery were significantly younger with less co-morbidity than the group as a whole (surgery group age 52.5 SD 16.0 years, non-surgical group age 61.6 SD 17.6 years, difference 9 years, 95% CI: 4.8 to 13.2, $p<0.001$, unpaired t-test). This age threshold is associated with a large difference in mortality (no. of deaths in participants aged <60 = 11/212 (5.2%), deaths in those

>60 = 87/242 (36%), difference = 30.8%, 95% CI: 24 to 37.5%, p<0.001. OR for death by age cut-off = 10.3, 95% CI: 5.3 to 19.9).

Thus, surgical drainage of pleural infection remains a vital therapy in those not responding to medical treatment, but whether it has a role earlier in the treatment pathway is unclear. It is possible that early surgery will result in better outcomes in the short and longer term, and that this vital therapy is avoided in those who may need it most (such as the elderly).

**Reasons for failed medical therapy**

Standard 'medical' therapy for pleural infection (chest tube drainage and antibiotics) often fails; this may be due to several reasons:

1. The presence of thick infected pleural fluid which cannot easily drain down the pleural catheter. Infected fluid is thick due to free, uncoiled, DNA liberated from dead leukocytes that forms 'tangles' in the abscess fluid, creating a gel.
2. The presence of locules which partition the fluid into separate and undrainable pockets. Locules are due to the development of fibrinous septations within the infected collection.
3. The presence of resistant collections of infecting organisms in bacterial structures known as "biofilms". A biofilm is a community of micro-organisms attached to a surface, producing extracellular polymeric substance (EPS). The organisms exhibit an altered phenotype compared with their corresponding planktonic cells and the EPS is a complex matrix, made up of both fibrin and free DNA, which serves as a storage facility for nutrients and entraps other microbes and non-cellular materials. Biofilm bacterial cells withstand host immune responses and are much less susceptible to antibiotics than their non-attached individual planktonic counterparts.

Potentially, each of these problems is amenable to therapeutic intervention with intrapleural adjunctive therapies. The fibrinous septations can be disrupted by fibrinolytic agents, the thick pleural fluid can be thinned with Deoxyribonuclease (DNase) and there is ex-vivo experimental data in support of DNase as a biofilm disruptor and an agent capable of decreasing biofilm formation in a number of bacterial infections, including several key microbiological organisms in pleural infection (*Strep Pneumonia*, *Enterococcus*, *Staph Aureus*, *Staph Epidermidis* and *Pseudomonas Aeruginosa*) (38-42).

**Evidence for intrapleural adjunctive therapies**

For many years, intrapleural streptokinase alone was advocated as a treatment with which to improve drainage from infected pleural collections. Case series and small randomised studies suggested improved drainage with streptokinase. However, the

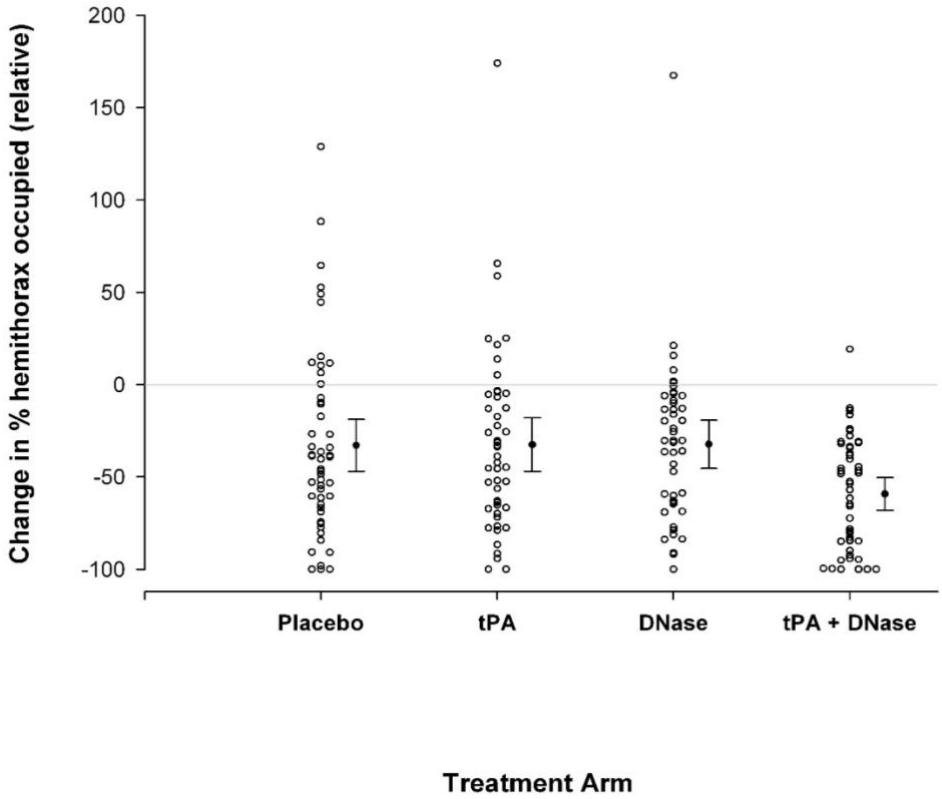
largest randomised study to date (MIST1 (4)) including 454 participants, and a meta-analysis of the 5 methodologically sound fibrinolytic studies (43) suggested no benefit from the use of intrapleural fibrinolytic on important clinical outcomes.

Based on this negative result, the MIST2 (44) study was conducted as an initial randomised assessment of the use of combination fibrinolytic (tPA) with intrapleural DNase. 210 participants were randomised in a 2 x 2 factorial double blind placebo-controlled study, with radiographic drainage as the primary outcome measure. The results of this study demonstrated that tPA alone or DNase alone were no better than placebo in improving the chest radiograph. However, combination therapy (tPA + DNase) resulted in significant treatment interaction and was significantly better than placebo in improving the chest radiograph (relative improvement in % hemithorax occupied by pleural fluid versus placebo = 22.8%, 95% CI: 7.1 to 28.9, p=0.002) (Figure 1). This treatment effect appeared to be independent of pleural fluid purulence, which was a minimisation factor for the study, well balanced between the treatment arms and a pre-planned subgroup analysis (p value for interaction between pleural fluid purulence and treatment effect = 0.95).
The improvement in the primary outcome measure was associated with strong signals toward an improvement in some clinically important outcomes which were secondary outcome measures for the purposes of the MIST2 study. There was evidence at 3 months post randomization that combination (tPA + DNase) treatment was associated with a decrease in surgical rate (placebo surgical rate 9/50 (18.0%), tPA + DNase surgical rate 2/47 (4.3%), estimated odds ratio for surgery vs placebo = 0.20, 95% CI: 0.02 to 1.02, p=0.052). This suggests that although MIST2 was underpowered to accurately assess change in surgical outcome, combination tPA + DNase therapy was associated with a potentially large reduction in need for surgery. This reduction, if proven in a larger study, would be highly clinically relevant (80% reduction in surgical rate) and potentially decrease treatment costs and morbidity for this disease.

In addition, combination tPA + DNase therapy was associated with a reduction in hospital stay compared to placebo (duration of hospital stay in days; placebo mean 14.9 days (SD 14.6), combination mean 11.0 (SD 9.4), difference -4.8 days, 95% CI: -10.4 to 0.1, p=0.06). The MIST2 study was not powered to accurately estimate this treatment effect, but if real would represent a substantial decrease in hospital stay (30% absolute reduction) with the attendant savings in cost and morbidity.
The cost of combination tPA + DNase treatment is not trivial, estimated at around £960 per participant. However, should the decrease in surgical rate and decrease in hospital stay prove to be true in larger trials, there are potential cost savings using this treatment.

Thus, intrapleural combination tPA + DNase therapy has been shown to improve the standard clinically used surrogate (chest radiograph) in pleural infection and may have important beneficial effects on reducing surgery rate and hospital stay.

**Figure 1. Primary outcome measure (radiographic improvement) from the MIST2 study.**




## Rationale for this study

Assessing the early use of VATS or IET requires a phase III randomised controlled trial to directly compare the early introduction of these treatments to conventional care. The recent MIST 2 trial concluded that IET improves drainage and reduces the need for surgery and hospital stay but has not been compared directly with surgery. The study will also consider the selection bias of previous trials and will randomise all participants enrolled, despite fitness for surgery, to any treatment arm. The analysis will be performed as per intention to treat, despite a proportion of participants in the surgical treatment arm who are likely not to be fit enough to undergo surgical intervention.

Before undertaking a large trial, it is important to establish the key outcome measures which are important to participants to allow for relevant outcomes in a subsequent randomised controlled trial (RCT). Information also needs to be collected on feasibility of recruitment, participant acceptability and the ability to collect

outcome data. The trial proposed will address the feasibility of randomising participants to standard care, early VATS or early IET by undertaking qualitative interviews both with a proportion of participants who have participated in the trial but also with those who have refused.
This combination of outcomes and objectives will establish whether a larger RCT can be undertaken with participant focussed outcome measures established through detailed interviews with people who have been directly involved in any process of care for a pleural infection.

### PPI Input and Feedback

A PPI group has been convened for the trial. An introductory meeting took place in October 2017 to gather the views and thoughts of patients, partners and carers who have undergone similar treatment to the MIST3 trial. This proved to be an extremely positive day with lots of interesting feedback. Consequently, these views were considered in the writing of this protocol and the accompanying trial paperwork.
Since then, the PPI group met again when the trial paperwork was established.
The trial team explained the rationale of the trial and invited the group to ask questions and provide feedback. The group were given the document pack to take away and comment on. Most comments were returned informing the trial team a questionnaire chosen to ask participants during the trial was felt not to be suitable due to limited ability, therefore this has now been replaced. We plan to meet on a frequent basis throughout the course of the trial.

## 4. OBJECTIVES AND OUTCOME MEASURES

| | Objectives | Outcome Measures |
|---|---|---|
| Primary | To assess the feasibility of randomising 75 participants with pleural infection to standard care, early VATS or early IET. | Recruitment rate, retention rate and the proportion of participants screened, who consented to be randomised, who consented to be interviewed. |
| Secondary | 1. Explore the risks/benefits from a participant/carer perspective of a referral to standard care, VATS or IET treatment strategy as well as which outcomes of pleural infection are most important to the participants.<br><br>2. Understand the acceptability of randomisation in a surgery versus non-surgery trial. | 1. Perform structured qualitative interviews with a selection of participants who have had pleural infection and their carers (carer interviews at Oxford recruiting site only)<br><br>2. Proportion of participants who accepted/did not accept to be randomised. Conduct structured interviews with a proportion of participants to collect information about their concerns and reasons |

| | | |
|---|---|---|
| | | for accepting/not accepting randomisation. |
| | 3. Establish feasibility of collecting accurate long-term (6 month) outcomes in randomised participants including mortality, hospital stay, readmissions, lung function (optional), further surgery, functional ability, participant reported outcomes and quality of life. | 3. Review completeness of data collected up to 6 months from randomisation, regarding mortality, length of hospital stay (time from starting intervention until discharge), number of hospital readmissions, completion of lung function tests (FEV1/FVC) (optional), proportion of participants requiring further surgery. Assess the number of qualitative assessments completed such as functional assessments, questionnaires, and visual analogue scores. Collect data on quality of life. |
| | 4. Assess feasibility of trial interventions | 4. Record type of surgery (VATS, thoracotomy) and time surgery (from randomisation to surgery point of surgical intervention) in the surgical arm and details of compliance (proportion initiating treatment/completing treatment/requiring dose reductions/missed doses) in each interventional arm along with the reasons for non-completion. |
| | 5. Establish treatment costs including standard care, intrapleural drugs, surgery, initial and subsequent hospitalisation, outpatient, A&E and primary care contacts. | 5. Costs of surgery will be assessed using a micro-costing study evaluating staff time, theatre time and consumables. Other healthcare resource use will be obtained from participants' trial records; hospital records; and participant self-report through questionnaires. Resource use will be costed using appropriate unit costs. |
| | 6. Assess which outcomes of pleural infection are most important to the participants. | 6. Perform structured qualitative interviews with a proportion of participants who have had pleural infection to collect information on their priorities of care. |
| | 7. Proportion of adverse events for the intervention arms | 7. Record agreed adverse events |

## 5. TRIAL DESIGN

Multi-centre, open-label, randomised three-arm parallel arm, feasibility study to determine whether randomising participants to standard care, intrapleural enzyme therapy or early VATS is possible and acceptable to participants with pleural infection and a prospective cohort of participants refusing randomisation.

### Design and Randomisation

*Participant Population*

Participants will be approached initially by the clinical team as in-patients, who are suspected of having pleural infection. The aim is to enrol all participants with pleural infection and then assess who would be willing to undergo randomisation. It will be explained to participants that, if pleural infection is confirmed, they will be randomised to receive either referral to standard care, referral for IET or referral for Early VATS (as per the local surgeon's clinical view) via the agreed pathways.

*Confirmation of diagnosis and randomisation*

Participants may be consented prior to pathological confirmation of pleural infection as the diagnostic procedure is often performed at the same time as a chest drain is inserted, but randomisation will only occur once pathological / radiological confirmation has been obtained, with randomisation occurring within 24 hours of confirmation of diagnosis.

As fluid may drain effectively after initial drain insertion, it is permitted, according to local investigator preference, to wait for an initial drainage period before offering entry to the trial (which includes up to 24 hours as above). All participants will initially receive a small-bore chest tube (<15F) and antibiotics once diagnosis is confirmed (standard care as per current national guidelines) to prevent a delay in treatment initiation and those participants in whom drainage occurs successfully will not be randomised (and not counted towards the denominator for this feasibility study), but outcomes kept with their consent.

*Follow up*

All participants randomised will be carefully followed up as per the follow up schedule and outcomes collected to permit assessment of the feasibility of randomisation and trial recruitment, and retention through until final follow up. Factors which affect acceptance of randomisation will be explored by specific structured interviews in a proportion of participants randomised/not randomised

during the trial period. However, in the participants who decline randomisation and interview but consent to follow up, this will be restricted to a short telephone call at 2 weeks asking the participant their reasons for declining randomisation, and a further telephone follow up call at 2 months to document death or need for surgery. These telephone calls will be conducted by the sites and recorded on the specific CRF.

Some participants may be considered to require immediate surgery (for example, in the presence of solid pleural material on ultrasound where the physician does not consider a chest tube drainage attempt would be reasonable). Similarly, some patients may drain effectively within 24 hours. The frequency of this scenario will be captured on the screening logs, but they will not be randomised and will not be followed up as part of the trial.

### Surgical and IET Exclusions

Specific consideration was given to the possibility of excluding participants who are considered “unfit for surgical intervention” or who may be “unsuitable for IET” from this randomised trial. However, including these participants is particularly important in this study for a number of reasons. Firstly, the study is assessing the feasibility and acceptability of randomising to surgical versus IET versus standard treatment, rather than the actual performance of surgery or IET. Secondly, IET has the specific advantage over surgery that it is applicable to “all comers” with pleural infection (25), including the frail and elderly in whom clinical outcomes are the poorest, but IET may not be used in certain circumstances where surgery is preferable (for example, in those with major haemorrhage). As we envisage the larger phase III trial to include all comers with pleural infection, inclusion of all participants in the feasibility study is therefore scientifically required for consistency.

This study therefore randomises participants for a surgical opinion (rather than for surgical intervention), with the receiving surgeon deciding on what intervention (if any) is required or possible. Surgical intervention will be according to the surgical SOP developed by the trial team. Similarly, participants in the IET arm will be randomised to “IET intended treatment” with the local physician considering if it is safe to give this treatment. All analysis will be by intention to treat.

### Interventions for randomised participants

Participants will be randomised 1:1:1 to the three treatment arms.

### 1. Standard Care

As per current treatment guidelines (BTS 2010 (5)), participants will be admitted to hospital and started on broad spectrum antibiotics as per local guidelines and until results of any positive microbiology. A chest tube (minimum 12F in size) will be inserted using image guidance and local anaesthetic, and the participant will be

monitored with radiology, blood, and clinical parameters to assess for treatment failure. This will be assessed at 3-5 days and be according to objective decision-making criteria which will be documented (please see below).

As not all participants with pleural infection are considered fit enough to undergo surgical intervention, objective criteria for “medical treatment failure” will be recorded in all cases using objective criteria. These will be measured at 3-5 days post study inclusion, will be recorded on the CRFs, and are:

- The presence of a residual and clinically significant pleural collection as judged by the local PI, based on current radiology (chest radiograph, ultrasound and/or CT); and at least one of the following:

    1) Clinical evidence of ongoing sepsis as manifested by factors such as otherwise unexplained persistent fever, tachycardia, and hypotension (on clinical discretion)
    2) A serum CRP (C-reactive protein) that fails to fall by more than or equal to 50% compared to the baseline value prior to initiation of medical treatment
    3) A lack of significant response in the peripheral blood white-cell count as judged by the local investigator.

**Standard care is received by thousands of patients in the UK each year with a mean inpatient hospital stay of 5 days before consideration of additional treatments in the form of IET or surgery. Most patients will require chest tube drainage with regular saline flushes for the duration of this period. All these patients will have access to additional treatments if medical treatment failure is confirmed, as defined by the criteria above, as is normal care.**

*** Crossovers from the standard care arm to the IET arm or VATS arm will be permitted once participants have been deemed to require additional treatment after a further 48 hours of standard care.** Any crossover prior to completing a further 48 hours of standard care post randomisation would constitute a protocol deviation.

**2. IET arm**

Through the chest tube inserted during usual clinical care, intrapleural tPA (10mg bd) and DNase (5mg bd) will be administered as per our previous randomised trial protocol (44) 12 hourly over 72 hours, to start as soon as possible after randomisation as per recruiting sites’ local administration protocols.

Sites will be able to determine doses on a participant-by-participant basis but must not exceed these doses. Centres will be permitted to use lower doses than this as per their local guidelines, and doses will be recorded on the CRFs. Recent studies

have demonstrated the safety and feasibility of administering the two agents in a single session (i.e., DNase and tPA in one intrapleural administration, followed by 1 hour of clamping, then repeating the procedure 12 hourly) and this will be the schedule used in this randomised trial, to ease pragmatic delivery of the protocol.

Some participants may not be considered suitable to undergo IET treatment – the reasons for this will be recorded in the CRFs and the participants will remain in this treatment group. In which case after a further 48 hours of standard care, if still deemed to be required additional therapy, these patients can be offered alternative intervention including large volume saline pleural irrigation therapy or surgical treatment as clinically necessary. Any crossover prior to completing a further 48 hours of standard care post randomisation would constitute a protocol deviation.

**3. Early VATS arm**

Participants assigned to VATS will be referred immediately post randomisation to local surgical services, and VATS conducted according to standard surgical standards (defined as a trial specific instruction for this trial). As above, the decision on requirement for and safety of conducting VATS will be at the discretion of the receiving surgeon, and according to the surgical SOP. Variation in timing of surgery, surgical bed, and operation room availability (from randomisation to surgical event), and the proportion of participants considered “fit” enough for surgery on surgical review (i.e., the number who undergo a surgical procedure) will be collected as part of the study, as these variations are key outcomes of this trial.

Not all hospitals have access to surgery in the same hospital, and these participants will need to be transferred to achieve a surgical treatment – hence the rationale of minimising by centre to ensure that balance is achieved across the randomised groups across all centres. All participants in the VATS arm will be referred for prompt surgical review; if the participant is considered not fit for surgery, the surgical team will dictate further management which may include a number of treatments (including for example an increase in the size of the chest tube). If after 48h no treatment on the surgical TSP has been found to be suitable, these patients may continue on the standard care arm with interventions such as large volume saline pleural irrigation. IET may be given if no other treatment is deemed clinically appropriate. If required, these patients can be discussed with the trial team.

***In the event of disruption or restriction of surgical services due to COVID-19 pressures, eligible patients can still be randomised. If they are allocated to the surgical arm, and receive a prompt and favourable surgical opinion i.e., early VATS would have been feasible outside of COVID then please indicate this on the CRF.**

**Standard Treatment in all arms**

In the IET and “control” arms, the size of the chest drain inserted is at the discretion of the local clinicians but at least 12F in size is generally recommended. To ensure high quality care, all participants will be treated with antibiotics according to microbiological sensitivities (where available – estimated positive cultures in 60% of cases according to our previous published data (28)) and with empirical antibiotic therapy according to local prevalence and national guidelines (8). All participants will be treated with thromboprophylaxis and supported nutritionally according to best practice, guided by standard operating procedures which will be written for this study.

The use of imaging (such as thoracic CT or ultrasound) is at the discretion of the local physician/surgeon, but it is recommended that all participants planned for surgery undergo a CT prior to VATS.

In the IET and standard treatment arms, if there is insufficient clinical response on the objective “medical failure” criteria listed above at 72 hours post randomisation, surgical referral as per national guidelines is recommended, and will be recorded on the inpatient CRF, including type of surgery undertaken.

**Data collection**

Data collection will be performed by the research team on the participant’s clinical condition, pathology results and outcomes. The participant’s radiology will be anonymised and transferred to Oxford as part of the analysis. Participants will complete questionnaires, supported by members of the research team when necessary. Pleural fluid samples will be collected and sent to Oxford for analysis. All data will be identified by a unique patient identifier.

**Follow up (post discharge)**

Follow up visits will be undertaken alongside normal clinical care. This is commonly:

- Within the first 2 (</+ 2 week) weeks post discharge, (face to face recommended)
- At approximately 2 months (+/- 2 weeks) (face to face optional)
- Optional 6 months (</+ 2 week)

Specific to this trial, a follow up point at 2 weeks is suggested post discharge / intervention to assess response to ongoing antibiotic therapy. The responsible clinician is permitted to stop antibiotic therapy at the two week follow up point if adequate response (regardless of assigned treatment group), with a general recommendation for 4 to 6 weeks of antibiotic treatment. If the participant is deemed to be progressing well and would clinically not require any further face-to-face follow up, in light of the COVID-19 pandemic, it would be reasonable to conduct further follow ups (i.e., at 2 months and 6 months) remotely.

### In-depth Participant Interviews

Qualitative interviews will be performed on a proportion of participants after the participant has recovered from their acute illness regarding their priorities of care. These interviews will either be performed by trained members of the ORTU team or by Oxford Brookes University. In addition, a proportion of those participants, who refused randomisation but consented to be interviewed, will also be approached to take part, and any themes arising from these two groups will be incorporated into the design of the subsequent randomised controlled trial. A proportion of carers from Oxford participants randomised or refused randomisation but consented to interview will also be approached. All interviews will be performed by a trained member of staff based at the Oxford Respiratory Trials Unit/Oxford Brookes University, the interviews will be performed either face to face, over the phone or via Skype. The interviews will be audio recorded and these recordings will be stored electronically on the ORTU network drive. Interviews performed by Oxford Brookes University will be transferred to ORTU via Oxfile. Audio files will be sent securely to a professional transcription company, with whom the University has a contracts and confidentiality agreements. The transcriptions will be anonymised, and the transcriptionist will delete the recording when they have completed their work and returned the transcript.

## 6. PARTICIPANT IDENTIFICATION

### 7.1 Trial Participants

All participants with pleural infection fulfilling the inclusion / exclusion criteria are eligible for the trial. Screening logs will be kept, documenting reasons for non-inclusions.

### 7.2 Inclusion Criteria

1) A clinical presentation compatible with pleural infection
2) A pleural collection with a chest drain in situ
3) Has pleural fluid requiring drainage which is either:
    - purulent **or**
    - gram stain positive **or**
    - culture positive **or**
    - acidic with a pH <7.2 **or**
    - low pleural fluid glucose (<2mmol / L) in the absence of accurate pH measurement **or**
    - septated pleural fluid on ultrasound which is likely secondary to pleural infection (based on local investigator view).
4) Residual collection or ongoing sepsis after 24 hours of standard care
5) Willing and able to give written informed consent

### 7.3 Exclusion Criteria

- Age <18 years
- Pleural collection not amenable to chest tube drainage
- Chest tube already in place for >= 72 hours
- Has previously received intra-pleural fibrinolytics and /or DNase for this empyema
- Has a known sensitivity to DNase or tissue plasminogen activator
- Has had a previous pneumonectomy on the side of infection
- Participants who are pregnant or lactating
- Estimated survival less than three months from a different pathology to this empyema, (e.g., metastatic lung carcinoma)

## 7. TRIAL PROCEDURES

### 8.1 Recruitment

Participants with either confirmed or suspected pleural infection will be identified by any member of the clinical team. Due to the nature of the trial, the participants will all be under inpatient care at the time and can be offered participation early in their admission. The clinical team will approach participants and either the clinical or research team will then provide the participant with the participant information sheet and be available to answer any questions. Participants will be identified through respiratory and general wards or from outpatient referrals, clinics, and ambulatory care.

### 8.2 Screening and Eligibility Assessment

There is no maximum duration between screening and randomisation but due to the nature of the disease, treatment must not be delayed, so it is likely that participants will have less than 24 hours to consider enrolment. Day 0 should be considered as being **first contact** with the PI team (</=3 days from first signs of pleural infection), and a decision to randomise needs to be made by the end of Day 1. If the participant remains eligible and the drain stays in, then randomisation is possible. The pleural fluid samples which are necessary to confirm eligibility are taken as part of clinical care and are not trial specific and thus do not require prior consent. If a participant is consented prior to pleural fluid samples being obtained these samples will be transferred to the central site for storage and analysis as per the consent form.

### 8.3 Informed Consent

Consent can be obtained **prior** to confirmation of pleural infection in participants who are likely to have a pleural aspiration and chest drain insertion in the same

procedure. These participants will be randomised once the eligibility criteria have been confirmed. If pleural infection is not confirmed the participants will not need to participate further in the trial.
The participant must personally sign and date the latest approved version of the Informed Consent form before any trial specific procedures are performed.

Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants detailing no less than: the exact nature of the trial; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is under no obligation to take part in the study and is free to withdraw from the trial at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal.

Although it is usually a requirement in clinical studies that a participant is offered 24 hours in which to decide whether to take part in a study, the nature of the disease process in question (pleural infection) and the intervention (intrapleural agents which improve drainage of infected material) suggest that delay of more than a few hours in administering the medication may be detrimental to participant care. On this basis, a shortened period of reflection will be offered to participants considering participation in the study, although no form of coercion or pressure will be used. This strategy has proved robust in previous clinical studies of pleural infection (MIST1 and MIST2) and will be specifically addressed in the ethics application.

Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Principal Investigator and have been delegated this responsibility. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained at the trial site with a copy emailed to ORTU to a trial specific inbox.

### 8.4 Randomisation, blinding, and codebreaking

Randomisation will occur via a web-based system with minimisation for centre and a validated score of risk in pleural infection (the RAPID score, scored in 3 categories = low, moderate, and high).
Randomisation will occur once pleural infection has been confirmed by the documented inclusion criteria. This may occur after the initial aspiration or once a chest drain has been inserted.
Participants will be randomised 1:1:1 to standard care, IET or early VATS surgery. All participants will require chest tube insertion; therefore, randomisation can occur after tube insertion (up to 24 hours post insertion).

The trial will not be blinded so no un-blinding procedures are required.

## 8. BASELINE ASSESSMENTS

***Some of the study assessments and visits have been made optional to streamline the trial pathway, following the slow recruitment due to COVID-19. This will reduce the data collection burden on sites and focus on the essential data required to meet the study outcomes. The maximum follow up time has been shortened to 2 months to facilitate a 4 month recruitment extension (Apr – Jul 2021) due to COVID-19. The 6 month follow up visit is now optional. Those participants randomised after 1st June 2021 will only be required to have a 2 month follow up visit in keeping with the trial timelines.**

### 8.1 Baseline data collected will include:

1) Participant demographics including co-morbidities (at enrolment)
2) Recent blood test results as part of usual clinical care including RAPID parameters where available (within 1 week) (see trial specific instructions)
3) Recent radiology results (within 1 week)
4) Details of the symptoms the participant has had for the current pleural infection (at enrolment)
5) Details of the treatment the participant has had for the current pleural infection (at enrolment)
6) Previous spirometry if available (within 12 months) (optional)
7) Details of any previous intrapleural treatment or thoracic surgery
8) Ultrasound findings (one image at enrolment)
9) Vital signs (first set of observations recorded in hospital including blood pressure, heart rate, temperature, respiratory rate, and oxygen requirement)
10) Patient weight (in kilograms)

### 9.2 Initial intervention

All participants will have 20mls of blood and 20mls of pleural fluid taken for standard care, and 20mls of blood and 20mls of pleural fluid to be sent to the coordinating centre for storage future use with the participants consent (i.e., total of 40mls blood and 40mls pleural fluid) *. These samples should be taken on the day of enrolment (+24 hours if needed).

***As a result of the COVID-19 pandemic, laboratory processing of research samples has been suspended. Therefore, no research samples for future storage are being collected currently. Once restrictions are lifted these will be**

**reinstated. The period of suspension will be documented within the trial master file.**

Data should be collected on:

a) Fluid purulence
b) Biochemical results (including LDH, pH and glucose)
c) Chest drain size (if required)
d) Pleural fluid microbiology results (gram stain and culture) (once only)
e) Blood culture results (if available) (once only)

### 9.3 The initial inpatient period

1) Data should be collected on:
2) Cumulative volume of pleural fluid drainage
3) Blood results including renal function and inflammatory markers – see below for frequency
4) Antibiotic treatment
5) Duration of drainage
6) Any chest tube displacement or blockage
7) Details of trial procedure – e.g., whether all intrapleural treatment was completed, any missed doses, date and type of surgery, reason surgical intervention was not undertaken, time from randomisation until surgery.
8) Details of subsequent pleural interventions
9) Requirement for surgery due to treatment failure on objective criteria
10) Adverse events (for surgery using the modified Clavien-Dando classification (Appendix A3.6), and all others on standard criteria)
11) Pain score (100mm VAS) every day until chest drain removal and at discharge (optional).
12) IPAQ-S7S and EQ-5D-5L questionnaires*
13) Hospital Anxiety and Depression Scale (HADS) score (once only - within 72 hours of admission) *

***These questionnaires can be completed remotely (over the phone) to minimise patient contact in light of the COVID-19 pandemic**

Clinical assessments will be conducted by a member of the clinical or research team.

The ultrasound image can either be a baseline image (prior to drain insertion) or randomisation image (showing residual collection following initial period of drainage prior to randomisation). This should be captured and uploaded onto the image CRF.

Chest x-rays may be performed at varying points throughout admission to guide clinical care. For the purposes of the study, as a minimum, 2 chest x-rays are required – the admission chest x-ray (day 0) and the last chest x-ray prior to discharge (appropriately labelled 'day X' when labelled onto image CRF).

Blood tests including inflammatory markers are to be taken as part of routine clinical care, and therefore are not specifically required for the trial if not clinically indicated. These tests will then be repeated at outpatient follow up appointments as detailed in the trial flow chart (see Appendix A).

VAS booklets will be completed once a day by the participant (optional).

The data collection should last until drain removal or day 7 if chest drain still in situ.

### 9.4 Discharge

Length of initial hospital stay from diagnosis to discharge including any social care through patients' Electronic Patient Records (EPR), and information should also be collected on specialty wards, diagnoses, and procedure codes.

At discharge, data will be collected on treatment received and completed, death as well as whether or not any serious adverse events occurred, related to pleural infection. Spirometry and pain score (100mm VAS) should be performed at the time of discharge*. If spirometry is not performed for any reason, this should be recorded on the discharge CRF.

Participants will ideally be provided with the Home VAS booklet questionnaire at discharge*. As an alternative, this can be posted out to the participant following discharge.

*Inpatient VAS, Home VAS and spirometry are optional but encouraged where possible

### 9.5 Qualitative Interviews

Participants (all approached during the study who agree to be randomised or agree to be interviewed) and their carers (Oxford only - with consent) will be approached for participation in qualitative interviews regarding their experiences during the trial or their reasons for refusing randomisation. This will aim to establish priorities of care and therefore important outcomes in the planned multicentre randomised controlled trial. It is anticipated that the interviews will not take place until the participant is discharged and appropriately recovered (i.e., at one of the early out-patient reviews). The interviews will be undertaken by members of the research team trained in qualitative methodology.

### 9.6 Follow up Visits post randomisation

Follow up will occur at 2 weeks, 2 months, and then at 6 months. +/- 2 weeks for all visits.

Data collected will be:

1. Height to be measured at 2 week follow up*(optional)
2. Weight (at each visit) *(optional)
3. Spirometry (FEV1 and FVC) at 2 weeks and 6 month follow up*∞(optional)
4. Duration of antibiotic therapy in total since discharge from hospital
5. Further hospital admission(s)
6. Date of death (if applicable)
7. Details if participant suffered side effects possibly attributable to the trial intervention since initial hospital discharge
8. Further interventions needed, including further surgery
9. Evidence of malignancy
10. Exercise ability (via the IPAQ-S7S questionnaire) .
11. Specific questions suggested by the MIST3 participant group, including:
    a. Do you feel back to normal?
    b. Time to return to normal work / function at home
12. Generic health-related quality of life (QoL) as measured using the Euroqol 5 Dimensions 5 Levels (EQ-5D-5L) questionnaire.
13. Information on subsequent hospitalisations (including ward transfers, and diagnoses and procedure codes will be obtained from participants' EPR records. Information on out-patient, A&E and primary care contacts will be obtained from participant questionnaires administered at each follow-up).
14. Chest x-ray will be performed at all visits as part of standard care*

Participants will be asked to complete a Home VAS booklet once a week post discharge until their 2 month follow up appointment (optional).

It is preferable if follow ups to 2 months occur face to face, to allow assessments such as chest x-ray and ultrasound. If this is not possible an attempt will be made to contact the participant to complete the follow up CRF by telephone. A 6 month telephone follow up is optional.

*These will not be expected if the follow up appointment was conducted remotely. For all other data items, these should be obtainable remotely.

∞ Spirometry may not be available due to the COVID-19 pandemic. If it is not possible to be performed this should be recorded in the CRF.

### 9.7 Sample Handling

Samples for routine clinical care will be conducted as per local hospital practice.

The additional 20mls of blood and pleural fluid will be put into transport tubes and sent to the coordinating centre (as per a trial specific procedure) and process / stored as per established Oxford Respiratory Trials Unit Standard Operating Procedures and will be stored for future research separate to this protocol with the consent of the participant.

**As a result of the COVID-19 pandemic, laboratory processing of research samples has been suspended. Therefore, no research samples for future storage are being collected currently. Once restrictions are lifted these will be reinstated. The period of suspension will be documented within the trial master file.**

#### 9.8 Discontinuation/Withdrawal of Participants from Trial Treatment

During the study a participant may choose to withdraw early from the study treatment at any time. This may happen for several reasons, including but not limited to:

- The occurrence of what the participant perceives as an intolerable AE.
- Inability to comply with study procedures
- Participant decision

Participants may choose to stop treatment and/or study assessments but may remain on study follow-up.

Participants may also withdraw their consent, meaning that they wish to withdraw from the study completely.

Participants have the right to withdraw from the trial at any time without having to give a reason and this will not affect their future care.

a) Withdrawal of a participant from the trial should be under the guidance of the principal investigator (in liaison with the ORTU team as appropriate). Withdrawal details will be recorded on the relevant CRF.

b) Participants are only withdrawn if they specifically request no further data collection. In the event of participants not wishing to attend visits, or to discontinue treatment, they are not considered withdrawn, but this will be recorded as a file note/protocol deviation. Should a participant decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible.

c) For participants moving from the area, every effort should be made for the participant to be followed up at another centre, or for follow up via GP.

d) Participants have a right to request the destruction of samples upon request.

### 9.9 Definition of End of Trial

Trial closure will either be when the last medical note review is performed at 12 months or at the direction of the Trial Steering Committee (TSC).

## 10. TRIAL INTERVENTION

### 10.1 Treatment Description

Recombinant human deoxyribonuclease is a sterile solution already licensed for use in nebulised form for the reduction of sputum viscosity in participants with cystic fibrosis. The standard dose is 2.5 to 5mg once or twice daily. It is well tolerated; rash, voice alteration, chest pain and laryngitis are the main reported side effects when administered as in inhaled solution. In animal studies it appears to be well tolerated in inhalation doses 180-fold higher than routinely used doses. It requires storage at 2-8$^{O}$C.

Recombinant human tissue plasminogen activator (tPA, Alteplase) is already licensed for use in myocardial infarction. The standard dose is <100mg. With this use, its main side effects are the risk of systemic bleeding associated with systemic fibrinolytics. With intra-pleural use, such adverse events are not reported and another fibrinolytic (Streptokinase), with a similar adverse event profile when used systemically, does not cause an excess of bleeding when used in the pleural space.

Use of combination tPA + DNase in the MIST2 study was not associated with an increased incidence of serious adverse events compared to either placebo or individual DNase or tPA. Bleeding events were captured as serious adverse events for the purposes of the MIST2 study, and no excess of bleeding events was seen compared to placebo in any group.
The solutions will be made up and administered by clinical staff as per local protocols.

### 10.2 Storage of Trial Treatment

Trial medication for this trial will be from the usual clinical supplies used in hospitals taking part in this trial (the MIST2 regime is used as standard care in selected patients in all the recruiting centres). Each course of trial treatments will be pre-prepared and dispensed to the ward as per local guidelines and the normal use of these medications.

### 10.3 Compliance with Trial Treatment

All the trial treatments will be administered whilst the participant is in hospital so it will be possible to accurately document participant compliance. If there are compliance issues the reasons for these will be collected as part of the feasibility assessment.

### 10.4 Accountability of the Trial Treatment

Trial drugs used will be those available via the NHS system (manufactured by Roche UK and Boehringer Ingelheim UK) and thus trial pack preparation is not required. Compliance will be recorded on the CRFs (number of completed doses) with no need for drug vial accountability.

### 10.5 Concomitant Medication

Participants may not receive any intra-pleural therapy other than the trial drugs and simple saline flushes to maintain chest tube patency (if required – this does not include irrigation with large volumes of saline (>120mls per day) which is not permitted in this study). Specifically, intra-pleural antibiotic therapy, or fibrinolytic or DNase therapy other than the trial drugs may not be given. Participants may not receive intra-pleural fibrinolytic therapy other than the trial medications without discussion with the chief investigator or deputy. It will be recorded whether the participant was anti-coagulated with therapeutic doses of warfarin or heparin (or its derivatives) or received any systemic fibrinolytic therapy on the report forms.

### 10.6 Post-trial Treatment

The trial treatment will not be continued outside the trial, with a maximum of 3 days' worth of dosing in all cases.

### 10.7 Other Interventions

There are no other specific interventions expected in this trial, other than surgical treatments which are specified in the surgical SOP.

## 11. SAFETY REPORTING

### 11.1 Definitions

| | |
|---|---|
| Adverse Event (AE) | Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product. |
| Adverse Reaction (AR) | An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant.<br>The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. |

| | |
|---|---|
| | All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions. |
| Serious Adverse Event (SAE) | A serious adverse event is any untoward medical occurrence that:<br>• results in death<br>• is life-threatening<br>• requires inpatient hospitalisation or prolongation of existing hospitalisation<br>• results in persistent or significant disability/incapacity<br>• consists of a congenital anomaly or birth defect*.<br><br>Other 'important medical events' may also be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.<br><br>NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.<br><br>*NOTE: Pregnancy is not, in itself, an SAE. In the event that a participant or his/her partner becomes pregnant whilst taking part in a clinical trial or during a stage where the foetus could have been exposed to the medicinal product (in the case of the active substance or one of its metabolites having a long half-life) the pregnancy should be followed up by the investigator until delivery for congenital abnormality or birth defect, at which point it would fall within the definition of "serious". |
| Serious Adverse Reaction (SAR) | An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided. |
| Suspected Unexpected Serious Adverse Reaction (SUSAR) | A serious adverse reaction, the nature and severity of which is not consistent with the Reference Safety Information<br>for<br>the medicinal product in question set out:<br>• in the case of a product with a marketing authorisation, in the approved summary of product characteristics (SmPC) for that product<br>• in the case of any other investigational medicinal product, in the approved investigator's brochure (IB) relating to the trial in question. |

NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above.

Any pregnancy occurring during the clinical trial and the outcome of the pregnancy should be recorded and followed up for congenital abnormality or birth defect, at which point it would fall within the definition of "serious".

### 11.2 Assessment results outside of normal parameters as AEs and SAEs

As pleural infection patients are generally unwell, no specific blood parameters will be considered to constitute an AE or SAE, with the exception of deranged clotting which in the judgement of the investigator is due to IET therapy and of sufficient abnormality to justify reporting. If any subset of coagulation profile more than doubles after IET treatment, the trial fellow will review.

### 11.3 Causality

The relationship of each adverse event to the trial medication must be determined by a medically qualified individual according to the following definitions:

**Unrelated** – Where an event is not considered to be related to the IMP / intervention

**Possibly Related** – although a relationship to the IMP / intervention cannot be completely ruled out, the nature of the event, the underlying disease, concomitant medication, or temporal relationship make other explanations possible.

**Probably Related** – the temporal relationship and absence of a more likely explanation suggest the event could be related to the IMP / intervention

**Definitely Related** – the known effects of the IMP, its therapeutic class or based on challenge testing suggests that the IMP / intervention is the most likely cause.

All SAEs labelled possibly, probably, or definitely related will be considered as related to the IMP.

### 11.4 Procedures for Recording Adverse Events

All AEs occurring during the initial trial period (to 7 days post treatment (whilst an in-participant)) will be recorded to ensure all data on adverse outcomes from the IET or surgery or standard care are captured. Known and well recognised complications of pleural infection, surgery or IET therapy will be recorded as part of the CRFs for the study, but (even if serious) are not subject to SAE reporting timelines if a known and documented complication of therapy (see section 11.5.1).

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to trial medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.

The severity of events will be assessed as one of the following: mild, moderate, or severe.

AEs considered related to the trial medication as judged by a medically qualified investigator or the Sponsor will be followed either until resolution, or the event is considered stable.

It will be left to the Investigator's clinical judgment to decide whether or not an AE is of sufficient severity to require the participant's removal from treatment. A participant may also voluntarily discontinue from treatment due to what he or she perceives as an intolerable AE. Normal follow up within the trial will continue.

## 11.5 Reporting Procedures for Serious Adverse Events

The safety reporting period is for 7 days post treatment (whilst an in-participant) (or 7 days post initial intervention for the pleural infection if surgical treatment is delayed). Serious adverse events which are not in the foreseeable natural history of complications of pleural infection or treatment for this condition (which includes all the complications listed above) are reportable in the first to 7 days post treatment (whilst an in-participant) (or 7 days post initial intervention for the pleural infection if surgical treatment is delayed).

All serious adverse events are recorded on the CRFs as part of the study in the first to 7 days post treatment (whilst an in-participant) (or 7 days post initial intervention for the pleural infection if surgical treatment is delayed).

Thereafter, only those serious adverse events which are considered directly attributable (related) to the treatment for pleural infection (not including any of the mentioned foreseeable complications) according to local Investigator opinion will be recorded over the further 6 month follow up period. There will be no adverse event reporting beyond this, but outcomes collected on CRF's.

### *11.5.1 Events exempt from immediate reporting as SAEs*

Specific SAEs which do not require immediate reporting in this trial are those associated with the natural history of pleural infection or treatment for this condition.

Foreseeable complications of pleural infection are mortality (approximately 20% at 6 months), respiratory failure, admission to intensive care, complications of antibiotic therapy, worsening sepsis, requirement for emergency or other surgery, deep vein thrombosis and death due to progressive infection, as well as readmission with infection within a month. If these known complications occur and are judged to be due to sepsis or as a direct result of infection, this does not need to be immediately reported but will be recorded on the CRFs.

In addition, each treatment arm has foreseeable complications (as listed here) and do not require expedited reporting:

a) For the standard care arm, these are related to the chest tube insertion procedure and include:

*Bleeding, wound site infection, pain, major organ perforation, bronchopleural fistula.*

b) For the IET arm:

*Intrapleural bleeding, allergic reaction, systemic bleeding, and pain.*

c) Surgical arm:

There is list of well-established surgical complications which will form part of the surgical SOP. These include complications during the procedure requiring conversion of the 'keyhole' surgery into an open surgical procedure, such as *uncontrollable bleeding and failure of the lung to fully re-expand*. Post-operative complications include *pain, wound infection, prolonged air leak, repeat operation, blood transfusion, respiratory failure, and the need for a tracheostomy.*

Similarly, further interventions for pleural infection at any stage (including the need for surgery, or further surgical or pleural intervention) will be recorded on the CRFs and not as an immediately reported SAE.

### *11.5.2 Procedure for immediate reporting of Serious Adverse Events*

All SAEs (other than those defined in the protocol as not requiring reporting) must be reported on the ORTU SAE reporting form to ORTU as soon as possible of the Site Study Team becoming aware of the event. ORTU will perform an initial check of the report, request any additional information, and ensure it is reviewed by a nominated Medical Reviewer (including Expectedness Assessment). It will also be reviewed at the next Trial Safety Oversight Group meeting. All SAE information must be recorded on an SAE form and scanned and emailed, to ORTU respiratorytrialsunit@ouh.nhs.uk Additional and further requested information (follow-up or corrections to the original case) will be detailed on a new SAE Report Form and scanned/emailed to ORTU.

## 11.6 Expectedness

Expectedness for the IET arm is determined according to the Summary of Product Characteristics.

Expectedness for the surgical arm is determined according to the surgical SOP.

Expectedness for the standard care arm is determined according to the following list of expected events: *Bleeding, wound site infection, pain, major organ perforation, bronchopleural fistula.*

### Related and Unexpected SAE

In the event of an SAE (defined as reportable in this protocol) that is assessed as being 'related' to a trial intervention and 'unexpected' will be reported to the REC that gave a favourable opinion of the study.

Reports of related and unexpected SAEs should be submitted within 15 working days of ORTU becoming aware of the event, using the HRA report of serious adverse event form (see HRA website).

## 12. STATISTICS

### Statistical Analysis Plan (SAP)

The outline of the statistical analysis is included here. A separate Statistical Analysis Plan will not be drafted for this study. All statistical analysis will be conducted by the Centre for Statistics in Medicine, University of Oxford. All results will be reported according to the CONSORT 2010 statement: extension to randomised pilot and feasibility trials (Eldridge SM et al, BMJ 2016;355:i5239)

### Description of Statistical Methods

The feasibility outcomes (recruitment rate, acceptability of randomisation, retention rate) will be reported as proportions together with 95% confidence intervals. These will be used to assess whether a definitive trial is feasible. Descriptive statistics will be used to describe the demographics between the groups. For categorical variables, the number (and percentage) will be reported for each treatment group and overall. For continuous variables, means and standard deviation (or medians in interquartile range) will be reported for each treatment group and overall. Comparisons between treatment arms for the clinical outcomes will be reported using descriptive statistics only as this feasibility trial is not powered for definitive conclusions to be drawn. No statistical tests will be undertaken. These will be based on multivariable linear (for continuous outcomes), or logistic (for binary outcomes) regression adjusted for stratification factors and important prognostic factors and will be reported as an adjusted difference in means (for continuous outcomes) or in proportions (for binary outcomes). Treatment comparisons will be reported for the intention-to-treat population (all randomised participants will be analysed according to their allocated treatment group irrespective of which treatment they receive) as treatment effects together with 95% confidence intervals for the two main comparisons: (1) VATS vs IET; (2) VATS vs Standard Care.

Compliance to the interventions will be reported.

To establish the feasibility of collecting accurate long-term outcomes in randomised participants, we will present the completeness of the outcomes across the duration

of the trial. The outcome measures collected in this trial will be used to inform the sample size for the future definitive phase III RCT, if it is feasible to be undertaken.

Adverse events and serious adverse events will be reported by treatment arm on the safety population only (all patients who received the allocated treatment).

It is anticipated that STATA (StataCorp LP) or other appropriate validated statistical software will be used for analysis.

Interviews will be digitally audio-recorded, transcribed verbatim, and anonymised before being uploaded to NVivo data management software. The interview data will be analysed using Thematic Analysis. Audio-recordings will be listened to, and transcripts read and re-read for familiarisation, then open-coded to develop an initial code list. Codes will then be grouped into categories, and data explored to identify connections and to develop a descriptive account of the dataset. The analysis will focus on the acceptability of trial processes to patients, individual and group equipoise, and the patient experience of pleural infection and treatment.

**Sample Size Determination**

As a feasibility trial, no formal sample size calculations were performed or possible. However, the primary purpose of this study is to assess if recruitment to a larger, definitive trial is feasible, and the recruitment target of 75 randomised participants in a number of UK centres over 18 months has been chosen based on this aspect, and recent current recruitment to our observational study in pleural infection (PILOT which recruited 20 participants per month in 20 centres). Extrapolating this data to be obtained from this study, if 75 participants can be randomised in 18 months from 5 centres to this surgical trial, a future phase III study will be able to recruit 480 suitable participants from 20 centres over 2 years.

This number of participants is sufficient for a definitive two-arm trial comparing IET and VATS, whose primary outcome is hospital stay, in which our current data suggests a total sample size of 432 participants are required randomised 1:1 (rationale: using information encompassing a clinically meaningful difference in hospital stay of more than 3 days (mean hospital stay in IET arm = 11.8 days,(3), mean hospital stay in VATS arm = 8.5 days,(21, 22), assumed both arms has the same SD of 10 days, 90% power, 5% significance level), randomising 1:1 between IET and VATS requires 194 participants per arm, totalling 388 participants. Allowing a 10% attrition rate, the estimated total sample size for the larger definitive trial is 432 (216 in each arm) participants randomised).

Thus, demonstration of successful randomisation of 75 participants over 18 months of recruitment from a number of centres would demonstrate that a phase III trial of this size, in this population and with similar randomised groups, is feasible. The primary outcome(s) of a future phase III trial will be informed by work conducted in

this feasibility trial.

All participants who consent to interviews but not to randomisation into the study will be included in the analyses of the relevant qualitative outcomes.

Crossovers from the standard care arm to the VATS arm or to the IET arm will be permitted once treatment on the standard care arm has been deemed to have failed after a further 48 hours (as is current BTS guideline standard practice). Crossovers will be recorded, and a per-protocol analysis will be conducted.

### 12.4 Analysis Populations

The study will be analysed on intention to treat, with included populations as specified above.

### 12.5 Decision Points

No interim analysis will be conducted. The Trial Steering Committee will review the recruitment rate regularly throughout the trial.

### 12.6 Stopping Rules

No formal stopping rules are planned.

### 12.7 The Level of Statistical Significance

Not applicable

### 12.8 Procedure for Accounting for Missing, Unused, and Spurious Data

Missing data will be reported for the key feasibility and clinical outcomes, but no adjustment will be undertaken.

### 12.9 Procedures for Reporting any Deviation(s) from the Original Statistical Plan

Any changes/deviations from the statistical analysis outlined here will be described and justified in the final statistical report.

### 12.10 Health Economics Analysis

Initial Health Economic Analysis will be undertaken, to inform a potential larger trial, and will be the subject of a specific Health Economic Analysis plan to be written during trial recruitment, using the parameters collected.

### 12.11 Criteria for the Termination of the Trial

No specific premature closure / ‘stopping rules’ are defined for the TSC. However, it is anticipated that the TSC will only advocate trial closure where there is proof

beyond reasonable doubt that one treatment arm is clearly superior to the other such that continuation in the trial would result in significant participant disadvantage.

### 12.12 Cost-effectiveness analysis

This is a feasibility trial, the main aim will be central monitoring to assess whether we can obtain the resource use, cost, and main outcome data. As part of the central monitoring procedures by ORTU, where appropriate, queries necessary to perform a cost-effectiveness analysis in a full definitive trial will be recorded.

We will assess, the response rates to the EQ-5D-5L and resource use questionnaires administered to patients and evaluate patterns of missing data.

Reason for missing data, a pilot of the micro-costing study used to evaluate the costs of trial surgical intervention and assessments of whether we obtain reliable costs for participants undergoing surgery will be recorded.

In addition, we will assess if we can obtain all the relevant information required to generate costs of hospitalisation from participants' EPR records, including dates of hospitalisation, dates of ward transfers, and diagnoses and procedure codes. In addition, we will assess if we can obtain all the relevant information required to generate costs of hospitalisation from participants' EPR records, including dates of hospitalisation, dates of ward transfers, and diagnoses and procedure codes.

Crossovers from the standard care arm or the VATS arm to the IET arm will be permitted and recorded.

## 13. DATA MANAGEMENT

### 13.1 Source Data

Source documents are where data are first recorded. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be obtained), clinical and office charts, laboratory and pharmacy records, and medical imaging.

Data required for the conduct and analysis of this trial will be collected on Case Report Forms (CRFs). This may be transcribed or summarised from source documents or may be collected directly in trial CRFs. CRF entries will be considered source data if the CRF is the site of the original recording (e.g., there is no previous written or electronic record of data).

### 13.2 Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits, and inspections.

### 13.3 Data Recording and Record Keeping

Data will be entered into a secure, validated, GCP-compliant electronic data management system. All staff performing data entry will be appropriately trained prior to access being granted. Access is controlled by individual user accounts, and a full audit trail is kept of all modifications made to data.

Standard Operating Procedures (SOPs) will be followed to maximise completeness and accuracy of trial data. The processes for quality assurance of study data will be detailed in the study monitoring plan, data management plan, and other associated documents.

Participants will only be identified in all trial documents and datasets (other than the signed consent form) by a unique trial-specific number or code. The name and any other identifying detail will NOT be included in any trial data electronic file.

All trial documents will be stored securely. Both paper and electronic trial data will be retained through an archiving service for a period as described in the Data Management Plan.

## 14. QUALITY ASSURANCE PROCEDURES

### 14.1 Risk assessment

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures. A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities.

### 14.2 Monitoring

Regular monitoring will be performed according to the trial specific Monitoring Plan. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents as these are defined in the trial specific Monitoring Plan. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted, and data are generated, documented, and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

## 14.3 TRIAL COMMITTEES

### 14.3.1 Trial Management Group

Trial Management Group (TMG) will meet regularly throughout the trial to discuss the day-to-day management of the trial, a TMG charter will be written detailing all the requirements.

*Members of the TMG:*

CI
Research Fellow
Trial Manager
Data Manager
Clinical Trials Assistant

### 14.3.2 Trial Steering Committee

The Trial Steering Committee (TSC) will meet on a 6 monthly basis throughout the trial to assess the progress of the trial. A TSC charter will be written detailing the requirements of this committee and its members.

*Members of the TSC:*

Independent Chair
CI
Independent Member
Non-Independent Member
Independent Member
Research Fellow
Trial manager
Data Manager
PPI Rep

### 14.3.3 Safety Monitoring Committee

The Oxford Respiratory Trials Unit (ORTU) will conduct a review of all SAEs for the trial reported during the reporting period and cumulatively. The aims of this committee include:

- To pick up any trends, such as increases in un/expected events, and take appropriate action
- To seek additional advice or information from investigators where required
- To evaluate the risk of the trial continuing and take appropriate action where necessary

## PROTOCOL DEVIATIONS

A trial related deviation is a departure from the ethically approved trial protocol or other trial document or process (e.g., consent process or IMP administration) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and filed in the trial master file.

The Oxford Respiratory Trials Unit has Standard Operating Procedures for deviations and breaches which will be used throughout.

## ETHICAL AND REGULATORY CONSIDERATIONS

### 16.1 Declaration of Helsinki

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

### 16.2 Guidelines for Good Clinical Practice

The Investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice.

### 16.3 Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), HRA (where required), and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

### 16.4 Other Ethical Considerations

Eligible participants will be given detailed information and the opportunity to discuss the trial further with a member of the trial team. Participants are generally given 24 hours ‘thinking time’ thereafter to consider enrolling in a trial. It is recognised that clinical circumstances in this trial are likely to make this impossible. The participants will be asked to consent to trial entry, the collection of information about their care, and collection of subsequent data sheets. All will be appropriately anonymised.

The safety profile of the intra-pleural medications appear reasonable from the previous study, however, are not fully defined and this is an outcome of the trial. This risk will be covered by specific consent.

### 16.5 Reporting

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the REC, HRA (where required), host organisation and Sponsor. In addition, an End of Trial notification and final report will be submitted to the same parties.

### 16.6 Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number on all trial documents and any electronic database, with the exception of the CRF, where participant initials may be added. All documents will be stored securely and only accessible by trial staff and authorised personnel. The trial will comply with the General Data Protection Regulation (UK GDPR) and Data Protection Act 2018.

For further information on how UK GDPR and associated data protection legislation impacts on research please, University of Oxford researchers see https://researchsupport.admin.ox.ac.uk/policy/data/checklist and https://researchsupport.admin.ox.ac.uk/policy/data/practical and OUH researchers see

https://www.ouh.nhs.uk/privacy/default.aspx

Participants consenting to be interviewed, will have their details sent to Oxford from nhs.net email accounts at sites to the trial specific nhs.net email account. Oxford Brookes University staff will also have access to trial specific inbox to obtain these details, but research passports will be in place.

### 16.7 Expenses and Benefits

Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, or a mileage allowance provided as appropriate.

## FINANCE AND INSURANCE

### 17.1 Funding

Funding is provided in full by a NIHR Research for Patient Benefit Grant.

### 17.2 Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the

research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment that is provided.

## PUBLICATION POLICY

The preparation of a manuscript for rapid publication will be the sole responsibility of the trial's Chief Investigator. High priority will be given to this. Any detailed reports of the study prepared by Boehringer or Roche for internal use and for submission to regulatory authorities will be submitted to the Steering Committee for review within an appropriate period of time, prior to their dissemination and will not be submitted without approval from TSC.

The primary report is planned to be with all co-investigators and recruiters named in the author list, but subject to specific journals which limit the number of authors, this may be in the name of the "MIST3 investigators group" with the trial fellow(s) as specified by the CI, and Chief Investigator named, and all other contributors listed with their roles in the acknowledgment section.

## DEVELOPMENT OF A NEW PRODUCT/ PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

No specific IP is expected in this trial.

## ARCHIVING

All trial documentation will be archived at Restore Datacare, ORTU's archiving facility.

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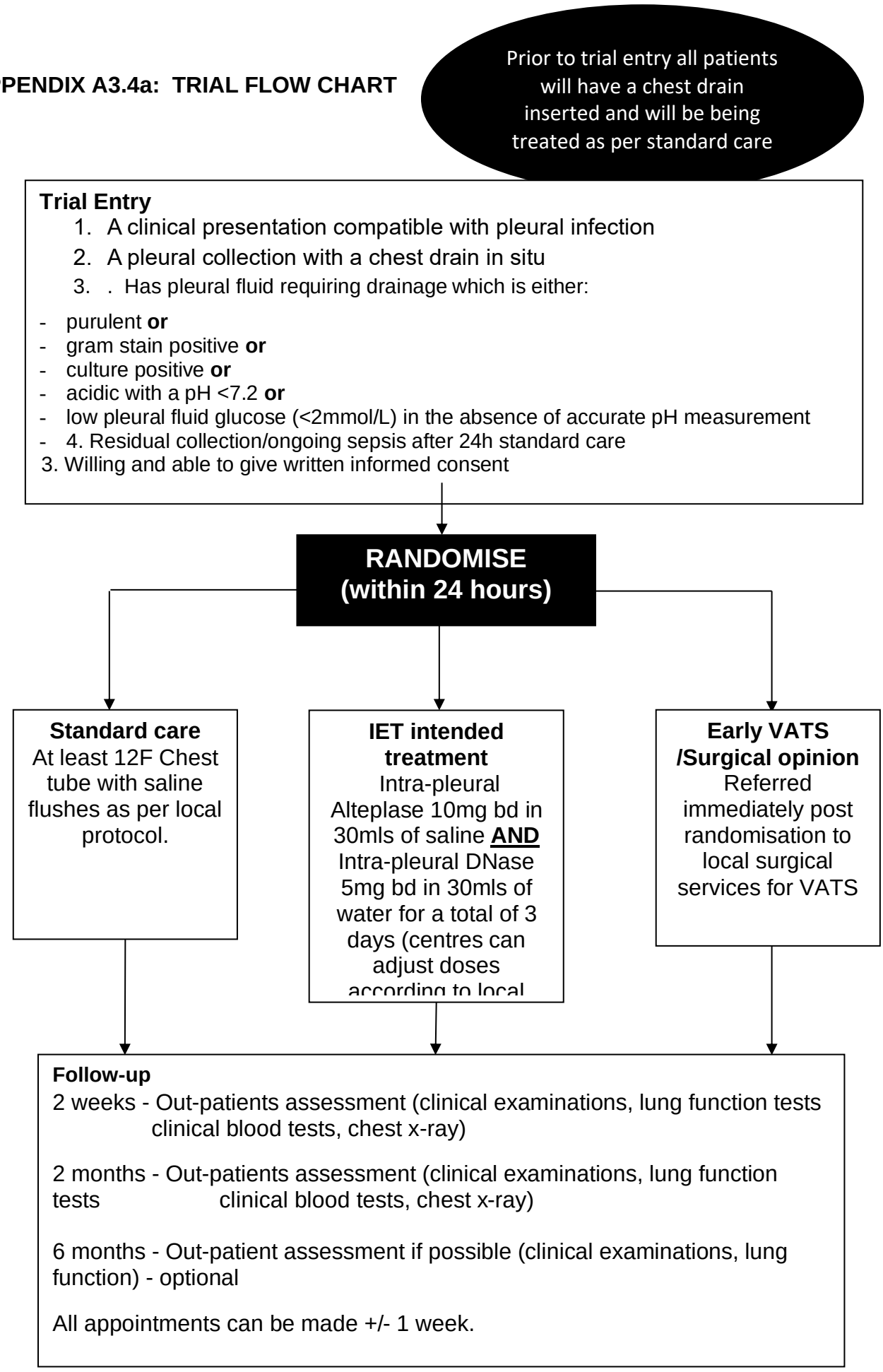
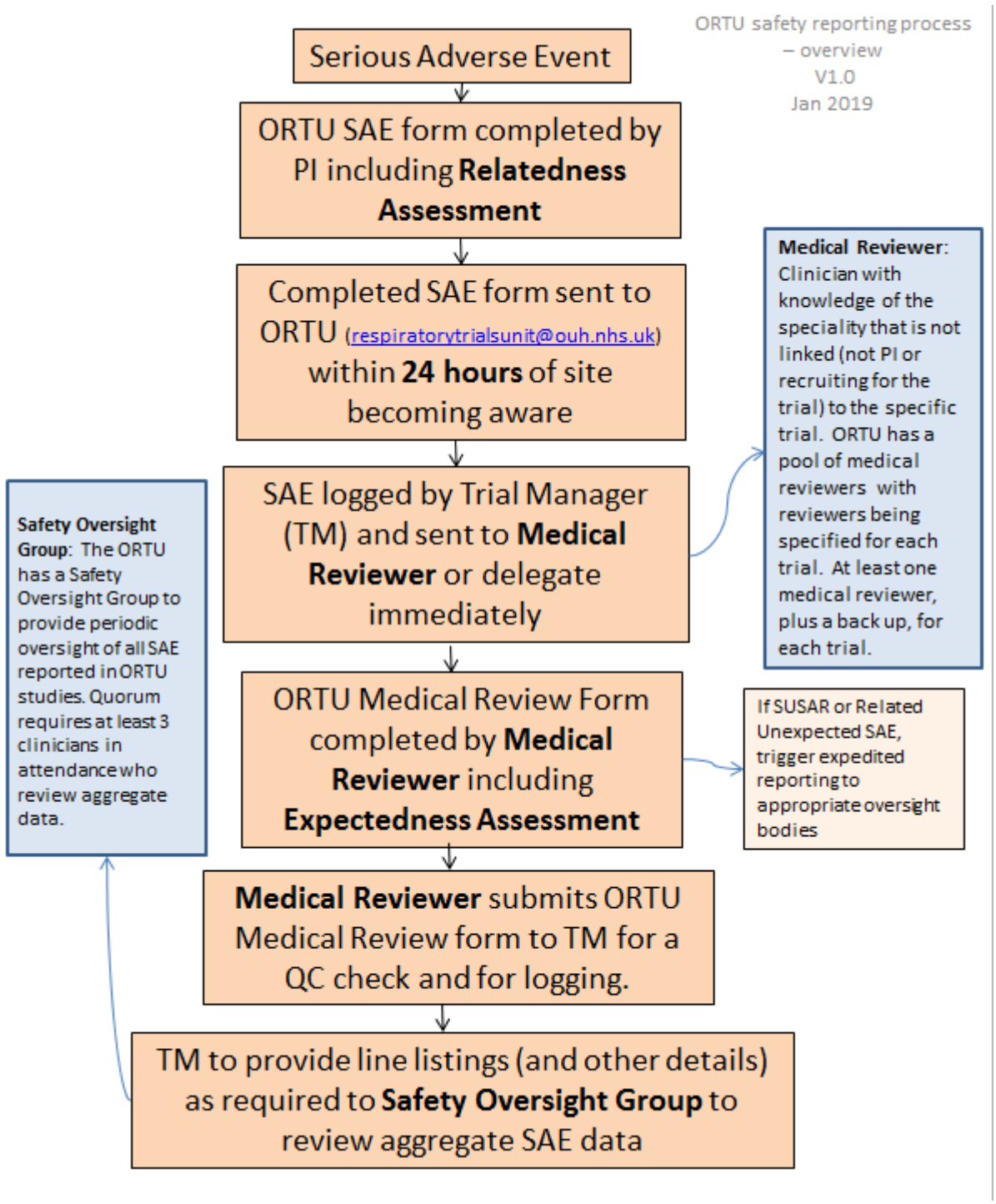
**APPENDIX A3.4a: TRIAL FLOW CHART**



## APPENDIX A3.4b: SAE REPORTING FLOW CHART



**APPENDIX A3.4c**

**MIST-3 PROTOCOL AMENDMENT HISTORY**

| Amendment No. | Protocol Version No. | Date issued | Author(s) of changes | Details of Changes made |
|---|---|---|---|---|
| Minor 1 | V3.0 | 31Jul2019 | Dr Eihab Bedawi | Minor change to clarify that the trial intervention solution will be made up and administered by clinical staff as per local protocols and not as per TSPs. |
| Minor 2 | V4.0 | 21Jan2020 | Dr Eihab Bedawi | Minor changes made through the protocol bringing it in line with the information being collected during the participant visits on CRF. Clarification on how the participant's interviews will be performed and the addition of pain to the post-operative complications of the surgical arm in the safety section. |
| Minor 3 | V5.0 | 13Feb2020 | Dr Eihab Bedawi | Clarification on the use of a transcription service provider for the qualitative interviews.<br><br>Clarification on randomisation arm crossovers, standard care arm can cross to VATS or IET if required.<br><br>Additional inclusion and exclusion criteria added. |
| Minor 4 | V6.0 | 22Jul2020 | Dr Eihab Bedawi | Clarification that research samples have been suspended during COVID pandemic. Change to how questionnaires are completed and how follow up appointments can be undertaken remotely, detailing which assessments can and cannot be undertaken. |
| Minor 7 | V7.0 | 21Oct2020 | Dr Eihab Bedawi | P8 and 8.2 inclusion of another inclusion criteria "A pleural collection with a chest |

| | | | | |
|---|---|---|---|---|
| | | | | drain in situ". Minor typos picked up by our PPI rep. |
| Sub 8 | V8.0 | 05Nov2020 | Dr Eihab Bedawi and Professor Rahman | Inclusion of using Oxford Brookes University as a collaborator to perform the qualitative interviews. |
| Minor 9 | V9.0 | 21Jan2021 | Professor Rahman and Dr Eihab Bedawi | Amendment to the trial design section, updating the information on cross over of treatment between the 3 treatment arms. |
| Minor 10 | V10.0 | 14Apr2021 | Professor Rahman and Dr Eihab Bedawi | Amendment to make some of the trial visits optional to streamline the process, following the slow recruitment due to COVID-19, this will enable sites to recruit to target but adding less burden to complete all visits and assessments, focusing on the essential data to meet the trial outcomes. Appendix A flowchart updated in line with previous amendment for inclusion criteria. |

## APPENDIX A3.5: CLASSIFICIATION OF SURGICAL COMPLICATIONS BASED ON THE MODIFIED CLAVIEN-DINDO SYSTEM

| Grade | Subgrade | Definition |
|---|---|---|
| I | | Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions. Allowed therapeutic regimens are drugs as antiemetics, antipyretics, analgesics, diuretics, electrolytes and physiotherapy. This grade also includes wound infections opened at the bedside |
| II | | Complications requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusions and total parenteral nutrition are also included |
| III | | Complications requiring surgical, endoscopic or radiological intervention |
| | a | Intervention not under general anesthesia |
| | b | Intervention under general anesthesia |
| IV | | Life-threatening complications (including CNS complications) requiring IC/ICU management |
| | a | Single organ dysfunction (including dialysis) |
| | b | Multiorgan dysfunction |
| V | | Death |
| Suffix "d" | | If the patient suffers from a complication at the time of discharge, the suffix "d" (for disability) is added to the respective grade of complication. This label indicates the need for a follow-up to fully evaluate the complication |

**REFERENCE**

Clavien, Pierre A. MD, PhD*; Barkun, Jeffrey MD†; de Oliveira, et al. The Clavien-Dindo Classification of Surgical Complications: Five-Year Experience. Annals of Surgery 250(2):p 187-196, August 2009. | DOI: 10.1097/SLA.0b013e3181b13ca2

# CHAPTER 4

# MIST-3 Qualitative sub-study

## 4.1 INTRODUCTION

The main purpose (primary outcome) of the MIST-3 RCT was to establish whether a larger scale definitive RCT can be completed. However, there is a major gap in the literature relating to pleural infections, namely that to date, no studies have adequately assessed the optimal participant focused outcome measures in pleural infection. In addition, there is a sparsity of literature on recovery and quality of life measures after discharge from hospital.

A recent study from Meggyesy et al (presented at ATS 2020, currently in submission) found that 53/56 patients (95%) who were in regular employment prior to being treated for pleural infection were only able to return to work after a median of 4 weeks post discharge. Of these patients, 45% reported that their effectiveness at work was impacted by their pleural infection recovery and 9% reported that they had to change their occupation as they were unable to continue with their previous role as a result of their pleural infection (Meggyesy et al., 2020). These important if somewhat limited findings indicate that pleural infection has a major and sustained effect on quality of life following discharge from hospital. However, there is little if any data on patient experience during the period of hospitalisation and how this impacts on decision-making regarding treatment options and discharge planning.

Primary outcomes in pleural infection trials over the last decade have been varied. There is currently no published qualitative research in the domain of pleural infection and research in this area to date has mainly focused on patient centred outcomes chosen by clinicians. This is going to be the first study to examine what outcomes are important to patients and through semi-structured interviews, will be the first study using qualitative methods to establish patient-reported experience measures (PREMs) specific to pleural infection.

A prospective qualitative sub-study of the MIST-3 trial was therefore planned to explore, through semi-structured participant interviews, the pleural infection inpatient experience, specifically what patient priorities are with regards to care and treatment, as well as to gain insights into the post-treatment recovery course. The interviews also aimed to explore the experience of participating in a pleural infection RCT, specifically being randomised to different interventions, with the aim of informing the design and conduct of a future definitive RCT of early medical versus early surgical intervention for empyema.

Qualitative research was a new area to the ORTU so a unique opportunity for me to lead on and develop new research skills for the unit. I obtained the relevant background from published literature and arranged meetings with an experienced colleague in Qualitative Health Research, Professor Catherine Henshall at the Oxford Brookes University. Once we had discussed MIST-3 and the aims of the qualitative data, we agreed on the semi-structured interview approach (DeJonckheere and Vaughn, 2019). She suggested I draft an interview guide (Kallio et al., 2016) to be used as a prompt by the interviewer during the interviews and we agreed on 3 broad themes to be explored (Appendix A5.1). Myself and another other member of the research team (Jack Seymour) received specific training in qualitative methodology including management, analysis, interpretation and reporting of qualitative data by attending a 2-day course (Appendix A5.2) and undertook the interviews alongside Sophie Harrad, a postgraduate student who had undertaken prior qualitative research with oversight from Prof Henshall.

## 4.2 METHODS

This was a prospective, multicentre, exploratory qualitative study conducted as a sub-study to the third Multicentre Intrapleural Sepsis Trial (MIST-3), which ran between November 2019 and June 2021. These semi-structured interviews were designed to explore the experiences of MIST-3 participants during the trial, or their reasons for refusing randomisation if that was their informed choice. The aim was to establish patient-focussed priorities of care, thereby informing important outcomes in the potential future multicentre randomised controlled trial. All participants who consented

to interview but declined randomisation into the study were included in the analyses of the relevant qualitative outcomes.

The interviews were conducted at the 2 week post-discharge follow-up appointment to allow a balance between minimising the effect of recall bias, reducing the number of appointments the patients need to attend, and allowing appropriate time for patients to recover from their acute illness. The interviews were conducted by either myself or one of 2 other members of the research team who received the same training outlined above. The interviews were designed so that the participant would not know the professional role of the interviewer, who introduced themselves as ‘a member of the MIST-3 trial team’. The interviewer was blinded as to the participant’s randomisation arm or their treatment course. As I was likely to have been involved in the recruitment or treatment of the participants recruited from the Oxford site, I was not involved in their interviews to reduce risk of bias, as I felt participants may not be as forthcoming if they know they are speaking to the trial fellow or one of the doctors who treated them however, I interviewed the majority of the non-Oxford participants.

The interviews were performed either face to face (Oxford) or through a video call via Skype, Zoom or equivalent video-chat platform (non-Oxford sites). All interviews were standardised and followed a ‘MIST-3 Interview Questions’ prompt document created by myself, which was included in the ethics approval (Appendix A5.1).

The interviews were digitally audio-recorded, deidentified and sent securely to an external transcriber, with whom the University of Oxford has a written contract and confidentiality agreement. The transcription was carried out verbatim and securely returned by the same method. The transcriptions were anonymised and the transcriptionist deletef the recording when they had completed their work and returned the transcript.

An initial early review of the transcripts was undertaken after the first 5 interviews had been conducted, in the presence of a researcher with extensive expertise in qualitative research methodology (Prof Cathy Henshall) to ensure that the participant interviewees were appropriately conducted and adequately stratified according to treatment received, geography, gender, age, comorbidities and to ensure that the interviewees were not obviously skewed in terms of demographics or outcomes

**Ethical Considerations**

Ethical approval for the MIST-3 qualitative substudy was embedded within the the wider investigation (MIST-3) which had NHS REC and HRA approval (Reference: 19/EE/0174). No incentive to participate in the qualitative sub-study was offered.

**Informed Consent and Confidentiality**

Written informed consent for the qualitative interviews was obtained in hospital by myself as part of the Trial Fellow (or another authorised member on the delegation log) as part of the consenting process for the main MIST-3 trial (Appendix A5.3). Patients were informed that interviews would be planned at approximately 2 weeks post discharge and they could withdraw their consent to participate in these at any time. The transcripts provided for this secondary data analysis were anonymised, with participants identified by a participant ID number.

**Primary objectives**

- To understand the patient perspective regarding priorities of care in pleural infection
- To use patient perspective to inform patient-centred study outcomes in pleural infection
- To explore the understanding of the risks and benefits of being randomised to standard care, early VATS, or early IET treatment, from a patient/carer point of view.

*Participant interviews*

We planned to undertake a total of up to 30 semi-structured qualitative interviews in up to 20 participants across the study and up to 10 carers from the Oxford cohort only. Participants (who either agreed to be randomised, or didn't agree to randomisation but agreed to be interviewed) and their carers, were invited to participate in qualitative interviews. The participants were asked questions regarding their experiences of being approached about the trial including reasons for refusal to be randomised, experience going through the trial itself and its related activities and finally experience of having pleural infection, its treatment and subsequent recovery (full details of the interview structure are in appendix A5.1).

**Table 4.1 – Eligibility criteria for MIST-3 Qualitative Interviews**

| Eligibility Criteria | |
|---|---|
| ***Inclusion Criteria*** | ***Exclusion Criteria*** |
| Meets the eligibility criteria for the main MIST-3 trial | Readmission or significant medical relapse following discharge from hospital |
| Able to provide informed consent | Did not consent for interviews as part of the MIST-3 consent process |
| Ability to speak sufficient English | Significant language barrier |

## Qualitative analysis

The analysis was supported by Professor Cathy Henshall (Oxford Brookes University). Transcripts were read and re-read for familiarisation, then open-coded to develop an initial code list. Codes were then grouped into categories, and data explored to identify connections and to develop a descriptive account of the dataset as a whole. The analysis was aimed to focus on the acceptability of trial processes to patients, individual and group equipoise, and the patient experience of pleural infection and treatment.

The interviews were analysed following the framework method (Gale et al., 2013), a method of qualitative analysis that has become increasingly utilised in medical and health research.

## Coding

I coded the transcripts assisted by Miss Sophie Harrad (BSc student researcher from Oxford Brookes University) by applying codes to words or phrases that could be important. The aim of coding is to classify all of the data so that it can be compared with other parts of the data set (i.e. so that each interview can be compared) (Gale et. al., 2013). Coding allows for the development of an analytical framework that can be used for each interview and the same codes/categories applied.

## Charting

A matrix was then generated using Microsoft Excel, and the data were charted into it (Appendix A5.4). This enables summarising the data from each transcript by category.

The aim is to maintain the meaning of the data while cutting down the volume so that it is easy to interpret (Gale et. al., 2013).

**Interpretation of data**

Through observation of the framework matrix, we were able to identify similarities and conflicting opinions within the data and draw conclusions, which can be compared with previous studies and data.

**Extenuating circumstances**

The COVID-19 pandemic presented significant challenges to the MIST-3 trial overall (see Chapter 4). Specifically with regards to this qualitative study, the redirection of research resources to support urgent public health (UPH) studies meant that coordinating the interviews with the surrounding logistics was no longer feasible. Staff trained to conduct qualitative interviews were redeployed to COVID wards either in a clinical capacity or to recruit patients to COVID-19 treatment and vaccine trials in a research capacity.

**Mitigating strategies**

There were no deviations from the planned enrolment and consent procedures to mitigate against the effect of the pandemic. Where a participant consented and it was feasible for their interview to be conducted within the required timeframe, interviews were conducted via Skype only (including the Oxford site) to limit patient/clinician exposure and no face-to-face interviews were performed. All trial modifications were agreed by the trial steering committee (TSC) prior to implementation and protocol amendments submitted accordingly.

## 4.3 RESULTS

In the four month period that the trial was open to recruitment before the pandemic (November 2019 – March 2020) and the period between the first and second lockdown (August 2020 – November 2020) we were able to conduct a total of 15 interviews (Table 5.2). A sample of the transcripts were reviewed independently by Prof Cathy

Henshall who was satisfied that these had reached data saturation (i.e no new data or themes were emerging) (Saunders et al., 2018) and so the collected transcripts could inform a robust analysis.

The fifteen interview transcripts were analysed using the Framework Method. Key themes were identified. A table of the matrix generated through coding each transcript, and highlighting each key theme is shown as an appendix (A5.4). The five key themes identified were:-

1. Participant emotions
2. The level of explanation they received/their understanding of this information
3. Their reaction to being randomised
4. Impact on physical wellbeing
5. Overall experience within the study.

Each theme has been summarised below.

**Theme 1. Emotions**

*Pre-admission phase*

When discussing emotional responses in the period leading up to the presentation there was a feeling of regret (for leaving it too late), concern and worry (that there was something serious) and pain. Interestingly, pain seemed to be the most common symptom that prompted seeking medical attention. When discussing reasons for delayed presentation or if they felt they should have presented sooner, patients described not wanting to be a ‘bother’ or trouble their busy doctors. Patients recognised their own illness but in most cases, patients felt they would ‘blame themselves’ for delayed presentations. There was also a significant COVID-19 factor which would have significantly impacted both patients’ perceptions and GP advice where shared symptoms initially prompted patients to self-isolate at home and made them reluctant to seek hospital treatment.

*Inpatient*

Participants were *shocked* at the severity of their illness when often in the context of being treated for a throat or chest infection in the community. Terms such as

‘pneumonia’ or association with ‘possible cancer’ (as a differential of an undiagnosed unilateral effusion) were quite *distressing*. One participant remarked that they were particularly shocked at the amount of infected fluid that came out of their chest drain in their initial visit to hospital: “more than a litre of fluid came out straightaway, and that’s a huge volume.” Another commented that ‘you know things are serious when people are talking about surgery’. The theme of *fear and anxiety* during the inpatient experience was markedly worse during the pandemic, as aside from the pleural infection itself, one participant in particular (MI3-A-013) described the fear that they might catch COVID-19 on top of their already significant illness; "So I, literally, had a massive panic attack because they rolled me in through a door that said: COVID: NO ENTRY”. This was also reflected in the Hospital Anxiety and Depression Scale (HADS) scores reported in the data from the main trial (mean score at day 1-2 post-randomisation 16.7 (SD 9.3, 95% CI 14.2-19.2) 0-7 (Normal) 8-10 (Mild) 11-15 (Moderate) 16-21 (Severe)).

Another subtheme that arose from this domain was that the pleural infection treatment pathway within hospitals often feels disjointed to patients. There were comments of inconsistent clinician communication about the treatment plan, duration of treatment and/or inpatient stay, particularly at the ‘front door’ (likely due to a lack of familiarity Emergency Department or other non-specialist physicians may have with empyema compared to, for example, treating community acquired pneumonia), which led to *reduced confidence* and *increased anxiety*. This was however offset by a consistent mention of confidence within the trial recruitment teams. There was also a theme of *insufficient carer communication/involvement* during the inpatient period, and this is likely to have been exacerbated by the restrictions on visitation during the COVID pandemic. Surgery was a notable subject of significant anxiety and is likely to have impacted on recruitment.

**Table 4.2 – MIST-3 Qualitative Interview Record**

| MIST3 Trial Qualitative Interview Record | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| **Study ID** | Participant | Date Consented for Interview | Date Interview Taken Place | How was the interview performed | Date recording sent to transcriber | Interviewer | Gender | Age | Treatment Arm |
| **MI3-A-001** | Participant | 09/01/2020 | 04/02/2020 | Face to face | 13/02/2020 | JS | M | 43 | VATS |
| **MI3-A-002** | Participant | 01/01/2020 | 11/02/2020 | Face to face | 13/02/2020 | JS | F | 72 | IET |
| **MI3-A-004** | Participant | 27/01/2020 | 18/02/2020 | Face to face | 04/03/2020 | JS | F | 56 | Standard |
| **MI3-A-007** | Participant | 08/02/2020 | 25/03/2020 | Skype | 26/03/2020 | SH | M | 64 | Standard |
| **MI3-E-001** | Participant | 21/02/2020 | 08/07/2020 | Skype | 08/07/2020 | EOB | M | 39 | VATS |
| **MI3-A-013** | Participant | 08/06/2020 | 13/07/2020 | Skype | 14/07/2020 | SH | F | 48 | VATS |
| **MI3-A-010** | Participant | 03/04/2020 | 14/07/2020 | Skype | 14/07/2020 | SH | F | 63 | VATS |
| **MI3-B004** | Participant | 21/05/2021 | 23/06/2021 | Skype | 06/07/2021 | EOB | M | 51 | Standard |
| **MI3-A033** | Participant | 07/05/2021 | 24/06/2021 | Skype | 06/07/2021 | SH | F | 70 | IET |
| **MI3-A032** | Participant | 07/05/2021 | 25/06/2021 | Phone | 06/07/2021 | EOB | M | 54 | IET |
| **MI3-A030** | Participant | 28/04/2021 | 28/06/2021 | Phone | 06/07/2021 | SH | M | 77 | IET |
| **MI3-A030** | Participant | 29/04/2021 | 28/06/2021 | Skype | 06/07/2021 | SH | F | 68 | VATS |
| **MI3-A028** | Participant | 05/04/2021 | 05/07/2021 | Phone | 06/07/2021 | SH | M | 50 | VATS |
| **MI3-G005** | Participant | 15/06/2021 | 05/07/2021 | Phone | 06/07/2021 | EOB | F | 73 | Standard |
| **MI3-H-001** | Participant | 29/06/2021 | 13/07/2021 | Phone | 14/07/2021 | EOB | M | 71 | IET |

Interviewers: JS – Jack Seymour, SH - Sophie Harrad, EOB – Eihab Bedawi

*Recovery and expectations*

Participants felt nervous at the point when they were discharged and worried that they may relapse as they knew their treatment hadn't been completed (most were going to complete antibiotics at home). The most common persisting symptoms were *fatigue and breathlessness*, which patients described were the *biggest barriers to resuming baseline function in terms of ADLs (**A**ctivies of **D**aily **L**iving) and return to work*. There was a common theme of taking longer than expected to get better after discharge, but it was apparent that this was more manageable if patients had been appropriately informed prior to discharge.

One participant (MI3-A-004) specifically recalled a clinician on the ward telling him 'it's going to be a long haul to feel well again' and he felt that expectation (or lack thereof) was vital in his recovery. Unfortunately, a significant proportion of patients felt that they had not been given any advice or support throughout their recovery process. As a result of this, one participant explained "To tell you how anxious I became, I slept for the first time in 5 weeks after I saw him (the doctor) on the Tuesday." Participant MI3-A-013 became visibly upset during their interview when recalling how upset they had felt during their recovery when no one had checked up on them or given them any support. When discussing their recovery one patient (MI3-A-007) remarked "I was scared and a little stressed out because this is something, because of this whole affair, that has taken a month and a half out of my life, that scares me totally." Despite this, it was apparent that there was a consistent prioritisation of *'eagerness to be discharged and get back home'* and patients generally felt *happy to be home*.

"Grateful/Lucky"

Most participants felt gratitude towards the hospital staff and the research team involved in their hospital admission. They felt well cared for and believe staff were honest with them. One participant (MI3-A-002) stated: "I knew the NHS was amazing and I am moved by the amount of care I've been given."

"Compassion/Empathy"

Participant MI3-A-002, on numerous occasions mentioned how much empathy and compassion they felt they had been given during their hospitalisation and how grateful they were for that: "you can teach someone to be a doctor and a surgeon, you can't

teach somebody empathy and compassion…Pretty much everybody I met had that gift".

*Pain*

Pain was a significant feature through the pleural infection experience. This was either pain of the condition (secondary to the pleural inflammation) that was part of the presenting symptoms, pain of the chest tube insertion or having the chest tube in-situ as well as pain with IET which was often associated with decreased, or in some cases, non-compliance with this treatment arm. Lack of or insufficient pain relief was a recurrent theme. Recollections of the chest tube insertion included “immensely uncomfortable” (MI3-A-002) and “extremely painful” (MI3-A-007). Participant MI3-A-004 mentioned that the pain of having the IET administered was so painful they could only withstand one dose and refused a second one.

**Theme 2. Level of explanation/understanding**

*The Trial enrolment experience*

All participants explained that they were given a lot of information about the study and the majority recalled receiving about four pages worth of written information as well as significant verbal information. The majority of participants understood roughly what each trial arm entailed, however a common theme among the participants was that they would have liked to have more information on the differences between, as well as the pros and cons, of each treatment. It was also common that the participants understood less about the IET arm than the other treatments, participant MI3-E-001 says: “I don't know if I had the enzyme thing explained very well. I don't know if I would know now what that would have meant.”

Participant MI3-E-001 also mentioned that the vocabulary of the medics was sometimes difficult to understand: “words that medics use are quite sort of specific to the medical profession and they’re not easily understood”. Participant MI3A004 mentioned that with lung problems, the words “chest infection” are often used and it seems more generalised than being able to understand specifically what is going on: “There is too many words that look alike about what’s going on in the lung when you get an infection, and people can’t distinguish.”

Another common theme between the participants was that a lot of them recall being in a very bad physical state at the time of receiving all the information, therefore making their memory of contact with the trial leader cloudy or non-existent. Participant MI3-A-002 mentioned that it would be good to find a way in which you can get that level of information across to someone who is not in a well enough state to take it all in at that time; this patient had asked for his brother to be included in discussions. Almost all of them mentioned that a *visual aid* of some sort such as a diagram would be really useful in explaining the illness better.

The participants all felt that they understood pleural infection a lot better since they have recovered than they did before they underwent treatment and felt that there is a lack of awareness about the condition as most had never heard of it prior to their illness. They also shared a common trust in the trial recruitment team and one specific quote “Being in the trial felt like the best option as felt like care was going to be in the hands of experts"

**Theme 3. Reaction to randomisation**

*Acceptance/satisfied*

A common emotion from many of participants was a sense of being at satisfied or accepting of the treatment arm that they were randomised to. Those who were assigned to either standard treatment or IET were pleased because they did not want to undergo the surgery for various reasons such as COVID-19 and anaesthetic risks. One trial participant stated “when you’re in that situation, what you want is the best possible outcome, and the quickest possible outcome. So I was prepared to do whatever it took to come out positively on the other side.” Another surgical participant stated “I think we know there’s always a risk to having surgery”. Interestingly, one participant who made a good recovery with IET had no regrets about but was pleased he had not been well informed on how much pain their treatment was going to cause “I would have probably said, go and do it to somebody else".

However, the surgical arm did appear to split participants. In describing their reactions to being randomised to surgery, two participants stated:

“ I had a personal preference for being in the surgical group…because I felt that that had, potentially, the greatest chance of success …And that that might resolve my issue more quickly than perhaps, certainly, being in the control group, where I think that there were potential drawbacks.” (MI3-A-001)

“I think had I, although I’ve got nothing to base this on, had I been in the control group or perhaps the medication group, it might have taken longer, in terms of clearing the infection.” (MI3-E-001)

“I was happy because I felt I needed surgery anyway, because I had one drain put in but, obviously, when they did the ultrasound there was another two pockets. And it was felt that it would probably be easier just to go with the surgery, rather than doing it another two times, sort of thing.” (MI3-A-028)

Other patients were concerned about the risks and implications of surgery:

“I’m a believer that if you don’t have to have surgery it’s better” (MI3-A-010 – randomised to VATS)

“I think we know there’s always a risk to having surgery” (MI3-A-002 – randomised to IET)

“I was thinking, this in my mind, that opening up my side somehow and even just going in with a camera and a tube, was way more serious than these other ones.” (MI3-A-007 – randomised to standard care)

*Positive thoughts*

All participants were happy with the treatment arm they were assigned to. Participant MI3-A-001 was pleased to not be in the standard treatment arm:

“I was happy I wasn't in the control group because I knew that I'd be getting some form of positive intervention.”

*Negative thoughts*

Two of the participants (MI3-A-002 and MI3-A-007) had negative views about the surgery treatment arm because of the risks associated with having surgery. However, it was stated by MI3-A-002 that they would have ultimately had any treatment if it were to make them better. Participant MI3-A-001 had a preference to the surgery arm but

also felt they had not been given full information about the other treatments and therefore assumed that surgery would be the best option to make them better.

**Theme 4. Influences on physical wellbeing**

*Severity of illness before trial*

Most participants stated that the point at which they were approached for the trial was when they were at their worst stage of their illness.

Participant MI3-A-010 stated “I was just about at my most critical illness" and participant MI3-E-001: “was at a state in the progression of the infection in my pleural cavity, that meant that something had to be done.”

Participant MI3-A-013 described: “I've never felt that bad, I've had a heart attack and I didn’t feel as bad as I did this time round.”

*Response to treatment*

MI3-E-001, MI3A001, and MI3-A-013 who all underwent surgery experienced some pain and tenderness post op, however they all explained that they felt better straight after. Participant MI3-A-013 stated that they felt well straightaway: “It’s like somebody had pulled the plug and all the pressure had been taken off.”

MI3-A-007, who received standard care, described feeling somewhat better following insertion of chest drain, despite the discomfort of the chest tube,: “The initial pain, when I first came into the hospital, I told them this, it was an eleven on a scale of one to ten… and after they put in the tube, it was eight and a half to nine.”

*Recovery*

The participants recovering from surgery explained that they experienced a lot of fatigue and even doing the smallest tasks drained them, MI3-A-013 says: “By the time you've got dressed, had your breakfast, you, literally need to go and have a little siesta"

The participants who underwent standard care or IET appeared to recover quicker.

Participant MI3-A-032 explained that since returning home: “my energy levels are back up and I'm back doing fitness training.” For participant MI3-A-013, who underwent surgery, the recovery process was a highly stressful time, and due to ongoing cough and fears that the condition was not better they had to call a GP for reassurance.

Interestingly these signals were replicated in the formal QOL data from the MIST-3 data, with quicker recovery being seen in the IET arm.

**Theme 5. Overall experience**

*Positive experiences*

Participants' reflection of their time in hospital was generally positive.

Participant MI3-A-002 stated: "I am moved by the amount of care I've been given. Not just the professionalism, that immense compassion and love really, to be honest with you."

Participant MI3-A-013 also mentioned how they were made to feel like a priority. All participants also believed that the research and medical teams worked very well together apart from one participant (MI3-A-010) who believed that the research team could've been better integrated and didn't appear to have had much contact with them: "to be fair, they never made themselves clear as to who they were."

Participant MI3-A-004 made a point of mentioning that having one of the research team visit them was a lovely experience because they had been seen as whole being and not just some symptoms that needed to be treated.

*Negative experiences*

A few participants mentioned that it would have been nicer to be given more information on exactly what is going on/what is going to happen next. On occasion they felt as if they didn't know what was happening or why. Participant MI3-E-001 felt that there could be an improvement in care consistency, on a few occasions different medical professionals gave conflicting information by different staff who were checking up on them. Participant MI3-A-013 suggested some sort of *documentation to take home* that explains what to expect during the recovery process. Participant MI3-A-002 felt that they had left it too long before they finally went to the hospital, and mentioned that *more public awareness* about pleural infection generally and when is right to seek help could benefit a lot of people and prevent illnesses worsening.

## 4.4 DISCUSSION

Interpretation of the study findings above has identified a few common viewpoints from the key themes that need addressing. Emotions (theme 1) overlaps with the other themes and will be integrated in this discussion accordingly.

### Discussion of Theme 2 – Level of explanation / understanding

*Information regarding the randomised control trial*

The participants in this study shared a common opinion that they could've been given more information on the differences between each trial treatment arm. It was often the case the participants weren't certain what the differences between, and what the benefits/negatives of each treatment arm were. In particular, the IET arm was an area that very few participants were able to recall much information about.

There are many factors that can affect the transfer of information from medical professionals to patients, including use of medical terminology, patients' level of literacy, and unsatisfactory explanations from the medical professional (Klamen and Binder, 1996). A comment by one participant was that, often the language used by medical staff can be quite scientific, and difficult for someone who isn't a medical professional to fully understand. This could have been a factor affecting the participants full understanding of each treatment. It may be that the term 'enzyme' was not commonly understood (unlike concepts such as drainage and surgery), and it may be that a simplified framework of terms should be developed. However, it was also common that the participants had trouble remembering their contact with the research professional who first approached them, due to their illness at the time. Their lack of memory/knowledge could have been due to their decreased physical wellbeing, rather than the lack of information given. An interesting point raised by a participant was that it might be a good idea to find a way of being able to get that level of detailed information across to a patient who isn't in the best mental or physical state to understand it fully. Whilst I attempted to address this by creating a 'summary participant information sheet' in an intentionally larger font (as suggested by the MIST-3 PPI group) incorporated into the main PIS (Appendix A5.5), the incorporation of

visual aids, elaboration of treatment in plain English as well as translation of information into multiple languages are important take aways that that should be addressed and incorporated in future trials.

*Information regarding pleural infection*

The majority of participants had a brief understanding of what their illness actually was prior to the trial. However, the case with almost all of them was that they gained a better understanding of pleural infection in their follow up assessments post-treatment. Most participants were of the opinion that a visual aid of some sort would have helped with their understanding of the disease. A study showed that using infographics has a powerful effect and can facilitate a patient's understanding of their disease and the risks associated with it (Balkac and Ergun, 2018). Furthermore, using a well-designed visual aid can reduce the amount of intelligence required to achieve the same level of understanding (Wei, 2013). This could be a good solution to giving information to patients who are not physically or mentally at their best as was the case with many of the participants. I am currently planning the MIST-4 study and as a direct result of the qualitative data obtained from this study, have sought early involvement and expertise of Dr. Ciliein Kearns (*Artibiotics*) who specialises in Medical Illustration and research infographics to assist with the design of visual aids. These would also likely help those from whom English is not the first language.

*Information regarding patient recovery*

There were mixed opinions about how much the participants understood about the recovery process and its likely duration, with some feeling confident in what the road ahead entailed, and others not quite understanding what would happen. The signals toward a lack of support during the recovery period and the feelings of health anxiety surrounding this are notable and should be considered in future studies. It was clear that patients were eager to be discharged and whether or not treatment during the COVID-19 pandemic amplified this, it appears that *length of hospital stay* is a priority of care, and an important patient centred outcome. The majority of participants were surprised at how long their recovery process was. No two people will have the exact same recovery experience; therefore, it would be difficult for clinicians to give an exact, accurate time frame of recovery, and underlying comorbidity/pre-illness functional baseline as well as unpredictable complications would all have a significant impact on

this. However, as was felt by some of the participants, slightly more information on the process, and how they could contribute to their own 'active' recovery, would have been a valuable addition thus highlighting our need for more longitudinal outcome studies in pleural infection; data beyond the first 6 months is usually lacking in the larger RCTs.

**Discussion of Theme 3 – Reaction to randomisation**

Randomised control trials have been suggested to provide the most reliable evidence for the success of treatments (Wasmann et al., 2019). However, a problem with randomising patients is that any patient may (understandably) have a preference to what treatment(s) they would like or would prefer not to have. As seen in this study, one of the participants expressed a strong preference to be in the surgical arm because they felt did not have all the information/understanding about the other two treatment options and felt more comfortable going with what they felt they understood best. If patients do not like the option they have been randomised to, they may decline to participate in the trial (Wasmann et al., 2019). This begs the question of how much patients' preferences based on the information they are given and the way it is presented to a potential participant impacts their decision to agree to be randomised, compliance with their randomisation treatment and their retention within the study. It is important to acknowledge that clinicians' own biases will become apparent in the way they present a study and its interventions. Generally speaking, there were reasonable levels of acceptability reflected in the primary feasibility (62% randomisation rate) but it is noteworthy that preferences to a specific treatment, and consequently lower recruitment rates, can reduce the external validity of a study (Mills et. al., 2011). Ensuring clinicians involved in the randomisation process give consistent information, particularly when multiple sites are involved in trials with several treatment arms, is an important part of study design and preparation. Written patient information leaflets are key to this and in retrospect, I would make significant changes to the patient information leaflet I made for MIST-3 (Appendix A5.5) knowing what I know now about patients' concerns, and this will be a valuable in the design of the patient facing documents for the MIST-4 study.

When analysing reactions to randomisation intervention, multiple participants expressed wanting the best treatment that results in the best outcome. Whilst

seemingly obvious, this brings to light the risk of ‘treatment failure’ as another important patient centred outcome, where one can conclude that that patients want the treatment that is associated with the least repeat interventions and readmission – the one that is least likely to fail.

Furthermore, the ITT (Intention to Treat) analysis strategy used in the main trial warrants a mention here. Patients who are unfit to undergo a specific treatment (for example, surgery), or participants who withdraw for other reasons, can lead to study bias. This is because the main rationale for randomisation is defeated and it is no longer certain that baseline prognostic factors between the treatment groups are similar (Akobeng, 2005). Hence, the ITT approach gives an unbiased estimate of treatment effect, accepting that non-compliance and protocol deviation are likely to occur in clinical practice (Gupta, 2011). However, in this qualitative sub-study, the participants were randomised, and their views collected, regardless of whether they received the treatment or not (for example, participants who were randomised to the surgery arm but were unfit for surgery, were still analysed with regards to the surgery arm). It is unclear how much this would have affected the results and had the sample been larger, perhaps a secondary ‘per protocol’ analysis would have been useful. Due to the impact of the COVID-19 pandemic on multiple arms of the work presented herein, it was not felt to be feasible to undertake further interviews but incorporating such methodology in future trials would be desirable.

**Discussion of Theme 4 – Impact on physical wellbeing**

*Severity of illness*

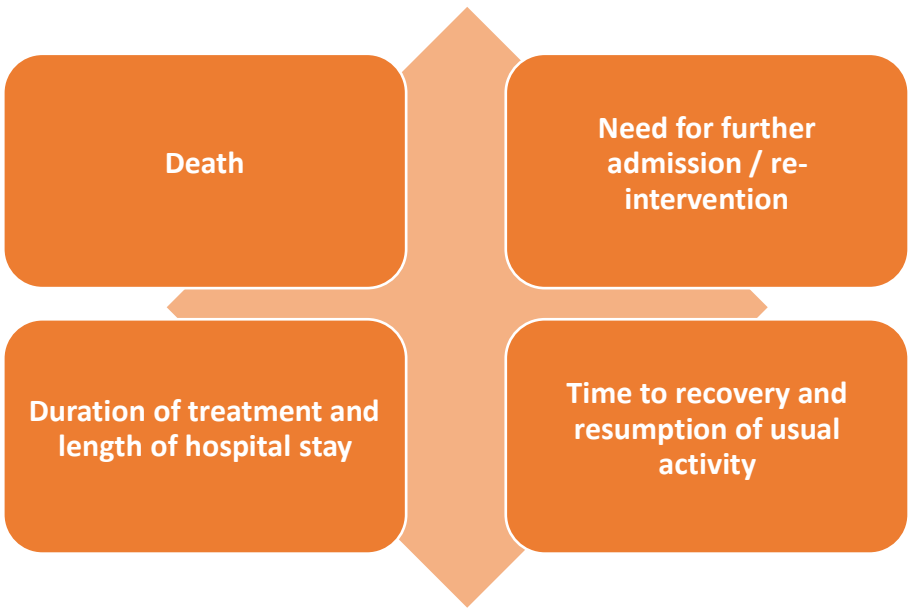
Almost all patients agreed that at the time of their first contact with the trial team, they wereat the worst stage of their illness. Most would have gone through almost any intervention to feel better.

What became apparent from analysing the transcripts was that, although breathlessness was common and fever and malaise not uncommon, it is pleuritic chest pain that most commonly leads patients to present to hospital. One patient notably compared the pain of their pleural infection to a heart attack, stating that the pain of the heart attack was not as bad. A challenge with early diagnosis is that both symptoms are non-specific, meaning that the differential is wide and prone to

misdiagnoses in primary care such as lower respiratory tract infections with pleurisy. This was likely exacerbated during the COVID-19 pandemic when many appointments were conducted by telephone, and fewer patients were willing to attend hospitals even for investigations such as X-rays.

Despite the pain associated with interventions such as chest tube insertion, administration of IET and post-operative pain following VATS, patients appear to respond promptly and the benefits clearly outweighed the negative impact of the pain from the patient perspective. It is notable that the qualitative data in this study reflected similar trends to a slower recovery with surgery, reflected in the QOL / EQ-5D-5L questionnaires used in the main study. Time to recovery and resumption of usual activity is also an important patient centred outcome to arise from this study.

**Figure 4.1 – Patient priorities in pleural infection treatment**




### Discussion of Theme 5 – Overall experience of taking part in a trial

Despite our apprehensions around patient perception and the acceptability of being randomised early to 3 completely different interventions, general reflections and experience of having been a part of the trial were positive, and this specific aspect did not seem to deter patients from agreeing to enter the trial. All participants had positive reflections of the care they were given throughout their hospital stay and the staff who

cared for them. It is also clear that the added attention and extra research nurse visits gained from being part of a trial was found to be advantageous rather than a burden. Previous studies have also shown that patients of RCTs feel that they receive special attention such as extra staff efforts or superior facilities, and ultimately, gained a better relationship with the healthcare providers (Naidoo et al., 2020). This extra level of care has obviously had a significant impact on the patients overall positive thoughts about taking part in the study. An important factor arisen from the patient journey is the communication between the research team and the clinical team and something that should be improved in future studies.

The conflicting information from different members of staff is a difficult aspect to control due to the multidisciplinary nature of the modern healthcare teams. The recollections of being taken for scans or treatments being administered 'abruptly' and without much communication about timeframes is probably, and unfortunately, a reflection of usual clinical practice not specific to pleural infection where healthcare teams are often stretched. Even if levels of staffing are considered 'safe' as a minimum standard, it is interesting to note that communication and personal attention clearly do impact greatly on the overall patient experience. Efforts clearly need to be made also with regard to communicated results of tests/scans and in a way that is comprehensible not only to a lay person, but a significantly ill lay person.

Reflections of patients feeling pleased to have been part of a research project as if they were part of a greater purpose were admirable. One participant (MI3-A-010) memorably commented that by making good response to standard care and not needing the surgical intervention he was randomised to, he felt as though they had been somewhat 'excluded' from taking an active part in the study.

.An intriguing observation raised by one participant was that while filling out the questionnaire on pain levels (by VAS – Visual Analogue Score), they noticed that there was no space or question to indicate whether the patient has been given pain relief or not. Whilst being a well-validated tool, the potential for differing levels of pain relief leading to bias in VAS pain reporting and subsequent interpretation has been reported (Jensen, 2003) and poses a specific challenge in the comparison of medical vs surgical treatment of pleural infection. Physicians may or may not administer

prophylactic doses of opiates prior to tube insertion or pre-IET administration, whereas patients wake up post VATS (performed under a GA) to a patient controlled analgesia (PCA) pump with or without a background infusion that often contains significant doses of opiate. Whilst we did not protocolise pain relief in the MIST-3 trial, pain is clearly an important (secondary) outcome and prospectively collected pain data comparing modern VATS to medical management (including IET) is lacking. This will need careful consideration in a future trial. What is clear from the patient experience in this study is that both pleural infection and its treatment are associated with significant pain, and particularly that this clearly influences compliance with IET, appropriate analgesia should be more proactively recommended as part of routine care.

Based on the data from this study, the psychological and emotional impact of pleural infection cannot be overlooked. This was mostly due to the severity of illness experienced, but undoubtedly the COVID-19 pandemic contributed to this. The pandemic severely impacted hospital admissions due to fears of contracting COVID-19 in the hospital setting (Mafham et al., 2020). Furthermore, it is apparent that this anxiety continues beyond discharge and the levels of psychological support required by patients post-discharge after pleural infection are probably underestimated. It is notable that in a large UK multicentre study of ICU survivors (n=21,000), over half of patients who responded to a postal questionnaire following treatment reported significant symptoms of anxiety, depression or PTSD (Hatch et al., 2018) and that depression following critical illness was associated with an increased mortality in the first 2 years following discharge. Given the high HADS scores reported in the MIST-3 study, supported by the qualitative data in this sub-study, psychosocial support warrants greater attention by treating clinicians and this aspect should be incorporated as part of research follow up in future large pleural infection studies.

**Limitations**

This analysis was limited by a smaller sample size than originally intended, although the transcripts were independently assessed as meeting data saturation. Due to the COVID-19 pandemic, the MIST-3 protocol was amended to reduce the burden on participating sites (prioritising main feasibility outcomes) and consent for interviews reduced substantially. This may limit the external validity of the findings. However, the

‘correct’ sample size for qualitative interview-based studies is debated. Guest et al. (Guest et al., 2006) analysed 60 interviews (of female sex workers) and found that 72% of themes emerged in the first 6 interviews and 92% in the first 12 interviews. Whilst this population and the subject of the interviews were very different from the MIST-3 sub-study, others have reported similar findings (Francis et al., 2010; Namey et al., 2016). Thus although it was disappointing not to be able to recruit to the initially planned sample size of 30, data saturation was assessed to have been reached by Prof Henshall and hence it seem unlikley that additional partcipants would have substantially altered my findings.

Secondly, the use of multiple interviewers was predominantly conducted to allow me to take part in the interviews withstanding the fact that I would not be able to, as the main clinician driving the main study, to conduct all the interviews as discussed above. The impact of using multiple interviewers has been reported as potentially having the benefit of reducing bias and different interviewers may pick up on different aspects based on gender and nature of interaction (Matteson and Lincoln, 2009). The use of an interview guide maintained uniformity of the interviews.

The timing of the interviews was arbitrarily set at 2 weeks post follow up to limit recall bias, but this was at the expense of understanding longer term recovery beyond 2 months. The majority (13/15) of these interviews were conducted via Skype video call medium and telephone due to the impact of the pandemic. The use of Skype as an interview method has been shown to enable discussion of a similar number of topics (codes) and derive similar numbers of words, but in-person study interviews have been shown to be marginally superior (Krouwel et al., 2019). Qualitative interviews over the telephone are likely to have impacted quality of the data but perhaps been more accessible to participants of an older age bracket or lower socioeconomic status who may have been less likely to consent had the interview medium been limited to Skype/Zoom.

Finally, we originally set out to obtain both patients’ and carers’ perspective, but the latter had to curtailed in view of the strained recruitment, obstacles and subsequent time pressures created by the pandemic. This would be something to consider if future qualitative studies.

## 4.5 CONCLUSION

This qualitative study is the first of its kind in pleural infection. It has provided valuable insights into the pleural infection patient experience and highlighted the factors that are most important to patients. Patients want the best treatment that enables them to recover speedily and completely, emphasising the unmet need for a paradigm shift in the current treatment pathway that escalates patients to sequential therapy over a period of several days from diagnosis. The design of the study was acceptable to patients and their experiences of being involved in the trial were mostly positive. Clinician communication to patients in the context of the study and along the treatment pathway including the use of visual aids are valuable learning points for a future study. Given the challenges of randomising acutely to complex interventions, it would be useful to incorporate recruitment training interventions (Mills et al., 2018) into a future RCT. Length of stay, time to complete recovery and treatment failure rates have emerged as important patient centred primary outcomes in pleural infection. Pain has likely been underestimated as a symptom of pleural infection and its intendent therapies and warrants specific attention in future studies. Finally, the high levels of fear and anxiety require further study and potentially incorporation of psychosocial support during and post treatment in clinical guidelines.

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# APPENDIX A4.1

# MIST-3 INTERVIEW PROMPT SHEET

| *Study Code:* | | | *Site ID Code &* | *Participant identification number:* | | | |
|---|---|---|---|---|---|---|---|
| **M** | **I** | **3** | | | | | **C / P** |

| | | |
|---|---|---|
| **Date of Interview:** | | |
| **Time of Interview:** | | |
| **Interviewer Name:** | | |
| **Consent Form Signed** | **YES** □ | **NO** □ |
| **Confirm consent to being recorded** | **YES** □ | **NO** □ |
| **Test Recording Equipment** | **YES** □ | **NO** □ |
| **Verbal Explanation of Semi Structure interview before starting** | **YES** □ | **NO** □ |
| **Check Participant/carer is ready to start the interview** | **YES** □ | **NO** □ |

This is a brief overview of the topics to be considered. It is likely that the content of the interview schedule will develop and may incorporate other areas as the researcher reflects on each interview as it takes place.

The 'Prompts' sections in *italics* will only be raised if not covered spontaneously by participants.

**Theme 1 – Approach for the trial**

1. How did you feel about/your partner being approached for the MIST3 trial?
   - *Who approached you/your partner?*
   - *Any initial concerns?*
   - *What information were you/your partner given? Written? Verbal? Was it easy to understand? Could this be improved?*

2. What made you/your partner want to be part of the trial / not want to be part of it?

3. What do you understand about the trial?
   - *The different treatment procedures?*
   - *Randomisation?*
   - *Pleural infection?*
   - *How did you feel about the treatment arm you/your partner were randomised to?*
   - *Why did you/your partner choose not to be randomised? Did something put you off?*
   - *What could be improved in how this was explained to you/your partner?*

- *What would have persuaded you/your partner to be part of the study if you/your partner did not want to take part?*

**Theme 2 – Experience in the trial**

1. What are your experiences of being in/involved the MIST3 trial?
    - *Example of a positive*
    - *Example of a negative*

2. How did you feel after the/your partners procedure?
    - *Any problems or complications?*
    - *Were you prepared – i.e. did the information you/your partner were given prior prepare you/your partner for the procedure?*
    - *Would you/your partner have preferred a certain treatment? Which one and why?*
    - *Were you/your partner happy with the treatment options offered in the trial?*

**Theme 3 – Experience of pleural infection**

1. Do you have any advice for the doctors and nurses who are part of the study?
2. Do you have any advice for doctors and nurses who are treating you/your partner for this condition?
3. How did you/your partner feel when you/they were sent home?
4. How did you feel when you/your partner were in hospital?
5. Any other comments?

| |
|---|
| **Thank Participant for taking part in the Interview** |
| **Assure participant of confidentiality of responses** |
| **Switch off recording device** |

## APPENDIX A4.2

Content of Qualitative course and certificate of attendance

OXFORD QUALITATIVE COURSES

# TIMETABLE

## Analysing Qualitative Interviews

| Day 1 -8th June | |
|---|---|
| Time and Format | Session |
| 09.00-09.15 | Log in via zoom link |
| 9.15-10.45 *(Live)* | 1.1 - Welcome and Introduction to thematic analysis<br>*Dr Charlotte Albury* |
| 10.45-11.15 | Break |
| 11.15-12.30 *(Live)* | 1.2 - Managing qualitative data 1: Coding interview data<br>*Dr Anna Dowrick* |
| 12.30-13.30 | Break |
| 13.30-15.00 (*Live)* | 1.3 - Managing qualitative data 2: Developing a coding framework<br>*Dr Anna Dowrick* |
| 15.00-15.30 | Break |
| 15.30-16.00 *(Pre-recorded)* | 1.4 - Introduction to NVivo<br>*Dr Charlotte Albury and Dr Susila Davis* |
| 16.10-16.30 *(Live)* | 1.5 - Questions<br>*Dr Charlotte Albury* |

| Day 2 – 9th June | |
|---|---|
| Time and Format | Session |
| 09.00-09.15 | Log in via zoom link |
| 9.15-9.30 *(Live)* | 2.1 - Introduction to day 2<br>*Prof Catherine Pope* |
| 9.30-10.30 *(Live)* | 2.2 - Interpreting qualitative data 1: Introduction to OSOPs<br>*Prof Sue Ziebland* |
| 10.30-11.30 *(Live)* | 2.3 - Interpreting qualitative data 2: Finding a story, from OSOP to published article<br>*Prof Sue Ziebland* |
| 11.30-12.00 | Break |
| 12.00-12.45 *(Pre-recorded)* | 2.4 - Finding and mobilizing theory<br>*Dr Caitlin Pilbeam* |
| 12.45-13.45 | Break |
| 13.45-15.00 *(Live)* | 2.5 - Small group practical and presentations<br>*Dr Charlotte Albury* |
| 15.00-15.15 | Break |
| 15.15-15.45 *(Live)* | 2.6 - Writing your thematic analysis<br>*Prof Catherine Pope* |
| 15.45-16.30 *(Live)* | 2.7 - Final questions and group discussion<br>*Dr Charlotte Albury and Prof Catherine Pope* |

NUFFIELD DEPARTMENT OF
**PRIMARY CARE**
HEALTH SCIENCES

OXFORD
QUALITATIVE
COURSES

Oxford
Qualitative Courses

# CERTIFICATE OF ATTENDANCE

## Eihab Bedawi

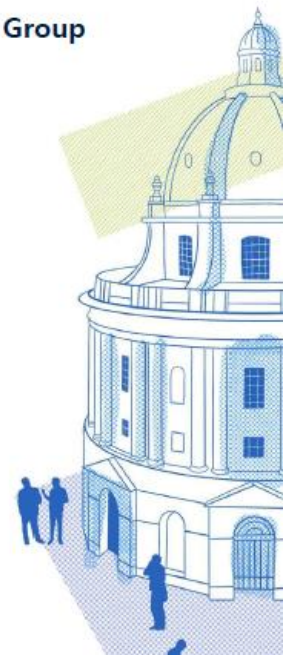
Attended the two day course

**Introduction to Analysing Qualitative Interviews**

on the

**8th & 9th June 2021**

Organised by the **Medical Sociology & Health Experiences Research Group**
at the Nuffield Department of Primary Care Health Sciences,
University of Oxford.



Vanessa Eade

**Vanessa Eade – Course Administrator**
**Date:** 9th June 2021

## APPENDIX A4.3



*Study Code:* *Site ID Code* *Participant identification number:*

| M | I | 3 | | | | |
|---|---|---|---|---|---|---|

# CONSENT FORM

**Title: The third Multi-Centre Intra-Pleural Sepsis Trial (MIST-3): Early Video Assisted Thoracoscopic Surgery (VATS) or Intrapleural Enzyme Therapy (IET) in Pleural Infection – a feasibility, randomised trial**

*Chief Investigator: Professor Najib Rahman*

| | *If you agree, please initial box* | |
|---|---|---|
| 1. I confirm that I have read the information sheet dated..... (version.....) for this study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. | | |
| 2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. | | |
| 3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from University of Oxford, from regulatory authorities and from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. | | |
| 4. I agree to take part in the trial. | | |
| | | |
| | Yes | No |
| 5. I agree to donate blood and pleural fluid samples I am aware that these will be stored to be used in research with appropriate ethical approval here or abroad in the future. I consider these samples a gift to the University of Oxford and I understand I will not gain any direct personal or financial benefit from them. | | |

| **Interviews:** | | |
|---|---|---|
| *6. I agree to be contacted by the University of Oxford, or Oxford Brookes University for interview regarding my participation in the trial* | Yes | No |
| | | |
| *7. I agree to the interview being recorded and agree to the use of anonymised quotes being used in research reports and publications.* | Yes | No |
| | | |

| Additional (***OXFORD PARTICIPANTS ONLY)***: | | |
|---|---|---|
| *8. I agree to be contacted about ethically approved research studies for which I may be suitable. I understand that agreeing to be contacted does not oblige me to participate in any further studies.* | Yes | No |
| | | |
| *9.I agree for you to approach my partner/carer for interview.* | Yes | No |
| | | |

| _____ | _____ | _____ |
|---|---|---|
| Name of Participant | Date | Signature |

| _____ | _____ | _____ |
|---|---|---|
| Name of Person taking Consent | Date | Signature |

**1 copy for participant; 1 copy for researcher site file; 1 (original) to be kept in medical notes*, 1 to be sent to ORTU.

# APPENDIX A4.4

# MIST-3 QUALITATIVE INTERVIEW DATA MATRIX

| Category | Sub-category | MI3-E-001 | MI3-A-010 | MI3A002 | MI3A007 | MI3A001 | MI3A004 | MI3-A-013 | MI3-A-028 | MI3-A-030 (pt) | MI3-A-030 (partner) | MI3-G-005 | MI3-A-033 | MI3-B-004 | MI3-A-032 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Emotions | Pleased/happy | This participant was pleased about the trial arm they were randomised to and the treatment they were to receive (pg2) | Participant was delighted to not have the surgery arm of the trial because of covid and for other reaons "I mean I'm a believer that if you don't have to have surgery its better." (Pg2); Participant was also delighted at the time when they were discharged despite being in quite a lot of | Participant was actually glad that they had not been so highly informed on how much pain the chest tube was going to cause because they would "have probably said, go and do it to somebody else"(Pg4) | Participant was glad that the people in charge of looking after him, were looking after him (Pg5). ; Participant was happy with the treatment arm they were randomised to due to the fact that they would not have to go through too much discomfort again (Pg3). "I was happy that they weren't going to have to | Participant was happy to have been approached to join the trial and take part due to their academic/ medical background working in the NHS (Pg1) "I was quite happy to participate in a medical trial." "I'm quite happy to participate in research that I think is going to | | | Pt was happy with their treatment arm: "I was happy because I felt like I needed surgery anyway" Pg1. | Participant was delighted when he had a check up call so soon after his procedure, and has been blown away by the support he has received since leaving hospital, from check up calls to calls offering advice and reminders to take | | | Pt felt good because they knew that they were in good hands and the doctors were always there if something wasn't right. (Pg5) ; Pt was happen to be discharged form hospital because theyd been there for over a month (Pg5). | Pt was happy knowing that they were "in the hands of experts" (Pg1) | |
| | Shock/unexpecting | shocked at the amount of infected fluid that came out of the chest tube on their first visit to hospital before the trial had begun (pg4) "The thing that I'm still shocked about, which is before the trial, is that when I had the drainpipe stuck in through my ribs into | Participant was shocked at the severity of their illness that required him to be in hospital for a week (pg6) | | | Participant was surprised at how tired they were after being discharged from hosptial (Pg6) "I could not get up a flight of stairs carrying a cup of tea without having to stop.. Made me think that my recovery might be slower than anticipated." | | | | | | Pt was concerned about the causes of his infection due to prior conditions that he thought may have returned. (Pg 2) - *'why did this happen' / association with cancer as a differential* | | | |
| | Disappointment | | | | | disappointed that his chest tube had not been inserted at an earlier time. The staff who originally did an ultrasound did not deem it necessary for the tube to be inserted however a day or so later the consultant said it was absolutely | | | | | Pts partner was disappointed that they werent able to be more involved in appointments etc with the current covid climate. (Pg2) | | | | |
| | Amused (frustrated) | Participant was amused at the fact that so many different doctors were involved and checking up on them that often the doctors gave contrasting information because they werent fully informed on the participants medical status (pg8) | | | | | | | | | | | | | |
| | Impressed/ confident | Participant was really impressed with how the doctors and staff who handled acted "I was actually very impressed with the professionalism of all the staff I deal with... I mean I was very, very impressed" (pg10) | | | Participant had great confidence in all the staff who handled him/ explained what was going to happen and that it was going to work (Pg3). "And so I am extremely confident, more so than I think I would be in the States." | | | | | | Partner felt confident in the explaination of the trial and the people who gave it. (Pg1) | | | Pt was happy knowing that they were "in the hands of experts" (Pg1) *(copied from pleased/happy theme above)* | |

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| | Afraid/anxious | Participant was worried that they may not be randomised to the best option (pg5) | | Participant was nervous to be discharged from hospital due to their heart condition (AF) and the worry that things weren't fully sorted "Yes, I think I did feel a bit nervy but not terrified" (Pg6/7) | at the prospect of maybe having surgery and the realisation that it was more serious than other minor surgeries they had had before (Pg3). "This whole affair, that has taken a month and half out of my life, and that scares me totally." ; Participant was afraid that the doctors would just walk in at any moment and say "surgery in an hour" due to a lack of communicatio n (Pg5). ; Participant was "scared to death" because of how quickly they were dealt with at the hospital - In America wehre they previously lived, you were only seen | | whole experience of having pleural infection scary because despite their medical knowledge, its always difficult to diagnose yourself (Pg8/9). "Even though, how much knowledge you have, you haven't got a clue. You haven't got a clue about what's going on and I am very bad at self-diagnosing." ; Participant was worried about the randomisation of the trial and whether they would be given the right option for them (Pg4). ; | incredibly anxious being in hospital during the current climate (Covid), especially when they got put on a covid ward because they needed the specialist care in that ward (Pg7). "Thats where all ym anxiety started. So I, literally, had a massive panic attack because they rolled me into the door where it said, Covid, don't enter.(Pg 7) ; "Participant was very anxious after being discharged from hospital as no one gave them much guidance on what to do, or phoned to | Pt felt relieved that his diagnosis was something manageable and not more serious: "I think I was relieved because the night before *I was told it might be cancer* " Pg 2. | Pt was worried about the treatment not working "I was always worried that the drain would sort of, would get higher than me, as it were, so it would backfill" (Pg 2). | | Pt was concerned about the causes of his infection due to prior conditions that he thought may have returned (patient had previously cancer). (Pg2) | Pt was frightened because they were in a lot of pain and didn't know what was going on. Further to this they were unable to take certain strong pain relief and weren't given any alternatives. "I felt really frightened actually because I didn't know what was happening really" (Pg3) | Pt was scared before the trial started because they were unsure about what was going on and were in a lot of pain (Pg2). | |
| | Grateful - *merge with confidence in care and expertise of treating team* | | Participant was grateful for the "calmness and clarity of the explanations" around the time and that the doctors and research team were always honest with her and told her how she was likely to feel. (Pg | Participant was "enormously grateful" for every person they had contact with. (Pg4); "I knew the NHS was amazing and I am moved by the amount of care I've been given" (Pg7) ; Participant felt | | | | | | | | | | | |
| | Embarrased - *merge with shock/unexpecti ng toreflect how unwell patient was 'uanble to recall any information'* | | | Participant was embarrased due to the fact that they were unable to remember much of the information they had been given before the trial started due to their physical unwellness (Pg 1) | | | | | | | | | | | |
| | Relieved - ?include | | | | to be discharged from the hospital and to be home with his family again (Pg5). "I felt relieved. I felt, I was so very happy that I was | | | | his diagnosis was something manageable and not more serious: "I think I was relieved because the night before I was | | | | | | Pt was relieved that there were options other than surgery that could be used to treat it (Pg1). |
| | Sad/upset | | | | | | Participant was sad in general at the fact that they had to go through the whole process due to being ill and gained another scar on their body. (Pg6) "Sad that I had to go through it, you know, another hospital admission." | Participant became emotionally upset during interview after speaking about the lack of support they felt after being discharged from hosptial (Pg4) - *dominance of fear/anxiety/st ress theme should indirectly emphasise importance of inpatient/post discharge emo/psych support* | | | | | | | |

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| Level of explanation/understanding | Randomisation | recalls that the trial had been explained "in quite good detail" (Pg1). However the IET arm of the trial "wasn't explained in huge detail". (Pg1) "I don't know if I had the enzyme thing explained very well. I don't know if I would know now what that would have meant." (Pg3). Participant believes that everything was explained well but couldnt remember because of their physical state at the time. Participant understood that he would be randomised but didn't have a great level of understanding | Participant was given 3 very comprehensive sheets of how the trial would work. Their understanding was very good due to reading these extensively. | Participant could not recall what she had been told because she was so ill at the time. Thought that maybe there could be an improvement on how doctors go about giving information to patients who arent in the best state to remember or know exactly whats going on (Pg1/3) | Participant was given a four page sheet which was extensive and gave lots of info on the trial. Participant explains that they understood well what was going to happen "they spelt it out very good" (Pg2) The particpnt also asked lots of questions to gain further knowledge. Particpnt said that a real positive was being able to read all about what was going to happen and that it was explained really well (Pg4). | Felt like all 3 arms couldve been explained a bit better with pros/cons of all of them to avoid prefernce bias (Pg1) was given a 4/5 page summary explaining background but didn't particularly differentiate between the three options. (Pg1) | that approached me was very clear in his information.. And left me some paperwork and gave me time to read it" (Pg1) participant also wrote questions down to remember to ask the doctors and all questions were answered (Pg2). Participant says that the study was discussed with the verbally too. (Pg2) Didnt feel they were given proper information about the IET arm of the study (Pg7) Participants first language is not english- causes language barrier | "Very informative" (pg1) "Received both verbal and written information which was very informative (Pg1) at time, participant was out of it due to the drugs theyd been given so they asked that their brother be called to relay the information ((pg1/3) because a lot of detail was gone into (Pg2) Wouldve liked more information on the pros/cons of each trial arm (Pg5) | Pt could recall a consultant discussing the trial information with them. | Pt recalls a consultant explaining the information about the trial. Pt understood the concept of randomisation well and was able to explain it to me. Pt had a general idea of the trial arms but wasn't too fussed about the details: "I didn't really go into any depth but I was quite Happy to go along with what was going on" (Pg1). | able to recall a lot mnore information than the pt, and had exceptional understanding of the trial as a whole and what it meant in a wider picture, and had a sound understanding of the three different treatment arms. " they explained the three different approaches, they explained why they needed to do a feasibility study before really, to establish whether a research programme was required or viable" (Pg1). Partner had also worked in | Pt gained a thorough understanding of the trial through reading the leaflets and literature given to him upon recruitment to the trial (Pg1). Participant also mentioned that at every stage of the trial it was explained what was going on and would be happening. | Pt was slightly confused about the concept of randomisation within the trial, thinking that is would depend solely on how they reacted to each and worked their way through them (Pg2) | Pt knew exactly what randomisation meant and how it would be used within the trial "The doctor sat and we went thorugh it together.. Everything was explained to me in detail and I was fully, you know, understanding of everything that was said" (Pg1) | Pt recalled beihng initially approached by the doctors and then it was followed up by the researchers who explained what it would entail. (Pg1). "I had no expectation.. I knew it could have been on of the other two plans as well" (Pg2) |
| | Pleural infection | Particiapnt felt that a visual model of the lung and what happens during pleural infection (Pg 4) " Maybe a model would be quite good. To say, look, heres a lung and this is the problem." | Participant explains that the information leaflet they were given was very good at explaining (Pg6). | Participant didn't understand much at the time but now that they've recovered they understand a lot more. In their check up most treatment it was explained a lot better (this could be due to the foggy memory though) (Pg2) Interviewer asks do you understand a lot better now? and participant replies "Much better, much better, oh very much so." (Pg2) | | | Understood roughly what pleural infection is but felt that a lot of the time the words "chest infection" are used and its quite generalised esepcially on the ward they were on. (Pg8/9). "There is too many words that look alike about whats going on in the lung when you get an infection, and people cant distinguish" (pg9) Felt that maybe a visual aid ike a drawing would be a good way to explain pleural infection (Pg9) | Participant didn't have much understanding of pleural infection while they were ill, but since recovering it was drawn out for them in a check up appointment and they understand muhc better (Pg6) | | Pt had a slight idea because his mother had suffered from a similar condition | | Pt had a solid understanding of PI (Pg2). | | Pt felt that it was all explained quite well by the doctors (Pg2) | Pt described PI as well as its risk factors and had a good understanding of all. (Pg2) |
| | Recovery process - *3 main points - patient expectations through how well they were informed and actual time to recovery and recovery experience (pain) - "longer than expected"* | Participant was not given much information on how long they would take to recover and what it entailed. | Participant gained a better knowledge of their recovery process from their discharge notes | | Participant didn't realise how long it would actually take to recover "I thought it was going to be over in a few days and I was going home, going back to work.. And then it went into 12 days" (Pg5) | Wasn't much info on what the recovery time periods were for each treatment arm (Pg2) ; Was given verbal information by the consultant about how painful it was going to be so they were expecting it to hurt (Pg4) | Participant was told "it would be a long haul to get well again" (Pg7) on top of this participant is anaemic which added on to the recovery time | Participant felt like they had no support and werent given anyinfo about their recovery when they were discharged (pg3) Participant had to look on google to find out what exercises they could do to aid their recovery (Pg7) | | | | | | | "All this was explained to me, that it's going to take a range of months rather than weeks to get through" (Pg4) |
| Reaction to randomisation | Positive thoughts | Participant was pleased with the treatment they were assigned to. | Participant would have been happy to have undergone the surgery arm they were randomised to- although this turned out to not be necessary (Pg2) | Participant was happy with their randomised group "I believe that I had the most suberb treatment and support in every way possible" (Pg2) Participant does mention that had they been randomised to surgery they wouldn't have known how theyd feel about that because they wouldnt have known what it was like to not have that treatment (Pg5). | Participant was happy with their treatment arm and that they didn't have to progress to different treatment because they were getting better (Pg3) | Was happy they were assigned to have the surgery because didn't have all info (Pg1) **"was happy I wasn't in the control group because I knew that I'd be getting some form of positive intervention"** (Pg1) | | | Pt was pleased as they thought that they would have required the surgery anyway: " it was probably easier to just go with surgery, rather than doing it another two times, sort of thing" Pg 2. | Pt felt "satisfied" (pg1) | Didn't fully know what the two other options what have entailed but the treatment arm seemed adequate. (Pg2) | | | | "It was the one I was looking for" (pg2) |

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| | Negative thoughts | | | Participant wasn't keen to have surgery, despite thinking they would have had whatever would make them better they were relieved not to have surgery (Pg5) | Participant was scared of the surgery because theyd only had minor surgeries before but this one seemed a lot more serious, which dawned a realisation of how serious their condition was/ could have been. (Pg3) | Participant had a preference to the surgery arm because they assumed that would be the best option- didn't have all the information (Pg1) | Participant was concerned about the chest drain due to a personal experience of watching their mother die of lung cancer "somewhere, I connected with awful things, to have things in your drain.." (Pg3) ; Patient was also worried about being randomised to the standard care because they ahd already tried that and it had not worked (Pg4/5) | | | | | Pt was in a way disappointed that they hadn't got surgery straight away **"If I just got the surgery in the first instance that would have dealt with the problem"** (Pg1) but the pt understood that this is a decision that shouldn't be arrived at lightly and understands that it could still be a possibility down the line. | | "To be honest with you, at the time it was about the only option I had" (Pg1) ; | |

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| Influences on physical wellbeing | Severity of illness before trial | "I was at a state in the progression of the infection in my pleural cavity, that meant that something had to be done" "the drain had been in for a week and I'd been on these antibiotics and.. The medics were saying, look, we're not getting to the bottom of this." (Pg1) | Participant explains that before the trial began they were "just about at my most critical illness" (Pg1). The participant explains about the pain that "I thought I'd broken a rib, it was so bad that I just couldn't move" and that not being able to breathe became a concern. The participant says that they proobably left it longer than they should have to seek help (Pg6). | Participant explains that she cannot remember any of the information she was given about the trial because she was so unwell (Pg1) "I think I was much more unwell than I thought I was." | | Highest pain level the participant experienced was when they were discharged when the antibiotics werent working before they had had the surgery. (Pg3) Had had back pain prior to the plerual infection, the pain was bad but participant believes had they not had the back pain that they were used to then they wouldve got help sooner (Pg5) | "I don't know how sick I was but I think I've been very sick, very sick" (Pg6) "It could have gone very, very wrong if I hadn't asked for help" (Pg6) "I got this horrendous pain from under my breast.. I've been ill, vomiting, that wad the movement that started my pain off again" | "I felt, I've never felt so ill in my life, if I'm honest." (Pg4) "I've never felt that bad, I've had a heart attack and I didn't feel as bad as I did this time round" (Pg10) | Pt explained that they felt feverish, tired and was sweating a lot prior to admission to hospital. Pt had aching pains in legs + discomfort in lung which only eased when he lay on that side of his body: "I was literally taking Beechams max strength, no stop the aches in my legs so i could function." (Pg 3) ; I'm not someone that normally goes to hospital or the doctors and I thought, it was that bad, I need to go" (Pg3). | Pt complained of having a cough and some shortness of breath and didn't see it necessary to see a doctor. After speaking to his GP his was sent straight to A&E for an Xray (Pg2). | Partner was relieved when pt was diagnosed and that the GP's/ doctors had got it completely right first time (Pg2) " | "I was in a significant amount of pain in the right lung area and it ocuid be quite unbearable at times" (Pg2). Pt mentions that due to his oxygen levels being so low the GP instantly referred him to hospital (Pg2). | Pt had covid and felt absolutely fine, then a day or so later they were in excruciating pain "In myself I felt absolutely awful, the pain was horrendous" (Pg2) ; "I woke up with a terrible back ache I could hardly move" "I had to call an ambulance because I just, the pain was horrendous" (Pg3). | "I was in absolute agony, I couldn't breathe and..I couldn't stand up, I couldn't sit down, there was no position I could be in where I felt any comfort whatsoever" ; "I was pretty scared that I was suffocating" (Pg2). | Initially started as a cold, and then progressed a week after the original pneumonia diagnosis when it didn't get better. "I started to feel quite nauseous.. I was feeling very very flat, and afatigued" "The day after I was brought into hospital, then things started to get sort of progressively worse quite quickly" (Pg3) |
| | pain level during treatment | | Participant was due to have surgery buut responded so well to initial treatment that it was not required.(Pg2) | Participant mentions that the only thing they can remember being unpleasant was having the chest drain in which was "immensely uncomfortable but, obviously, very necessary" (Pg4) Participant wasn't informed about the pain they were going to feel, but ended up being glad about this (pg4) | Particiapnt states that having the tube inserted and afterwards was "extremely painful" (Pg1) However it did relieve their previous pain. | | When they put the IET in through the tube the participant recalls having 5 hours of pain and could only take one dose "I only had one because it was so painful, I said, I don't want it." (Pg3) | Says it was very painful but they were expecting that (pg2) "I'll never forget that he kept saying, its going to be really painful" | | Pt was comfortable during treatment, just concerned that it may reverse and all the fluid would go back into his lung (Pg2). | "I think the first episode he had was pretty uncomfortable, they delayed giving him the second pumping" (Pg2). | "The drain took immediate presssure off of that (the lung pain) and helped to sort of improve my condition" (Pg2). ; "Every procedure was done with the maximum amount of care possible and thought" (Pg2). | Painful. It was very painful, yes." ; "The first one was fine (the IET), I didn't feel any pain at all when it was in, and the second one was fine, but then the third and fourth was horrendous pain" ; "The whole body on one side just felt it was on fire, it was awful" (Pg4) | | "The actual process was very painful.. Its very much like being punched in the kidneys by Mike Tyson several times" (Pg3) ; "I wasn't quite prepared for the amount of pain that I would be in during that initial four or five hours after putting the drug into you" (Pg3) |
| | response to treatment *(colour code with randomsiation arm)* | Sharp pain experienced Participant responded across the chest a few incredibly well to intial times post-op "It was antibiotics and chest almosrt like heart tube that surgery was no attack pain" (Pg6). longer required (Pg2). | Participant responded well to increased antibiotics and didn't need further treatments (Pg5) | "The initial pain, when I first came into the hospital, I told them this, it was an eleven on a scale of one to ten... and after they put in the tube, it was eight and a half to nine" (Pg1). | After surgical procedure their left side was very "tender" and patient wasn't able to lie on their left hand side or their chest. The drains weren't particularly uncomfortable. Having them removed was slightly uncomfortable but not massively. Slightly sleep deprived due to new sleeping position because couldnt sleep on normal side due to pain (Pg3/4) | Participant only had one dose of treatment because they couldn't bare another- Scans the next day showed that the treatment had worked a bit so it wasn't required again. (Pg4) | "I felt really well kind of straightaway" (Pg2) " its like somebodyhad pulled the plug and all the pressure had been taken off." (Pg4/5) - *which arm?* | Pt felt a great post-op "it was an immediate improvement" (pg3); Had an odd side effect of sweating in reaction to the pain relief "there were a few weird things, like sweating at night.. Was down to the tramadol" (Pg3). | | "It did its job... He began to recover very quickly after they initially started the programme" (Pg2). | The drain took immediate pressure off the pain (Pg2) | "The treatment worked a treat... I've never seen it clear a chest like that in one dose, so it was really good" (Pg9). - proably clinician quote | "The treatment, more of less, was very effective, I think it worked straight away" (Pg 3). | "The success of the treatment was fantastic.. The infection markers went down.. I was released very quickly.. Three or four days after my final dose" (Pg3-4). | |
| | Recovery | | Participants recovery has been successful and participant explains that "my energy levels are back up and I'm back doing fitness training." (Pg5) Participant still has some soreness on the damaged lung when taking deep breaths (Pg5) ; a week after returning home participant experienced a sinmilar pain on the other side of their chest which was concerning but this sorted itself out my itself (Pg7) | | | Was surprised at how tired they were completing the smallest tasks "my stamina has been sort of significantly reduced by this." (Pg6). Particpant also found it painful moving round corners due to having to move their arms (Pg6). "its been more of an impact than I thought it would in terms of fatigue, shortness of breath, and kind of my general ability" (Pg6) | Participant was very ready to go home for a few days before they actually got discharged (Pg10) "I knew I was going to cope. I had my medication, my antibiotics. The fluid had gone and I knew it will be a long haul to get better" (Pg10) | Participant despite lack of support still could feel themself getting better, mentioned how tired and drained they felt (Pg5) " By the time you've got dressed, had your breakfast, you, literally need to go and have a little siesta" Participant had a friend who was a good support throughout the recovery process. (Pg7) Had a horrible cough that was making them gag (Pg8) that was very normal but this was only explained to them after theyd rung a GP in worry. | "I felt better but I felt weak" (pg3) ; "If I went out I felt a bit frail walking, a bit light in the head, because obviously my body has had quite a lot of work done to it" (pg3) | | "He is insistent he's absolutely fine. I think he's lacking stamina but that's pretty normal at 77.. it's going to take a while to build it back up again" (Pg2). "He appears well and I'm very comfortable that they sent him away with a perfectly normal sort of response" (Pg3). | "I was still a bit short of breath when I came out, I live in the top floor of a tenant, so I'm still a bit short of breath coming up but apart from that I've been absolutely fine" (Pg3). | "It's taken it out of me, I cant, my breathings a bit short at times and I cant do what I was doing, like a lot of things" ; "When I came home I felt a bit rough and short of breath but things are really coming on much better" (Pg9). | "I didn't feel I was as fit as I was before I went in but that's always ging to take some time" ; "Just before I was discharged, I felt, you know, I'd never felt better to be honest..when I got home, I suddenly realised that..I wasn't as well as I thought" (Pg3) | "I'm just coping with the aftermath of it" ; "I do have back pain sometimes, quite a bit to be honest with you. I do struggle with fatigue still and not quite feeling myself.. I very quickly, I can get breathless" (Pg4). |
| Overall experience | Positive comments | Participant was very impressed with all the staff that were involved from the consultants to the surgeons to the nursing staff. "I felt very confident I was in the best possible hands" "The surgeon and the doctor who I was under were fantastic, absolutely fantastic." (Pg10) ; Participant felt that the research team (nurses) were present a lot throughout the process and that this was a positive thing (pg5) | | Participant stated that "Its been an incredibly positive experience from a really nasty, you know, a nasty shock" (Pg8) and that the response to their condition was immediate which was really comforting ; Participant felt that the research team and the clinical team worked together incredibly well. "I felt that the research staff were there alongside the clinical team and were very well integrated" (Pg3) | Participant on multiple occasions states how they felt they had been treated with empathy and compassion and how grateful they were (Pg1/3/7) "I am moved by the amount of care 've been given. Not just the professionalism, that immense compassion and love really, to be honest with you." (Pg7) | I see nothing but positives about being part of the study" (Pg5) "they were all kind, informative, put up with my stupid questions, and I was glad they were taking care of me" (Pg5) Participant explains that they were dealt with very quickly from the moment they arrived in the ambulance (Pg6). | On several occasions participant mentioned how amazing the nursing staff had been "the nursing staff were excellent throughout" (Pg5/6/7) also that the surgeons and registrars and consultants were very professional and clear (Pg6/7) | I was treated so nicely in that ward with everybody that approached me (Pg2) ; Participant talks about one of the research team who went to visit them and says "She had another approach to things than the doctors and nurses.. I felt, I was seen as a whole being and there was an interest in me as a whole person, not just, how are you today with your lungs and your mobility" (Pg5) | becaime they a priority "even to the point where I felt quite special in a time that was really horrible" (Pg2) The consultant that the participant saw in their check up was "amazing, so reasuring, everything he said, he knew exactly what had happened to me" (Pg10) definitely thought the procedure was worth it. (Pg11)overall was a good experience (pg13) ; Person who approached them for trial was really reassuring that it would help (Pg1) The team made them feel really special | Pt couldn't fault the treatment they were given whilst in hospital "Ten out of ten. Its been really good" (pg4) "I'm over the moon with the service" (Pg 5). | Pt has been overwhelmed with the support he received throughout the trial and even now during the follow up period: "twelve out of ten because they were absolutely brilliant... even down to the what Iwould call the tea trolley and the cleaners, everything else" (Pg3). Pt thought the contact and communicatio n he has received throughout couldn't have been faulted (Pg3). | "its been rewarding and satisfying to know a little bit more about what they were doing. I would've said it was a positive experience not a negative one" (Pg3). | | Pt had constant communication about what was being done and when "every single morning they were there.. And everything was explained and I asked questions, you know, I can't fault them at all" (Pg8). | "There's nothing I could criticise, I just can't fault it" (Pg3) |

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| | would coudve been improved | More consistency of care, there were a few occasions where the participant was given conflicting information by different staff who were checking up on them. (Pg8) | Participant ended up feeling as if they were not properly involved in the study due to the fact that they did not require the trial arm that they were randomised to as they had repsonded so well to initial treatment (Pg3) ; Participant wouldve liked more information on how they came to the decision that they no longer needed to do the surgery/ why did they not do it- couldve been more communication beforehand about what might happen if they respond well (Pg3/4). Participant would want to ask how they can prevent this sort of thing happening again and what the risks of having | Participant felt that they left it too long before they went to see the GP and that it worsened in that time. **Would like for there to be more information to the public about when you should see a doctor or GP** "If it wasn't for a friendhearing me not breathing properly and waking me up to see a paramedic... Im not entirely sure I wouldve made it through to the next day" (Pg5/6). "Its something about knowledge in the public arena about, when do you really need to check things out" (Pg6) ; Participant didnt fully grasp what the study was about in the beginning - physicaly | Participant was concerned about how long they would need to take off work because the time frames of how long it all would take/ how long theyd be in hospitall for hadnt really been explained to them. Maybe more info on time frames? (Pg4) Also a little more clarity while treatment is ongoing and what is going to happen next rather than it all being rather last minute information (Pg5) ; Participant felt that they weren't given much *info at the end when they were getting isut sent home and had to figure out for themselves that that was it; "If someone* | Filling out questionaires after being assigned a group - Doesn't leave space to indicate whether you've had painkillers or not at the time of talking about your pain levels- could be redesigned to take into account whether or not pain relief has been given at time (pg3) ; More feedback about what the Xrays/CTs show- felt like they weren't properly informed about what was actually going on. (Pg6/7) ; just explaining a bit more clearly so people understand the differences between the three trial arms (Pg7) ; After participants first | Drawings or sketches to show whats actually going on (Pg10) ; At times found it intimidating having so many different professional profiles all present and doing different things (Pg9) | Some sort of documentation to take home to know what to expect (Pg8) Would like for someone to come and explain what happens next after the treatment has been delivered. Just some contact to give some reassurance- more emotional support afterwards (Pg10/13) ; After they were discharged, no one checked up on them ofor weeks- until someone from the research team called 4 weeks later. (Pg3) Participant became very anxious and lost sleep. ; Felt that there was a lack of teamwork and | Pt had no comments to make about things that could have possibly been improved and couldn't find any faults. The only thing they mentioned was a sample that got mixed up in the lab resulting in them needed a generic course of antibiotics instead of more specific ones, which worked anyway. | The hospital food (lol). | Partner mentions that if the HCPs could speak a bit slower when explaining details etc to give the patients a chance to understand/ ask questions. "Sometimes speak slower because theyre dealing with seventy pulses" (Pg3). | Better communication between the doctors in morning MDTs and rounds as sometimes consistency in information about when and what will be happening (Pg3). | At the beginning of the trial pt was slightly confused aout the treatment path and whether it was what normally would be done or whether it was a conmpletely different treatment that may or may not work, and was unsure if the research team and MDT had differing opinions that contradicted each other "I wasnt sure if the two of them were on different sides..would the doctors have treated me like that?" (Pg1) ; Pt was in a lot of discomfort and the codeine made them feel even more sick, would've liked a different option for people who can't tolerate certain medicines (Pg3) ; Pt was never given a definitive answer as to | | Pt explained that maybe some pain relief to coincide with the IET treatment going in would be beneficial"to make that experience a little bit more comfortable" (Pg5) |

## APPENDIX A4.5

**The third Multi-Centre Intra-Pleural Sepsis Trial (MIST-3): Early Video Assisted Thoracoscopic Surgery (VATS) or Intrapleural Enzyme Therapy (IET) in Pleural Infection – a feasibility, randomised trial**

**SUMMARY PARTICIPANT INFORMATION SHEET**

You have been asked to participate in this trial because you have a condition that has caused infected fluid within your chest – called pleural infection. Successful treatment of this condition would normally require you to be admitted to hospital to receive antibiotics and to have the infected fluid drained out of your chest. There are three known treatments for this condition that are used in clinical practice. However, they have not been directly compared with each other. The objective of this trial is not to carry out this comparison, but rather to assess the possibility of asking participants like you to be randomly allocated to one of the three treatments. This will help us decide if a bigger research study actually comparing the three treatments can be done.

The three treatments are:

1) Standard care - Administer antibiotics and insert a chest tube to drain the infected fluid.
2) Standard care plus Intrapleural Enzyme Therapy (IET) – this involves additionally giving you two drugs through the chest tube (DNase and Alteplase).
3) Standard care plus keyhole surgery to drain infected fluid – known as Video Assisted Thoracoscopic Surgery or VATS.

By now, you may have already had or be awaiting a chest tube insertion and antibiotics, which is the current standard care. If you agree to take part in the trial, a computer will randomly select if you carry on this treatment alone or have one of the additional treatments. In order to not delay treatments and carry out a fair comparison, we aim for this to be decided within the first 24 hours of your condition being diagnosed.

The IET involves 2 drugs being injected through the chest tube to help break up any pockets of fluid with the aim of aiding the fluid drainage. This will occur twice a day during the first 3 days of your hospital stay. These medications are not yet licenced for use in pleural infection specifically but have proven safe and effective for it in a previous large trial and widely used in treating this condition with over 500 published cases from around the world. Possible side effects of IET include occasional bleeding, chest discomfort and allergic reactions.

If you are selected for keyhole surgery, a chest surgeon will assess your fitness and suitability and discuss the planned procedure with you first, so this does not necessarily mean you will definitely be having an operation. If you are suitable for an operation, potential side effects include bleeding, chest wall pain and chest or wound site infection.

During your stay you will have some blood tests, chest x-rays and chest ultrasound scans as part of routine care to assess how well your infection is responding to treatment. In addition to the samples of blood and pleural fluid that are taken routinely as part of your care, an additional 20ml (approx. 4 teaspoons) of each will be collected solely for the purposes of the research study. These will be de-identified to protect your confidentiality. A research team member may also visit you to go through some questionnaires if you are well enough, regarding your health, mobility, activities and pain.

Once you have recovered from your acute illness, a member of the research team may contact you to discuss your views on the treatment you received and your participation in the study through a series of standardised questions.

You will be required to attend the outpatient clinic, as you would normally at roughly 2 weeks, 2 months with an optional follow up at6 months after discharge. No additional visits are required for the purposes of the study. At these appointments, you will have similar tests to the ones you had in hospital to assess your recovery, as well as a breathing test.

Participation in the study is completely voluntary and you may choose to withdraw at any stage without any effect on your medical and nursing care. Unless you state otherwise, any samples taken up to that time can still be used for the research. You may also wish to withdraw but allow us to keep in contact to let us know your progress.

If there are any problems, your care and wellbeing will be the utmost priority and your hospital doctors will do what has to be done to help you. This will be reported to the study team and acted on accordingly.

# PARTICIPANT INFORMATION SHEET

**You are being invited to take part in a research study called MIST3. Before you decide to take part it is important for you to understand why the research is being done and what it will involve. Therefore, please take time to read the following information carefully and discuss it with others if you wish. Please feel free to ask us if there is anything that is not clear or if you would like more information.**

## What is the purpose of the study?

This study is called a feasibility study and it is designed to assess the possibility of asking participants like you to be randomly allocated to one of three treatments, which are detailed below, for the treatment of your pleural infection. During the study we will gather data on the percentage of participants willing, or not willing, to take part in order for us to run a much larger study in the future.

The three treatment modalities are:

1) Standard care - Administer antibiotics and insert a chest tube to drain the infected fluid that has collected within your chest
2) Standard care plus Intrapleural Enzyme Therapy (or IET) – this involves giving you two drugs through the chest tube (DNase and Alteplase).
3) Standard care plus keyhole surgery to drain infected fluid – known as Video Assisted Thoracoscopic Surgery or VATS.

All of these treatment options are known treatments for your condition (pleural infection).. However, they have not been directly compared before and this feasibility study is looking to assess whether a direct comparison in a research study is possible.

## Why have I been invited?

You have been invited to take part in this study because you have a condition that has caused infected fluid to accumulate around your lung. This forms a collection around your lung and it needs to be drained for you to recover.

This study is being conducted in hospitals within the UK and we will aim to recruit 75 patients to this feasibility study.

## Do I have to take part?

It is up to you to decide whether or not to take part. This information sheet is to help you make this decision. If you do decide to take part you will be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect your future medical care outside the study.

If you decide not to take part we may also ask for your consent for one of our study doctors to contact you to ask the reasons why you didn't want to take part as your views are very important to us and will be very valuable in adding to our understanding for the purposes of this trial.

If you are, or could be, pregnant or lactating, please let a member of your clinical team or the trial team know as you will not be able to take part in the trial.

**What will happen to me if I decide to take part?**

Firstly, you can be reassured that there will be no delay to your treatment. A chest tube will be inserted into your chest to drain the infected fluid and you will receive antibiotics in the usual way.

If you enter the study you will receive one of the following treatments in addition to standard antibiotics; to continue with the chest drain, which is the current standard of care, or alternatively you will receive two drugs through the chest tube, or be considered for a surgical procedure. This will be randomly selected by a computer. To be able to carry out a "fair" comparison, we aim for this to be decided within the first 24 hours of your condition being diagnosed.

Regardless of which treatment you receive, you will be admitted to hospital to treat the infection with antibiotics which is normal care, for a period of up to one week. This period may vary depending on the progress of your condition.

If you are selected to be in the group receiving the drugs through the tube (IET), these will be injected into your chest tube and left for one hour to mix with the infected chest fluid. This will be twice a day for 3 days.

If you are selected to be in the surgical group, a surgeon who specialises in chest surgery will be asked to assess your case. They will assess your fitness and suitability for an operation to be carried out safely and discuss the planned procedure with you. The surgical procedure itself is well established and not "experimental" in any way – during this feasibility study, we are assessing whether it is possible to compare these different treatment options.

However, if your chest tube drains successfully and your clinical team feels you have made a good response within the first 24 hours, you will not be eligible to be randomised so your inpatient treatment will continue as per standard care. A member of the trial team will contact you by telephone two weeks after discharge to see how you recovered and ask you about your experience during your stay. This will be followed up by one further telephone call at approximately 3 months after discharge, to see whether you have required any further treatment.

In all randomised cases in this study, during the first week of the study you will have routine daily blood tests to assess how well your infection is responding to the treatment. These tests are normal care for this type of infection and will be needed even if you do not decide to take part in the study. We will look at these blood tests to help us assess whether your infection is improving and to check for any side effects which may or may not be caused by the treatment. You will also have chest x-rays and sometimes chest ultrasound scans throughout your treatment as normal care. One chest x-ray will be done before entering the study and you may have already had one done as part of diagnosing your infection. The radiation risk from a chest x-ray is extremely low and this trial will not expose you to any more radiation than what would be undertaken as part of routine clinical care. During your stay in hospital, a research team member may ask you to go through some questionnaires if you are well enough. These questionnaires will be about your health, mobility, activities and pain.

After you have received the study treatment, if this was standard treatment or medication through the chest tube, your hospital doctors will decide whether the infected fluid has drained successfully. If it has not, they will advise you whether you then need an operation, to help remove any remaining infected fluid. This will be at the discretion of your own hospital doctors and is not decided by the organisers of the study. If you underwent the surgical treatment, normal surgical care after the operation will be conducted.

You will then need to visit the out-patient clinic, as you would routinely, within two to three weeks of discharge. This will be followed by a further appointment at two months which is routine care and at 6 months however this is optional. .

During these appointments you will have basic breathing tests, a blood test and a chest x-ray and a doctor will assess your progress.. As well as being seen by a clinician, a member of the research team may go through the same set of questionnaire you may have completed whilst in hospital.

**What should I consider?**

If you agree to take part, your doctor will arrange the study treatment. Once you have recovered from your acute illness, a member of the research team may contact you to discuss your views on the treatment you received and your participation in the study. Even if you decide not to take part in the randomisation process and continue with normal care, your views are still very important to us. Providing you are happy for us to do so, we may also contact you to discuss these. Interviews will be either performed by skype or over the telephone arranged at a convenient time for yourself and if you are asked to come to hospital for interviews, any travel expenses relating to these that are additional visits to normal care will be fully reimbursed. All interviews will be audio recorded.

**Are there any possible disadvantages or risks from taking part?**

The main risk of taking part would be any unexpected side effects from one of the drugs (IET) or a surgical complication. These will be described in detail below. Both these treatments (the drugs in to the chest and surgery) are already used in the NHS for patients, and so we know quite a lot about their complications and how to deal with them,

You will be monitored closely for any such events and should these occur, they will be promptly addressed by the team of nurses and doctors looking after you. The study team would also be notified. If any unforeseen complications are felt to be a result of the medications given through the chest tube, they will be stopped immediately. Your surgeon will be very experienced and will have performed many of these procedures, and as is standard, there will be a qualified team to help manage any complications, should they arise. At the end of your hospital stay, you will also be given contact details for any advice, should you have any queries or problems, once you are discharged.

<u>Chest tube medications (Combined DNase and Alteplase)</u>

DNase is routinely used in patients with cystic fibrosis (where it is inhaled into the bronchial tubes). There has been extensive testing to establish that this use is safe and it has been used in many thousands of these patients. Alteplase is routinely used in the treatment of patients with a stroke and occasionally in those who have suffered a heart attack. It is also known as a ‘clot busting’ treatment. In this context it is normally injected directly into the bloodstream.

Unlike in the conditions above, in pleural infection, these drugs are used in much smaller doses and are therefore likely to be of an even lower risk. They have been tested separately, and in combination, in a previous large trial of patients with pleural infection, where they were administered in exactly the same way they would be done in this trial; through the chest tube. This trial found their use in combination to be safe and beneficial in the treatment of your condition, but there were not enough patients for it to be formally licenced. The treatment has been safely used in over 500 published cases of patients with your condition. The main side effects include chest discomfort (20%), allergic reactions (3.8%) and bleeding (1.8%).

There may be other side effects that we do not yet know about and we will collect careful information on any such possible side effects. Your hospital doctor will be informed if any new, unexpected, or serious side effects are found which are thought to be related to the study treatment. Therefore reporting of any symptoms to your hospital doctor is an important part of the study assessments.

Surgery

The surgery used here will be video assisted thoracoscopic surgery, or VATS, which is a type of “keyhole surgery” and is much safer than open surgery. However, as with any procedure, there are risks and complications can occur in an estimated 3-4%. These include persistent collapse of the lung, requiring a prolonged chest tube drainage until it resolves, or bleeding, which may require a blood transfusion or another operation. Other problems that may occur include chest wall pain after the operation, or infection (5%), either in the chest (pneumonia) or in the wound site, which would be promptly treated with antibiotics. There are risks associated with a general anaesthetic but your surgeon and anaesthetist will go through these in more detail before any planned procedure.

Blood samples

Routine blood tests will be taken throughout the course of your treatment to monitor your progress. These will be carried out by fully trained healthcare professionals including doctors, nurses and clinical support workers trained in phlebotomy. Possible side effects include bruising and/or fainting. There is a very small risk of infection but blood sampling will be carried out in a manner that strict infection control procedures are followed.

Interviews/Questionnaires

These will be standardised questions that will be agreed by the study team and asked to all the participants in the same way. They will not include any personal questions or anything of a sensitive nature. The aim of these will be to reflect your experience during the course of treatment you had and your answers will be important of informing the researchers of how the patient experience differs through each of the different treatment pathways for this condition. Your responses will be audio-recorded and a member of the trial team will transcribe the data

**What are the possible benefits of taking part?**
Many people suffer with your infection all over the world. We have three treatment options available, which are being used in this study. By taking part in this feasibility study, you will

be helping to inform medical professionals whether a larger study to fully compare these treatment options would be possible in the future.

**Have any special considerations been made in light of the COVID-19 pandemic?**

**We have taken specific steps to ensure that your participation in this study does not put you at increased risk of contracting COVID-19. Firstly, we would like to reassure you that this condition involves in-patient treatment regardless of your participation of the study. We do not know that any of the treatment options used in management of pleural infection (standard chest tube drainage, chest tube medications or surgery) is associated with a longer length of stay in hospital and ultimately this will be determined by your individual condition and how well you respond to treatment.**

**In relation to the follow up appointments, the initial follow up (approximately 2 weeks after discharge) is always carried out in person (face to face) in routine care as it is important that we carry out an ultrasound scan, perform a chest x-ray and re-check your blood tests to ensure you continue to make a good recovery. There have been strict measures taken place to ensure all patients are screened for symptoms and have a negative COVID-19 test before they attend the clinic to ensure that fellow patients do not pose a risk to you by you attending your appointment. In addition, social distancing measures have been taken to ensure patients are appropriately spaced apart in waiting rooms with restrictions on numbers who share a waiting room at any single time. Patients attending 'face to face' appointments will be flagged to the doctors in clinic to enable you to be prioritised and be promptly seen by a doctor as soon as you check in on arrival.**

**We will endeavour to ensure you are not in the department for any longer than needed e.g. you will not be asked to be seen again once you have had your chest x-ray and blood tests; instead if there are any changes to your treatment plan in light of these tests, your doctor will contact you by telephone to discuss this. The research team will give you the option of completing any questionnaires with them remotely e.g. over the telephone should this be your preference. Moreover, if your treating doctor is satisfied with your recovery, any future follow up (at 2 months and 6 months) will also be completed remotely. However, if it is clinically preferable for you to be seen again in person, this will be made clear to you. No face to face appointments will be scheduled purely for any purposes of the research study.**

**Will my General Practitioner (GP) be informed of my participation?**

Your GP will not routinely be informed of your participation. Your participation in this study will not affect any other aspect of your clinical care. Your treatment and follow up will be provided in full by your clinicians in hospital or remotely by the study team. Any incidental findings or unexpected events will be handled directly by your treating doctors and the study team, and if any action is required outside the scope of the study or managing your condition, your GP will be informed.

**Will my taking part in the study be kept confidential?**

All the study information is stored in a secure electronic system and participants will only be identified by a unique code. Responsible members of the University of Oxford, Oxford Brookes University, regulatory authorities, and the relevant NHS

Trust(s) may be given access to data for monitoring and/or audit of the study to ensure that the research is complying with applicable regulations.

**Will I be reimbursed for taking part?**
Travel expenses for any visits additional to normal care will be reimbursed on production of receipts, or a mileage allowance.

**What will happen to the samples I give?**

If you agree and with your consent, a small sample of the infected fluid from your chest (about 20ml/0.6flozs approx. 4 teaspoons), will be taken from what drains from your chest tube and would normally be thrown away. We will also collect a small sample of blood (20ml/0.6flozs approx. 4 teaspoons). These samples will be frozen in the Oxford Radcliffe Biobank - identifiable by your unique code. This protects your confidentiality, but also means we will not be able to give you your individual results.

These samples will be used in the future for ethically approved research. Some of the tests on blood samples will be gene studies. For these gene studies we will look at many genes relevant to chest infections including newly discovered ones as they are understood. The infected chest fluid, blood samples and other information collected as part of this study may also be used in other research (some of which may be funded by commercial companies) with a view to developing medical diagnostic tools and new treatments for doctors to use to help other future patients like you. Your samples will not be used in the creation of immortal cell lines, animal studies or in the Human Genome Project.

If you agree to your samples being used in future research, your consent form will be held until the samples have been depleted or destroyed.

**What will happen to my data?**

Data protection regulation requires that we state the legal basis for processing information about you. In the case of research, this is 'a task in the public interest.' The University of Oxford is the data controller and is responsible for looking after your information and using it properly.

We will store any research documents with personal information, such as consent forms, securely at the University of Oxford for 5 years. If you agree to your samples being used in future research, your consent form will be held until the samples have been depleted or destroyed. We will keep any other identifiable information about you for 6-12 months after the study has finished.

The audio recordings of your interview will be stored electronically within the Oxford Respiratory Trials Unit at the University of Oxford and will be sent securely to a professional transcription company, and then to Oxford Brookes University for analysis, both with whom the University has a contract and confidentiality agreement. The transcriptions will be de-identified and the transcriptionist will delete the recording when they have completed their work.

The local study team from your hospital will use your name and NHS number to contact you about the research study, and make sure that relevant information about the study is

recorded for your care, and to oversee the quality of the study. They will keep identifiable information about you from this study for 6 -12 months after the study has finished.

Data protection regulation provides you with control over your personal data and how it is used. When you agree to your information being used in research, however, some of those rights may be limited in order for the research to be reliable and accurate. Further information about your rights with respect to your personal data is available at http://www.admin.ox.ac.uk/councilsec/compliance/gdpr/individualrights/]

You can find out more about how we use your information by contacting us at respiratorytrialsunit@ouh.nhs.uk

*If you agree to your details being held to be contacted regarding future research, we will retain a copy of your consent form until such time as your details are removed from our database but will keep the consent form and your details separate.*

**What will happen if I don't want to carry on with the study?**
Participation in the study is completely voluntary and you may change your mind at any stage. You can be completely reassured that your withdrawal from the study will not affect any care you receive from the medical and nursing team looking after you. Standard treatment of your condition will continue in keeping with current guidelines and you will be followed up after discharge in the usual way. If you withdraw from the study, unless you state otherwise, any blood or fluid samples which have been collected whilst you have been in the study will be used for research as detailed in this participant information sheet. You may also wish to withdraw but keep in contact with us to let us know your progress. Information collected may still be used. You are free to request that your blood or tissue samples are destroyed at any time during or after the study.

**What will happen to the results of this study?**
At the end of the study these results will be made available to all doctors, through publication of a medical “paper”, and presentation at medical conferences.

**What if we find something unexpected or new information becomes available?**
As already discussed, this study is looking to evaluate the possibility of comparing three established treatment modalities against each other and there is no new or experimental treatment being used.

The committee monitoring this study will continue to review all new research data. If any new information that influences the study becomes available, alterations will be made accordingly to the study (including patient randomisation, patient information etc. wherever appropriate). Patients will be contacted about new data via their hospital doctors at their recruiting centre.

**What if there is a problem?**
If there are any problems, your hospital doctors will do what has to be done to help you. They will let the study team know about any problems, and they will act on this information and pass the information on to others in the study as is needed. If the problem is serious and maybe due to a study drug, the study drug treatment will be stopped. The University of Oxford, as Sponsor, has appropriate insurance in place in the unlikely event that you suffer any harm as a direct consequence of your participation in this study. Indemnity (cover) and/or compensation in the event of a claim by, or on behalf of, participants for negligent or non-negligent harm will be provided by the University of Oxford. NHS indemnity operates in respect of the clinical treatment which is provided.

If you wish to complain about any aspect of the way in which you have been approached or treated, or how your information is handled during the course of this study, you should contact the chief investigator Professor Najib Rahman via email najib.rahman@ndm.ox.ac.uk or telephone 01865 225230. Alternatively, you may contact the University of Oxford Clinical Trials and Research Governance (CTRG) office on 01865 (6)16480, or the head of CTRG, email ctrg@admin.ox.ac.uk

The Patient Advisory Liaison Service (PALS) is a confidential NHS service that can provide you with support for any complaints or queries you may have regarding the care you receive as an NHS patient. PALS is unable to provide information about this research study.
If you wish to contact the PALS team please contact 01865 221473 or alternatively you can email PALS@ouh.nhs.uk

**How have patients and the public been involved in this study?**
Patients treated in the Oxford Pleural Unit helped develop the research topic and what research questions should be asked. A focus group of patients and carers were consulted with regard to the design of the study and reviewing this participant information sheet. We have taken into account patient opinions on the frequency of hospital visits and the tests that we will carry out. Potential participants were involved in describing the inclusion and exclusion criteria for people taking part in this study.

If this is something that appeals to you, the following links provide general information about taking part in research:

- www.crn.nihr.ac.uk/can-help/patients-carers-public/how-to-take-part-in-a-study/
- www.nhs.uk/Conditions/Clinical-trials/Pages/Introduction.aspx

**Who is organising and funding the study?**
The study is run by the Oxford Respiratory Trials Unit. The study is being funded in full by a grant from the National Institute of Health Research as part of their Research for Patient Benefit Programme. Independent experts will regularly monitor the progress of the study in terms of both safety and benefits from the study treatment.

**Who has reviewed the study?**
All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect participants' interests. This study has been reviewed and given favourable opinion by East of England - Cambridge East Research Ethics Committee.

**Participation in future research:**
Provided that you agree for us to do so, your personal details will be kept so that we may contact you regarding similar studies in the future. Agreeing to be contacted does not oblige you in any way to take part in future research.

*Thank you very much for reading this information and for considering taking part in the MIST-3 Trial.*

**Further information and contact details:**
(Local Principal Investigator):
(Research Nurse):

# CHAPTER 5

## Bleeding Risk With Combination Intrapleural Fibrinolytic and Enzyme Therapy in Pleural Infection – An International, Multicentre, Retrospective Cohort Study (RETROLYSIS)

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## Contributor statement

JA, EOB, NMR and LY conceived the study. JA curated the database. EOB performed the literature search. All authors contributed to the design of the study and/or collected data. EOB and NMR analysed the data. EOB wrote the first draft of the manuscript. All authors reviewed and approved the final manuscript. JA and EOB verified the underlying data and jointly act as guarantors.

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## 5.1 ABSTRACT

Background

Combination intrapleural fibrinolytic and enzyme therapy (IET) has been established as a therapeutic option in pleural infection. Despite demonstrated efficacy, there is a sparsity of studies specifically designed and adequately powered to address complications. The safety profile, the effects of concurrent therapeutic anticoagulation and the nature/extent of non-bleeding complications remain poorly defined.

Study Design and Methods

This was a multicentre, retrospective observational study conducted in 24 centres across the United States and the United Kingdom. Protocolized data collection on 1851 patients treated with at least one dose of combination IET for pleural infection between January 2012 and May 2019 was undertaken. The primary outcome was the overall incidence of pleural bleeding defined using pre-hoc criteria.

Results

Overall pleural bleeding incidence was 76/1833=4.1% (95%CI 3.0% to 5.0%). Using a half-dose regimen (tPA 5mg) did not significantly change this risk (6/172=3.5%; p=0.68). Therapeutic anticoagulation (AC) alongside IET was associated with increased bleeding rates (19/197=9.6%) compared to temporarily withholding AC prior to administration of IET (3/118=2.6%, p=0.017). As well as systemic AC, increasing RAPID score, an elevated serum urea and platelets $<100x10^9$ L were associated with a significant increase in bleeding risk. However, only RAPID score and use of systemic AC were independently predictive. Apart from pain, non-bleed complications were rare.

Interpretation

IET use in pleural infection confers a low overall bleeding risk. Increased rates of pleural bleeding are associated with concurrent use of AC but can be mitigated by withholding AC prior to IET. Concomitant administration of IET and therapeutic AC should be avoided. Parameters related to higher IET related bleeding have been identified which may lead to altered risk thresholds for treatment.

## 5.2 INTRODUCTION

Pleural infection is rising in incidence (Arnold et al., 2021; Bobbio et al., 2021; Mummadi et al., 2021) and remains associated with prolonged hospital stays and high mortality (Corcoran et al., 2020). Combination intrapleural fibrinolytic and enzyme therapy (IET) with tissue plasminogen activator (tPA) and deoxyribonuclease (DNase) has been established as a surgery sparing 'rescue' treatment option (Chaddha et al., 2021). This may be required in approximately 30-40% of pleural infection cases who fail to respond to standard medical care with chest tube and antibiotics (Corcoran et al., 2020). Given the increasing numbers of older patients, where frailty and comorbidities often preclude surgical options, IET has been an important addition to the therapeutic armamentarium. Despite data demonstrating efficacy (Rahman et al., 2011), the safety of IET, in particular the potential intrapleural bleeding risk, remains a major concern for clinicians in choosing between prompt IET initiation and surgical referral.

A recent Cochrane systematic review into the use of intrapleural fibrinolytics in pleural infection concluded that there was insufficient data to give a precise estimate of the overall risk of significant adverse events (Altmann et al., 2019). The MIST-2 study recruited 52 participants in the tPA/DNase combination arm and reported 2 bleeding events giving an overall bleeding rate of 3.8% (Rahman et al., 2011). Subsequently, a number of smaller studies have reported rates of pleural bleeding with intrapleural administration of tPA (with or without DNase) in the context of pleural infection of between 1.8 and 12% (Abu-Daff et al., 2013; Alemán et al., 2015; Kheir et al., 2018; Majid et al., 2016; McClune et al., 2016; Mehta et al., 2016; Piccolo et al., 2014). Other than the heterogeneity between all these studies, the key limitation is the small study populations and therefore low event rates.

Without a pre-hoc definition of pleural bleeding, it is easy for a bleeding outcome to be over- or under-reported, as the use of IET is known to cause hemorrhagic discoloration of pleural fluid. It remains unclear if dose reduction alters bleeding risk, as studies evaluating such strategies have reported higher incidences of pleural bleeding (4.9%) (Popowicz et al., 2017) compared to the MIST-2 study, but this was likely due to low event rates (n=61; 3 pleural bleeds) making accurate conclusions difficult. Adequate

evaluation of safety of this therapy, along with other clinically important outcomes, requires larger scale data to achieve a significant event rate, which is difficult to achieve in the context of a single prospective pleural infection RCT.

With these deficits in mind, this international multicenter project was designed to evaluate the indications, application, safety, and efficacy of IET for the treatment of pleural infection. The aim of this analysis was specifically to assess the overall bleeding risk and safety profile associated with IET use, including the effects of concurrent therapeutic anticoagulation, and the nature/extent of non-bleeding complications. The data are also used to identify predictors of bleeding from IET use.

## 5.3 METHODS

### Study design

This was a multicentre, retrospective observational study conducted from 24 centres across the United States (USA) and United Kingdom (UK). Using REDCap (Vanderbilt University, Nashville, TN, USA), a secure web-based application for building and managing databases, a global account was developed for each centre, allowing a de-identified dataset to be uploaded to the primary REDCap account at the University of North Carolina (UNC).

### Ethics

Ethical and regulatory approval was obtained before recruitment began by the UNC Institutional Review Board (UNC IRB 18-2906). Data was held and analysed by UNC and the University of Oxford.

### Eligibility

The inclusion criteria were adult patients (≥ 18 years) with a diagnosis of pleural infection based on standard, internationally agreed criteria (Davies et al., 2010) (identical to those used in large prospective RCTs) (Maskell et al., 2005; Rahman et al., 2011). These were:

- a clinical history compatible with pleural infection
- a pleural collection that was one of:

    - purulent or
    - gram stain/culture positive or
    - acidic with a low pH <7.2 or
    - low pleural fluid glucose (in the absence of an accurate pH measurement) or
    - septated pleural fluid on ultrasound (or CT) which is likely secondary to pleural infection.
- at least one dose of combination IET (both tPA and DNase) after standard medical treatment failure (as determined by the local investigator) as per local site IET protocol.

In cases where the same patient received 2 or more courses of IET, only data for the first episode was included. Cases where IET was administered for recurrence of pleural infection following surgical treatment were excluded.

**Data collection**

Fourteen main data categories were included in the data collection protocol (see Appendix A5.1). Specific to this analysis, patient demographics, comorbidities (including anticoagulation use), serum/pleural fluid analyses, RAPID score parameters and details of IET therapy were assessed. The latter included dosing schedule, compliance, administration regimens and complications. Data was captured on concurrent systemic anticoagulation use prior to and during IET administration.

Clinicians recorded all adverse events following IET administration. Interventions to manage pleural bleeds were captured and ranked according to a 4-tier system where each pleural bleed event was scored according to the highest-ranking intervention required from level 1 (L1) to level 4 (L4) (Table 5.1).

**Primary and Secondary Outcomes**

The primary outcome was the overall incidence of pleural bleeding. To capture only clinically significant events, the consensus definition of haemothorax was adopted in the absence of an agreed definition of ‘pleural bleed’ in the literature (Patrini et al., 2015). The protocol mandated that for a bleed event to be recorded, a change in

pleural fluid haematocrit (Hct) during therapy to ≥ 50% serum Hct or pleural fluid Hct 25-50% with clinical suspicion prompting intervention was required.

**Table 5.1 Ranking of interventions required to manage bleeding complications**

| Level | Management details |
|---|---|
| L1 | Conservative management (stopping/temporary withholding fibrinolytics and observing) |
| L2 | Blood product transfusion (including correction of coagulopathy) |
| L3 | Additional/upsizing chest tube to manage haemothorax |
| L4 | Surgical exploration and/or transfer to higher level of care (e.g., High dependency, Intensive Care) |

Secondary outcomes included the incidence of pleural bleeding in relation to varying dosing and administration regimens of IET, use of therapeutic systemic anticoagulation, platelets, and non-bleed adverse events. Exploratory analysis was conducted on potential associations and predictors of bleeding events, including the RAPID score, as the only validated baseline predictor of poor clinical outcomes in pleural infection.(Corcoran et al., 2020; Rahman et al., 2014)

### Statistical analysis

Data is presented as mean (SD) and median (IQR) according to normality of data. Comparisons of proportions were conducted using the Fisher's exact test (two-sided) and Chi-squared test for variables with more than two levels. Suitable parametric and non-parametric methods used as appropriate for other data. Data points that were missing were queried from each site and entered as available. For missing data where centres could not provide information on data cleaning, data points were left blank and only cases with complete data were included in the final analysis. The data was analysed using descriptive statistics and binary outcomes were analysed using logistic regression models. Multivariate regression models were used to identify independent predictors with variables chosen based on the RAPID score and clinical or biological plausibility of a link to the primary outcome. Where suitable, multivariate analysis was conducted using a backward elimination approach and including parameters which

were significant in univariate analysis ($p<0.05$) or of clinical significance. Data analysis was carried out using SPSS v27 (IBM, Armonk, NY, USA).

## 5.4 RESULTS

### Study population

In total, 1851 patients were enrolled in the study, and 1833 with complete outcome data were included in the final analysis of the primary outcome (data completion rate 99%). Baseline characteristics of the study population (Table 5.2) were comparable to previously published studies in pleural infection (Table 5.3) (Corcoran et al., 2020; Rahman et al., 2011).

**Table 5.2 Baseline characteristics of study population**

| Characteristic | n=1833 |
|---|---|
| Age (mean; SD) | 57.6 (17.4) |
| Male (%) | 1173 (64) |
| Hospital acquired infection n (%) | 372 (20.3) |
| Small (<15F) chest tube (%) | 1334 (72.8) |
| BMI (mean; SD) | 27.2 (7.35) |
| | |
| PLEURAL FLUID | |
| Culture positive (%) | 819 (44.7%) |
| Pus n (%) | 829 (45.3%) |
| PF pH [median (IQR)] | 7.12 (0.5) |
| PF LDH [median (IQR)] | 1985 (4128) |
| Radiological loculation (%) | 1501 (81.9) |
| COMORBIDITIES | |
| Respiratory | 472 (25.8%) |
| Cardiac | 361 (19.7%) |
| Liver cirrhosis | 89 (4.9%) |
| Diabetes | 370 (20%) |
| End stage renal disease (CKD 5) | 107 (5.9%) |
| Chemotherapy/immunosuppression | 297 (16.2%) |
| Active cancer | 323 (17.7%) |

The most administered dosing regimen was that used in the MIST-2 study (10mg tPA and 5mg DNase, given twice daily for 3 days). Reduced dosing of tPA (Popowicz et al., 2017) was used in 172 patients (9.4%), in whom the mean dose per administration was 5mg (SD 1mg).

**Table 5.3 - Comparison of demographics and baseline characteristics of the study population with that of the MIST-2 randomised controlled trial (Rahman et al NEJM 2011) and the Pleural Infection Longitudinal Outcomes Study (PILOT) (Corcoran et al ERJ 2020).**

| | MIST-2 (n=210) | PILOT (n=547) | IPOG (n=1833) |
|---|---|---|---|
| Mean age (SD) | 58.8 (18.1) | 60 (SD 18) | 57.6 (17.4) |
| Male | 151 (71.9) | 385 (71%) | 1173 (64%) |
| Hospital Acquired Infection - n (%) | 28 (13.3%) | 259 (48%) | 372 (20.3%) |
| | | | |
| Small (<15F) chest tube | NR | 309 (70%) | 1334 (72.8%) |
| | | | |
| Mean pH | 6.9 (SD 0.3) | 7.00 (0.5) | 7.12 (SD 0.45) |
| Pus n (%) | 102 (48.6%) | 222 (41%) | 829 (45.3%) |
| Median PF LDH (IQR) | NR | 1968 (4063) | 1984.5 (4128) |
| | | | |
| Mean WCC (SD) | NR | 18.2 (20.8) | 17.87 (11.7) |
| Median urea (IQR) | 5.0 (4.2) | 4.8 (3.95) | 6.4 (7.14) |
| Mean albumin (SD) | 31.5 (7.8) | 28.5 (7.5) | 27.5 (15.6) |
| Median creatinine (IQR) | 78 (66-97) | 67 (30) | |
| | | | |
| Comorbidities | | | |
| Respiratory problems | 51 (28.3%) | 150 (27.6%) | 472 (25.8%) |
| Cardiac problems | 56 (30.6%) | 84 (15.4%) | 361 (19.7%) |
| Cirrhosis | 23 (12.7%) | 28 (5%) | 89 (4.9%) |
| Diabetes | 29 (16%) | 77 (14%) | 370 (20%) |

Median length of tPA treatment across the entire study population was 2 days and 5 doses (Table 5.4). Justification of dosing regimen or length of course chosen were not specifically captured, but where these were voluntarily reported, the most common reasons for stopping treatment early were pain, resolution of pleural collection, decision to proceed to surgery or the occurrence of other complications.

**Table 5.4 - Intrapleural enzyme therapy regimen used in the study population.**

| Dosing regimen | tPA | DNase |
|---|---|---|
| Dose - mg (median (IQR)) | 10 (0) | 5 (0) |
| Duration - days (median (IQR)) | 2 (2-3) | 2 (2-3) |
| No. of doses (median (IQR)) | 5 (4-6) | 5 (4-6) |

### Incidence of pleural bleed

The overall incidence of pleural bleeding in all patients treated with IET was 76/1833 = 4.1% (95% CI 3.0% to 5.0%). To assess possible underlying associations of pleural bleeding the following analyses were performed:

a) Dosing regimen

Differences between dosing regimens were assessed in those with complete dosing details (n=1792). Those undergoing treatment with the MIST-2 dosing regimen had a bleed incidence of 66/1620=4.1% (95% CI0.98 to 1.04), in comparison to a dose reduction strategy, in whom the bleed incidence was 6/172=3.5% (OR 0.84; 95%CI 0.37 to 1.9); this difference was not statistically significant [p=0.47].

As dose reduction regimens may be preferred for patients with a perceived “higher” bleeding risk (Popowicz et al., 2017), we hypothesized that use of dose reduction was correlated with use of baseline anticoagulation (AC). In the subgroup of patients on AC at baseline, 44/308 (14.3%) received dose reduction regimens versus 128/1482 (8.6%) not on AC at baseline [p=0.006].

When bleeding rate was compared between dosing groups corrected for use of baseline anticoagulation, there was no statistically significant difference between the full MIST-2 dose regimen and reduced dosing [OR1.62, 95%CI 0.36 to 7.25]. This analysis did not consider whether anticoagulation was withheld prior to administration, which is addressed separately below.

b) Administration regimens

Concurrent (i.e., tPA and DNase given together) and sequential (given separately) instillation of IET agents were compared for association with bleed incidence (Table 5.5). There was no significant difference in bleed rate (concurrent 55/1388=4.0%, serial 17/398=4.3%, p=0.53).

**Table 5.5 - Intrapleural enzyme therapy regimen used in the study population.**

| Administration regimen | |
|---|---|
| **Concurrent** | 1388 (75.8%) |
| **Sequential** | 398 (21.7%) |
| **Unknown** | 46 (2.5%) |

c) Systemic Anticoagulation

AC status during treatment was known in 1825/1833 patients (99.6%). On admission, 315/1825 (17.3%) of the study population were receiving therapeutic anticoagulation (AC). Use of AC was significantly associated with increased bleed rate (no AC 54/1510=3.6%, AC 22/315=6.9%; p=0.015, OR 1.99, 95%CI 1.19 to 3.31).

Bleed incidence was explored with respect to the effect of withholding AC prior to commencement of IET. Of all patients on systemic AC, 197/315 (63%) patients continued AC while treated with IET. In patients in whom AC was withheld, median duration of withholding AC was 2 days (IQR 1-4). A significant increase in bleed occurrence was seen in those in whom AC was continued during IET treatment (bleed incidence 19/197=9.6%), compared with those in whom it was withheld (bleed incidence 3/118=2.5%) [p=0.008, OR 3.76, 95%CI 1.13 to 12.44].

To explore the population in whom withholding AC was deemed high risk, a further analysis of bleeding rate between the MIST-2 regimen (tPA=10mg) and a dose reduction strategy (tPA=5mg) was conducted, correcting for withholding/continuation of AC. In those patients in whom AC was continued, the MIST-2 dosing strategy was associated with a bleeding incidence of 16/165=9.7% compared with 2/32=6.3% when using a dose reduction strategy, but this did not reach statistical significance (p=0.48).

d) Antiplatelet agents

The use of therapeutic antiplatelet agents (excluding Aspirin 75mg / Acetylsalicylic acid 81mg) was documented in 29 patients. Of these patients, 19 continued antiplatelets during IET and in 10 patients antiplatelets were withheld. There were no bleed events within this cohort and therefore no comparative analyses were conducted.

e) Platelets

The median baseline platelet count in the study population was 275 $x10^9$/L (IQR 179-397 $x10^9$/L). Logistic regression analysis was conducted to assess for the association of platelets on pleural bleeding. The predictor variable was tested a priori to verify there was no violation of the assumption of the linearity of the logit. Baseline platelet count in the logistic regression analysis was found to contribute to the model (unstandardized Beta weight for the constant; B=-2.564, SE=0.234, Wald=120.517, p<0.01, unstandardized Beta weight for the predictor variable; B= (-0.002), SE=0.001, Wald=6.361, p=0.012). The estimated odds ratio favoured a decrease of 0.2% [Exp(B)=0.99 (95%CI 0.997, 1.00)] for pleural bleeding for every 1 unit increase in platelet count.

Analysis of this effect size in a clinically meaningful way was performed. It was assumed that clinician behaviour would be significantly altered at platelets <50 so this small group was excluded from analysis. In patients with platelet count 50-100, the incidence of a pleural bleed complication (11/84=13.1%) was significantly greater than when platelets were >100 (52/1390=3.7%) (p=<0.001, OR 3.50, 95%CI 1.90 to 6.45). Further breakdown of this data by dosing regimen is presented (Table 5.6).

**Table 5.6 - Difference in bleed complications by platelet count and IET dosing regimen.**

| IET Dosing regimen | Platelet count | n | Bleed events n (%) | p-value | OR (95% CI) |
|---|---|---|---|---|---|
| **Half dose** | 50-100 | 7 | 1 (14.3) | 0.16 | 4.03 (0.54 - 30.0) |
| | >100 | 141 | 5 (3.5) | | |
| **Full dose** | 50-100 | 77 | 10 (13.0) | <0.001 | 3.45 (1.81 - 6.56) |
| | >100 | 1249 | 47 (3.8) | | |

### Management of bleed complications

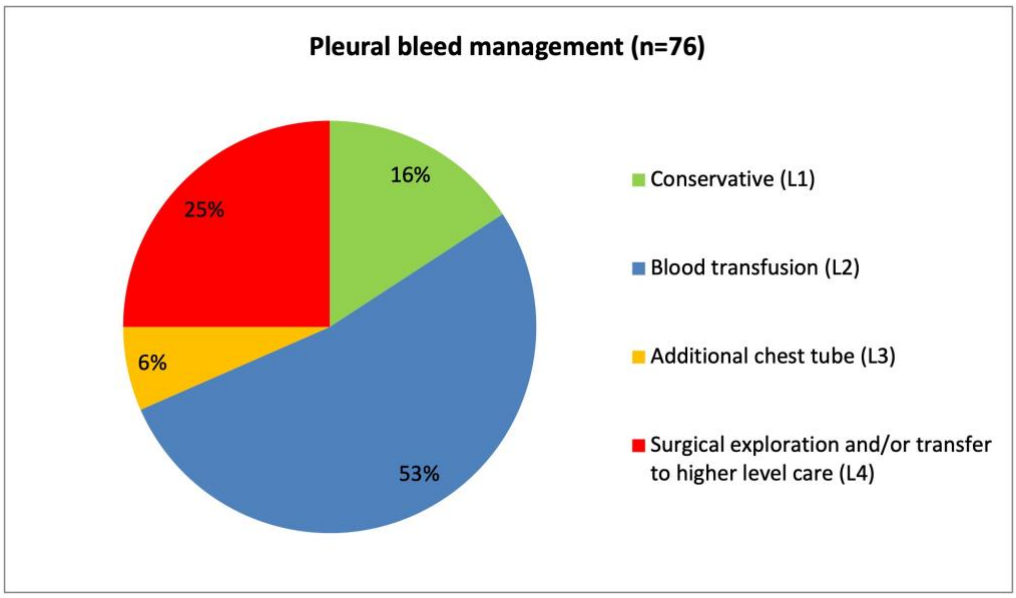
Over two thirds of the pleural bleed events were controlled by either withholding IET and observation alone and/or a blood product transfusion without the need for additional intervention (Table 5.7 and Figure 5.1). In 16/76 (21%) bleed events, the patient required surgical intervention specifically as part of their bleed management and where details of this were available, most cases included haemothorax evacuation as well as decortication or debridement as completion treatment of the pleural infection. There were no documented episodes of Interventional Radiology (IR)-guided attempted therapies.

**Table 5.7 – Classification of bleeding complications management**

| Level | Management details | n | % of pleural bleeds (n=76) | % of study population (n=1833) |
|---|---|---|---|---|
| L1 | Conservative management (stopping/temporary withholding fibrinolytics and observing) | 12 | 15.8 | 0.7 |
| L2 | Blood product transfusion (including correction of coagulopathy) | 40 | 52.6 | 2.1 |
| L3 | Additional/upsizing chest tube to manage haemothorax | 5 | 6.6 | 0.3 |
| L4 | Surgical exploration and/or transfer to higher level of care (e.g., High dependency, Intensive Care) | 19 | 25 | 1.0 |

A further analysis of whether bleed events occurring in the context of AC use were more severe and required higher level (L3/L4) management was conducted. Of the 22 bleeds that occurred in the context of AC use, 5/22 (22.7%) required L3/L4 management compared with 19/54 (35%) in the non-AC group. This difference did not reach statistical significance (p=0.36).

**Figure 5.1 – Management of IET related pleural bleeding**




## Other complications of IET administration

Adverse events following IET administration occurred in 561/1833 (30.6%) patients. A breakdown of the predefined non-bleeding complications is shown in Table 5.8a, and details of events listed as "other" is provided in Table 5.8b. Pain was the most frequently reported complication (n=224; 12.2%). No significant difference in pain was demonstrated. No episodes of major systemic bleeding secondary to IET were reported but death before hospital discharge was noted as an adverse event in 16/1833 patients (0.9%).

**Table 5.8a – Main categories of adverse events reported following IET administration.**

| Adverse event | n | % all adverse events (n=561) | % study population (n=1833) | 95% CI |
|---|---|---|---|---|
| Pain requiring escalation of analgesics | 224 | 39.9 | 12.2 | 11% - 14% |
| Increased oxygen requirement | 71 | 12.6 | 3.9 | 3 – 5% |
| Increased level of care | 44 | 7.8 | 2.4 | 2 – 3% |
| Death | 16 | 2.8 | 0.9 | 0 – 1% |
| Haemoptysis | 7 | 1.2 | 0.4 | 0 – 1% |
| Other* | 55 | 9.8 | 6.9 | 5 – 8% |

**Table 5.8b – Adverse events reported within 'Other' category.**

| 'Other' adverse events | n | % all adverse events (n=561) | % study population (n=1833) |
|---|---|---|---|
| **Tachycardia** | 12 | 2.1 | 0.7 |
| **Red/bloody discoloration of fluid not meeting pleural bleed criteria** | 11 | 1.9 | 0.6 |
| **Chest wall hematoma** | 8 | 1.4 | 0.4 |
| **Unexplained drop in Hb /acute anaemia without pleural bleed** | 5 | 0.9 | 0.3 |
| **Air leak / Bronchopleural fistula** | 5 | 0.9 | 0.3 |
| **Fever** | 4 | 0.7 | 0.2 |
| **Gastrointestinal Bleed** | 4 | 0.7 | 0.2 |
| **Hypotension** | 3 | 0.5 | 0.2 |
| **Allergic/hypersensitivity reaction** | 3 | 0.5 | 0.2 |

## RAPID score as a predictor of IET-related pleural bleeding

Complete RAPID score data was available in 1494/1833 (81.5%) patients. Distribution of the RAPID score was comparable to the external validation cohort (PILOT) (Figures 5.2 and 5.3). The association between RAPID categorization and bleed risk was explored using a multinomial logistic regression analysis using 3 RAPID risk categories as in the previous publications [low (RAPID score 0-2), medium (3-4) and high (5-7)]. Bleeding frequency was significantly associated with baseline RAPID risk category (Table 5.9) ($\chi^2$ 2df=15.4, p<0.0001).

Within components of the RAPID score, it was hypothesized that age, urea, and albumin were the most likely contributors to bleeding risk, based on biological plausibility. Analysis was conducted to assess the independent predictive ability of these variables using multiple logistic regression. The overall 3 variable model significantly predicted bleeding [F (3,1499) = 3.13, p=0.025], but urea was the only significant independent predictor (Urea $\beta$=0.068, p=0.009, Age $\beta$=0.027, p=0.299, Albumin $\beta$=-0.011, p=0.663).

**Figure 5.2 - Distribution of the RAPID score in the PILOT cohort (reproduced with permission from Corcoran et al ERJ 2020)**

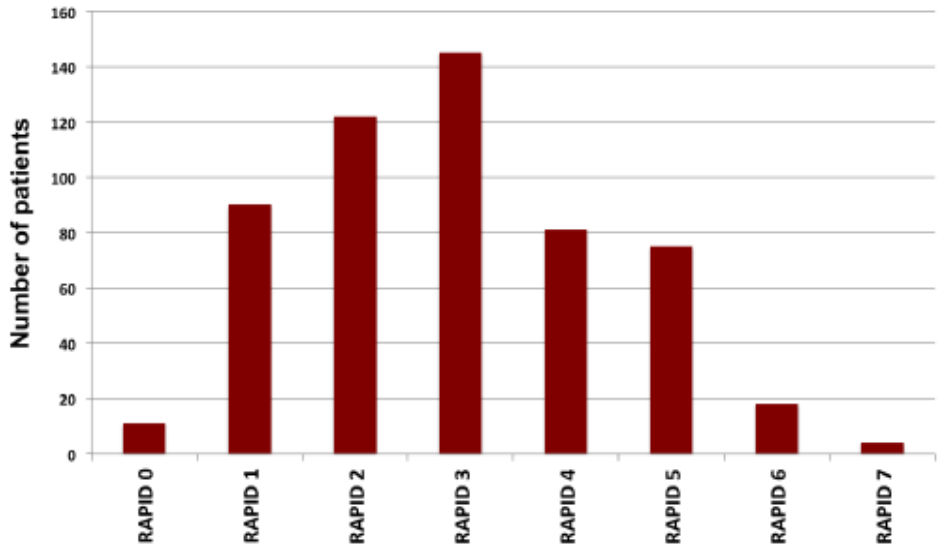



**Figure 5.3 - Distribution of the RAPID score in the RETROLYSIS cohort (reproduced from Akulian & Bedawi et al CHEST 2022)**

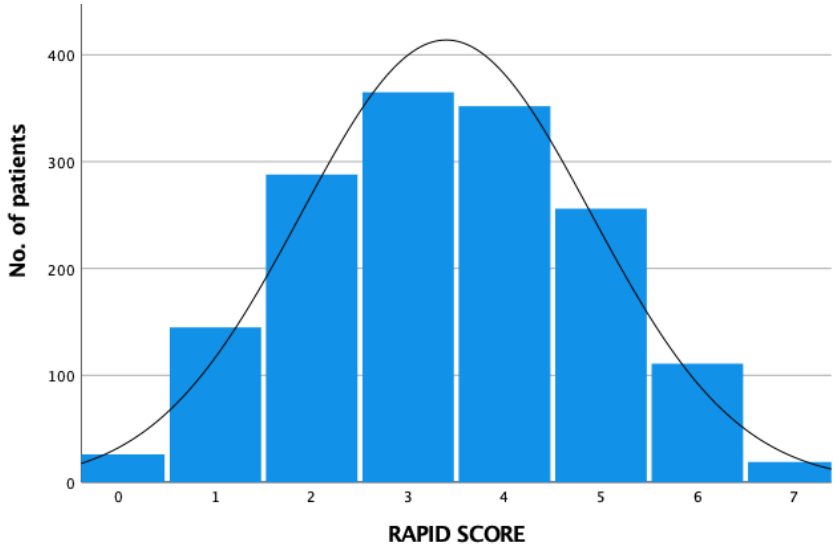



**Table 5.9 – Bleeding events by RAPID score (Low category as the reference group)**

| RAPID category | n | Bleed events (n) | Proportion of bleed events (%) | Odds Ratio (95% CI) |
|---|---|---|---|---|
| **Low (0-2)** | 447 | 12 | 2.6 | NA |
| **Medium (3-4)** | 692 | 25 | 3.5 | 1.35 (0.67 to 2.71) |
| **High (5-7)** | 355 | 31 | 8 | 3.25 (1.65 to 6.43) |

### Other predictors of pleural bleeding

A univariate regression analysis was performed for all factors where an association with pleural bleeding was biologically plausible including the individual components of the RAPID score (Table 5.10). Being on therapeutic anticoagulation on admission, serum urea, platelets and final RAPID score all predicted a greater likelihood of pleural bleeding. A multivariate logistic regression model was then performed (beginning with all univariate factors) and backward elimination ($p<0.1$) was used to identify independent predictors of a pleural bleed outcome. RAPID category and use of active anticoagulation were the only independent predictors of a pleural bleed outcome (table 5.11). The full model is shown in the appendix (A5.2).

**Table 5.10 – Univariate regression analysis of pleural bleed outcome predictors**

| Variable | df | p-value | Odds ratio |
|---|---|---|---|
| Patient age | 1 | 0.13 | 1.01 |
| BMI (kg/m2) | 1 | 0.61 | 0.99 |
| Liver cirrhosis | 1 | 0.17 | 0.18 |
| End stage renal disease | 1 | 0.14 | 1.83 |
| Active malignancy | 1 | 0.66 | 0.86 |
| Active chemotherapy | 1 | 0.19 | 0.45 |
| Active anticoagulation*$ | 1 | 0.01 | 1.99 |
| RAPID category*$ | 3 | 0.03 | - |
| Serum urea** | 1 | 0.003 | 1.01 |
| Serum albumin (g/dL) | 1 | 0.38 | 0.85 |
| Serum platelets* | 1 | 0.035 | 0.99 |
| Hospital acquired infection | 1 | 0.12 | 1.54 |
| Absence of pus | 1 | 0.70 | 0.90 |

*p<0.05 **p<0.01
$ Statistically significant in the multivariate model

**Table 5.11 – Independent predictors of pleural bleed outcome (final model of the multivariate regression using backward elimination)**

| Variable | p-value | Odds ratio | 95% CI |
|---|---|---|---|
| Active anticoagulation | 0.048 | 1.80 | 1.01 – 3.23 |
| RAPID category | 0.005 | 1.72 | 1.17 – 2.51 |

Further analyses were undertaken to explore bleeding incidence in patients with liver cirrhosis and end stage renal disease, correcting for dosing regimen used. Liver cirrhosis was not associated with a significant increase in bleeding regardless of dosing regimen (tPA 5mg, p=0.49; tPA 10mg, p=0.20). In the context of patients treated with full dose tPA, end stage renal disease was associated with an increased incidence of pleural bleeding [7/79 (8.9%) vs 51/1301 (3.9%); p=0.037; OR 2.4 95%CI 1.04 to 5.43]. However, in the population treated with a dose reduction strategy (tPA 5mg), end stage renal disease was not associated with a statistically significant increase in pleural bleed events (0/15 (0%) vs 6/142 (4.2%); p=0.42; OR 0.96 95%CI 0.92 to 0.99).

## 5.5 DISCUSSION

This is the largest study to date of combination intrapleural enzyme therapy (IET) in pleural infection, and the only study to use pre-hoc criteria to define pleural bleeding events. The bleeding risk of 4.1% found in this data is comparable to the original bleeding incidence of 3.8% reported in the MIST-2 study. The IET dosing regimen in MIST-2 was chosen empirically but nonetheless, to date, remains the only dosing regimen to have been tested in a randomized placebo-controlled trial. This data demonstrates that reducing the dose of tPA in routine use (all comers) is not associated with a decrease in pleural bleeding incidence, perhaps suggesting that the dose effect for bleeding is different in intrapleural versus intravenous use (Daley et al., 2015; Whiteley et al., 2016).

Systemic anticoagulation is associated with increased intrapleural bleeding but temporarily omitting treatment prior to IET (or an INR <2 in the context of warfarin) appears to mitigate this risk. Our data suggest that concomitant administration of systemic anticoagulants and intrapleural fibrinolytics requires careful consideration, as this increased the risk of intrapleural bleeding by 4-fold. There may be clinical scenarios where risks of withholding anticoagulation are unacceptably high (e.g., metallic heart valves or recent venous thromboembolic events). In such cases, a cautious approach should be adopted in the use of IET, such as more easily reversible AC e.g. heparin as opposed to DOAC or consideration of alternative interventions to IET such as surgical approaches, or intrapleural saline irrigation (Guinde et al., 2021; Hooper et al., 2015), accepting the inferior evidence base for the latter.

In cases where pleural bleeding occurred with IET, the majority resolved with observation or with blood product transfusion. Nonetheless, almost a third of bleed events did require pleural or surgical intervention and this information should inform clinical decision making and patient discussions in consenting to therapy. Of other complications, pain was the most frequent non-bleeding event hence consent for this likely side effect and pre-medication with appropriate analgesia should be considered to improve tolerability and compliance.

The analysis of predictors of pleural bleeding has shown that the RAPID score, as an independent predictor, allows clinicians to make a direct estimation of bleeding risk from IET at presentation and is not subject to the variability of serum urea and platelets. This is a novel use of the RAPID score derived from this study.

Our data provides reassurance that age does not appear to confer an increased bleeding risk. This is particularly relevant as IET use is targeted with increasing frequency at older, frailer patients, deemed to be high risk surgical candidates, and in whom IET may represent the only viable 'rescue' treatment option.

This study has several strengths. It is more than 10 times larger than the previous largest study evaluating the use of IET in the treatment of pleural infection. At this study size, we can provide precise estimates on frequency of events as reflected in the narrow 95% confidence intervals, and this data can now be used to provide precise information to clinicians for decision making and the consent process. Our study had very high rates of data completeness, and identical dataset collection across multiple centers capturing global practices, and therefore has strong external validity. The study population represents all-comers as opposed to the carefully selected patients enrolled into interventional clinical trials. This is the first study to our knowledge to use an *a priori* objective definition of the key clinical event (pleural bleeding), adding to robustness of reporting for the primary outcome. This aspect is important for future studies, as IET use is recognized to be associated with red/blood discoloration of drained pleural fluid as lysis of fibrin strands occurs, and IET use results in increased pleural fluid formation and drainage (Kanellakis et al., 2019). These factors in combination can cause alarm and may be mistakenly assigned to pleural bleeding.

There are limitations to this study. It was a retrospective study, and although the large study size and event rate mitigate against this to some extent, this is not equivalent to randomized controlled data with consecutive patient recruitment and will therefore be subject to some selection bias. This can be seen for example in the non-bleeding adverse events in which it is likely that some events e.g., death, although exceedingly rare, may have been caused by factors other than IET administration. Similarly, other adverse events (such as red discoloration of fluid) are likely to have been under-reported as most clinicians would not consider this to be a 'true' adverse event. In the

absence of non-IET treated controls, it is challenging to assign causation to some findings. Prophylactic anticoagulation was not addressed in this study, however previous studies have not shown an association with increased bleeding risk (Gervais et al., 2008). The low number of patients on antiplatelet agents precluded the ability to study this subgroup in detail and therefore until further data is available, the authors would suggest that antiplatelet agents (other than Aspirin 75mg / ASA 81mg) are held (if clinically appropriate) prior to IET administration.

## 5.6 CONCLUSION

This is the largest study to date of IET use in pleural infection, confirming a low bleeding risk. Although bleeding risk is increased with concurrent AC, withholding AC prior to IET reduces this risk and caution is advised for concomitant administration of IET and therapeutic-dose systemic AC. There is an increased bleeding risk with increasing RAPID score, elevated serum urea and serum platelet count $<100x10^9$/L. The RAPID score and the use of active systemic anticoagulation are independent predictors of IET-related bleeding risk in pleural infection.

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# APPENDIX A5.1

## RETROLYSIS STUDY PROTOCOL - Data points to be collected

Patient demographics

1. Age
2. Sex
3. Race
4. Date of hospital admission
5. Date of chest tube placement
6. Date of chest tube removal
7. Date of hospital discharge
8. Co-morbidities
    a. Lung Disease
        i. COPD
        ii. ILD
        iii. Cystic fibrosis
        iv. Lung Cancer
        v. Other
    b. Non-lung co-morbidities
        i. DM
        ii. Malnutrition
        iii. s/p Transplantation
        iv. Active Malignancy
        v. Use of Immunosuppressive Medication
        vi. Chemotherapy
    c. Anticoagulation
        i. Therapeutic Heparin
        ii. DoAC
        iii. Coumadin/warfarin
    d. Antiplatelets
        iv. Asa 325 mg
        v. Clopidogrel
        vi. Other (do not include Aspirin 75/Asa 81 or prophylactic LMWH)
9. Radiographic Features (CT): Review by either radiologist or data abstracter acceptable.
    a. Pleural Thickening (> 2 mm)
    b. Loculation
    c. Internal Septation
    d. Abscess or necrotizing pneumonia
10. Serum (Peak value for all but platelets. Nadir for platelets. Within 7 days of lytic administration)
    a. Bun
    b. PT/INR
    c. PTT
    d. WBC
    e. Platelets (lowest)
11. Pleural Fluid Analysis
    a. Diagnosis (empyema, CPPE etc…)
    b. Culture (+/- and results)
    c. Gram stain (+/- and results)
    d. Total Protein (g/dL)
    e. LDH (U/L)
    f. pH

g. Glucose (mg/dL)
h. Fluid description

12. Chest Tube and tPA/Dornase administration
    a. Date of Initial Chest Tube Placement
    b. Date of Initial Chest Tube Removal
    c. Initial Chest Tube Size (F)
    d. Doses TPA/DNAse
    e. Frequency of administration
    f. Total number of doses
    g. Timing of lytics (concurrent/serial)
    h. Total chest tube output
        i. Pre Lytics
        ii. After Initiation of Lytics
    i. Complications
        i. Pain (Requiring intervention)
        ii. Increased level of care
        iii. Increase O2 Requirement
        iv. Death
        v. Hemoptysis
        vi. Initial Tube Dislodgement
        vii. Hemorrhage (Pleural fluid Hct ≥ 50% of serum Hct, or 25-50% of serum Hct with clinical suspicion)
        viii. If Hemorrhage How Managed
        ix. Other
13. Outcomes
    a. Date of Hospital Admission
    b. Date of Hospital Discharge
    c. Additional chest tube placement
        i. #
        ii. Size(s)
        iii. Reason for additional tubes (Ongoing Sepsis, Significant Undrained Focus of Fluid)
    d. Additional doses of lytics (tPA+Dornase)
        i. #
        ii. Reason for additional doses of lytics (Ongoing Sepsis, Significant Undrained Focus of Fluid)
    e. Surgical referral
        i. Performed
            1. Open
            2. Minimally invasive thoracic surgery (MITS)
14. Mortality
    a. 30-day
    b. 90-day
    c. Alive > 90 Days

# APPENDIX A5.2

## Multivariate regression analysis for pleural bleeding following IET using backward elimination (standardized regression coefficients)

| Variables | Model | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Patient age | 0.004 | 0.004 | - | - | - | - | - | - | - | - |
| BMI (kg/m$^2$) | -0.008 | -0.008 | -0.008 | -0.008 | -0.008 | - | - | - | - | - |
| Liver cirrhosis | -1.646 | -1.646 | -1.642 | -1.631 | -1.611 | -1.602 | -1.597 | -1.573 | - | - |
| End stage Renal Disease | 0.243 | 0.244 | 0.230 | 0.250 | - | - | - | - | - | - |
| Active malignancy | -0.194 | -0.194 | -0.171 | - | - | - | - | - | - | - |
| Active chemotherapy | -0.747 | -0.746 | -0.740 | -0.863 | -0.871 | -0.865 | -0.895 | - | - | - |
| Active anticoagulation | 0.497 | 0.497 | 0.510 | 0.511 | 0.508 | 0.507 | 0.516 | 0.503* | 0.545 | **0.589*** |
| RAPID category | 0.388 | 0.394 | 0.448* | 0.443* | 0.436* | 0.442* | 0.493* | 0.502 | 0.485* | **0.540**** |
| Serum urea | 0.004 | 0.004 | 0.004 | 0.004 | 0.004 | 0.004 | - | - | - | - |
| Serum albumin (g/dL) | -0.012 | - | - | - | - | - | - | - | - | - |
| Serum platelets | -0.002 | -0.002 | -0.002 | -0.002 | -0.002 | -0.002 | -0.002* | -0.002 | -0.002 | - |
| Hospital acquired infection | 0.363 | 0.356 | 0.365 | 0.370 | 0.335 | 0.322 | 0.318 | - | | |
| Absence of pus | -0.066 | -0.072 | - | - | - | - | - | - | - | - |

*p ≤ 0.05 **p ≤ 0.01

# CHAPTER 6 –
# FINAL DISCUSSION AND FUTURE WORK

## 6.1 Overview

The earliest descriptions of pleural infection date back to Imhotep in Ancient Egypt more than 4,000 years ago, referring to ‘an abscess with prominent head from the breast’ suggestive of empyema with chest wall invasion (Peters, 1989), the condition that would be referred to as ‘empyema necessitans’ in modern practice. Around 500BC, Hippocrates began treating cases of pleural infection with open thoracic drainage (Somers and Faber, 1996), although it remained a fatal disease up until the introduction of closed tube drainage in the late 19$^{th}$ century, resulting in a substantial reduction in mortality (Meyer, 1989). Pleural infection is not limited to humans, and has been reported in domestic animals including cats and dogs (Stillion and Letendre, 2015), as well as horses (Raphel and Beech, 1982). Interestingly, in a case series of 101 dogs with ‘pyothorax’ (Eiras-Diaz et al., 2021), surgical management was associated with improved outcomes compared with medical treatment (closed tube drainage and antibiotics). In many canine empyemas, inhaled foreign bodies (principally grass seeds) were identified as the culprit. In humans, the aetiology and the microbiological agent associated with pleural infection are often uncertain, although it seems likely that next generation sequencing will increase the frequency of a microbial diagnosis as has been the case in recent small studies (Kanellakis et al., 2022; Xu et al., 2022).

In the last two decades, empyema mortality has remained unchanged at an unacceptably high figure of 15% at 1 year (Arnold et al., 2021). This is well in excess of the improvements seen in conditions such as myocardial infarction with mortality rates now around 5-7% (a reduction of 40-50% since the 1960s) as a result of improved front line care including primary percutaneous coronary intervention, admission into specialist coronary care units and modern pharmacotherapy (Laforgia et al., 2022). Pleural infection is a heterogeneous condition that can present along a spectrum of severity and rate of progression, with a diverse bacterial

profile (Kanellakis et al., 2022), and affects a diverse population particularly in terms of age (Arnold et al., 2021) and comorbidity (Cargill et al., 2019). Yet unlike many other serious acute conditions where risk stratification and phenotyping have advanced management strategies using a 'precision medicine' approach, e.g. using the peripheral blood eosinophil count to predict the severity of an exacerbation of COPD (Vedel-Krogh et al., 2016) and responsiveness to oral corticosteroid use (Bafadhel et al., 2012), or the identification of neutrophil NETs associated with disease severity and treatment response in bronchiectasis (Keir et al., 2021), in pleural infection progress has been limited.

## 6.2 Biomarkers in pleural infection - biology

We have long depended on a single diagnostic tool of pleural fluid pH using a binary endpoint, greater or less than 7.2, to instigate necessary chest drainage, based on it predicting a 'complicated' clinical course defined as requiring drainage for complete patient recovery (AUC 0.89) (Heffner et al., 1995). This is despite the major limitation acknowledged by the authors of the meta-analysis on which the pleural fluid pH was based (Heffner et al., 1995) being the quality of the primary studies (7 studies; total n=251) as well as lack of prospective validation following publication of the meta-analysis. It is also important to note that in the studies assessed, decision thresholds varied between 7.21 and 7.29 depending on cost-prevalence considerations (Heffner et al., 1995).

However, pleural fluid acidity as a result of bacterial invasion and subsequent bacterial metabolism and leucocyte phagocytosis producing high levels of lactic acid (except in the case of urea splitting organisms such as Proteus (Isenstein and Honig, 1990)) is only one piece of the complex infected pleural space puzzle. If we consider the pathogenesis of pleural infection further upstream, the intrapleural pathways that occur offer a number of inflammatory mediators and fibrinolysis-associated proteins as potential biomarkers of importance. Knowledge of the underlying biology of pleural infection, and specifically biomarkers that drive poor outcomes, has the potential to alter clinical management through targeted therapy for those at higher predicted risk. A recent small prospective study (Johansson et al., 2023) reported that pleural fluid lactate performed slightly better than pleural fluid pH in identifying

complicated vs simple parapneumonic effusions, but the increment was minor (receiver operating characteristic (ROC) area under the curve for pH 0.905 (CI 0.847-0.963), and for lactate 0.927 (CI 0.877-0.977)), and will need additional validation. Links to outcomes were not explored in this study.

Prior to the study reported in Chapter 2 (Bedawi et al., 2022a), one prospective study found that pleural fluid suPAR as a biological marker predicted the need for more invasive intervention but did not influence clinical outcomes such as length of stay or mortality (Arnold et al., 2020). Pending external validation, my findings suggest that PAI-1 is potentially the first pleural fluid biomarker that could be assessed in its clinical utility combined with validated clinical prediction models to identify patients at greatest risk of poor outcomes who may benefit from more aggressive intervention early in their clinical course. The findings also direct future research into personalised intrapleural fibrinolytic dosing regimens targeting PAI-1 levels. As reported by Komissarov and Idell in their editorial of the published manuscript in the American Journal of Respiratory & Critical Care Medicine, “the findings in this relatively large study of patients with pleural infection substantively advance our understanding of the relationships between PAI-1 and patient outcomes” (Komissarov and Idell, 2023). Of note, pharmacological neutralisation of PAI-1 in a rabbit model of empyema induced by inoculation of *S. pneumoniae* increased the efficacy of alteplase in achieving successful fibrinolysis (Florova et al., 2023). PAI-1 has been suggested as a therapeutic target in a range of conditions, as recently reviewed (Sillen and Declerck, 2021), but as yet this has not reached the clinic. In addition, potential modulation of PAI-1 in pleural infection needs to be considered with caution as in a mouse model of pleural injury (carbon black/bleomycin installation), PAI-1 over-expression promoted fibrin formation and was detrimental, PAI-1 deficiency promoted profibrogenic alterations of the mesothelium that ultimately exacerbated pleural organization and lung restriction (Tucker et al., 2014). Thus PAI-1 may play different roles at different stages of pleural infection.

The coagulation cascade and inflammatory pathways interact and synergise with, each other in a range of settings. In health they are restrained by anti-inflammatory and anti-thrombotic factors, for example produce by endothelial cells. A reduction in the production of anti-thrombotic factors and the prothrombotic effects of platelet and

leukocyte activation may unleash the coagulation pathways leading to ‘thrombo-inflammation’ in systemic infection, a term that reached high prominence during the COVID-19 pandemic (Perico et al., 2023). Pleural mesothelial cells can produce pro-coagulant factors such as tissue factor and PAI-1 in the context of inflammation (Bottles et al., 1997; Hsieh et al., 2019); whilst neutrophils and proteins associated with neutrophil extracellular traps are abundant in exudative pleural effusions (Twaddell et al., 2021). It is tempting to speculate that neutrophils may promote the local dysregulated of coagulation seen in pleural infection in addition to contributing to pleural fluid viscosity via the release of DNA, and further evaluation of NETs-associated proteins as prognostic biomarkers for response to IET would seem an interesting prospect.

## 6.3 Biomarkers in pleural infection – radiology

Another potential biomarker of outcomes in pleural infection is the associated radiology. In the last decade, there has been indisputable evidence around thoracic ultrasound increasing safety of pleural procedures (Diacon et al., 2003; Mercaldi and Lanes, 2013) such as the diagnostic aspiration of pleural fluid in the context of suspected pleural infection. The European Respiratory Society (ERS) Thoracic Ultrasound Taskforce (of which I was a member) has recommended the use of bedside thoracic ultrasound be mandatory in the assessment of pleural effusions and guidance of pleural intervention (Laursen et al., 2021). Thus, the use of a sonographic parameter such as septation presence and/or severity to predict outcomes becomes an attractive prospect. It is noteworthy that the only externally validated clinical outcome prediction in pleural infection, the RAPID score (Rahman et al., 2014), used the MIST-1 (Maskell et al., 2005) and MIST-2 (Rahman et al., 2011) studies respectively as its derivation and validation cohorts, both RCTs performed before the era of commonplace ultrasound. Thus, ultrasound parameters, specifically septations, were not included in the analysis of either study.

Studies to date, limited by their retrospective nature, have suggested that septations, as a radiological surrogate for intrapleural fibrinous organisation, are associated with poor clinical outcomes; the requirement for surgery and intrapleural fibrinolytics

(Chen et al., 2000), increased ICU admission and greater mortality (Chen et al., 2008). For the first time in a large cohort in the PILOT study (Corcoran et al., 2020), sonographic septation data was collected prospectively at the time of enrolment and using an objective septation score, my analysis in Chapter 2 (Bedawi et al., 2022a) represents the strongest evidence to date that septations, in isolation, despite being a robust diagnostic parameter (Svigals et al., 2017), do not predict subsequent clinical outcomes. The negative association may also speak to the findings of previous randomised trials where lone intrapleural fibrinolytic therapy did not result in improved clinical outcomes (Maskell et al., 2005). I hypothesised that septations are likely an epiphenomenon in the progression of pleural sepsis. Despite not predicting clinically important outcomes, septations may still have a role in predicting early response to chest tube drainage (which could equally be of value in the context of septated malignant pleural effusion), however I suspect that dynamic assessment of interlocular communication using novel ultrasound techniques such as contrast-enhanced ultrasound (CEUS) may be a more accurate approach (see section 6.6).

Computed tomography may be undertaken in the setting of pleural infection, but it is not routine worldwide and its role in outcome prediction is currently unclear. The classic CT signs regarded to be typical for pleural infection include pleural thickening, and enhancement of both the visceral and parietal pleura (“split pleura” sign), and pleural septations (although ultrasound is likely superior for the detection of septations. These signs have previously been reported to have good sensitivity, but low specificity (Porcel, 2018). However a recent retrospective study of 711 patients across 6 centres in Japan did link certain CT findings with outcomes (Shiroshita et al., 2023). These authors found the presence of bronchopleural fistula predicted a 13.8% increase in 90-day mortality, even when adjusted for RAPID score and early surgical intervention, whilst interlobar infection was associated with a similar reduction in mortality. Whilst these findings are from a retrospective cohort with significant missing data, they are of interest and warrant further investigation.

## 6.4 Beginning to impact treatment paradigms – early intervention and the surgery versus IET debate

Aside from predicting which patients are at risk of the poorest outcomes, improving outcomes in a meaningful manner ultimately requires redefining the treatment pathway. The management strategy defined by the BTS 2010 guidelines, recently updated this year, is one based on expert consensus. This largely involved a sequential approach of chest tube drainage, waiting 5-7 days, followed by consideration of intrapleural therapy (IET) and then surgery. Both the medical (Meyer et al., 2018) and surgical literature (Towe et al., 2019) have conclusively shown that treatment delays are associated with worse outcomes. This allows us to conclude that beyond prompt initial drainage, coined by the adage ‘the sun should never set on a parapneumonic effusion’ (Sahn and Light, 1989), the escalation of therapy also needs to happen earlier on in the treatment pathway. The question then becomes which intervention?

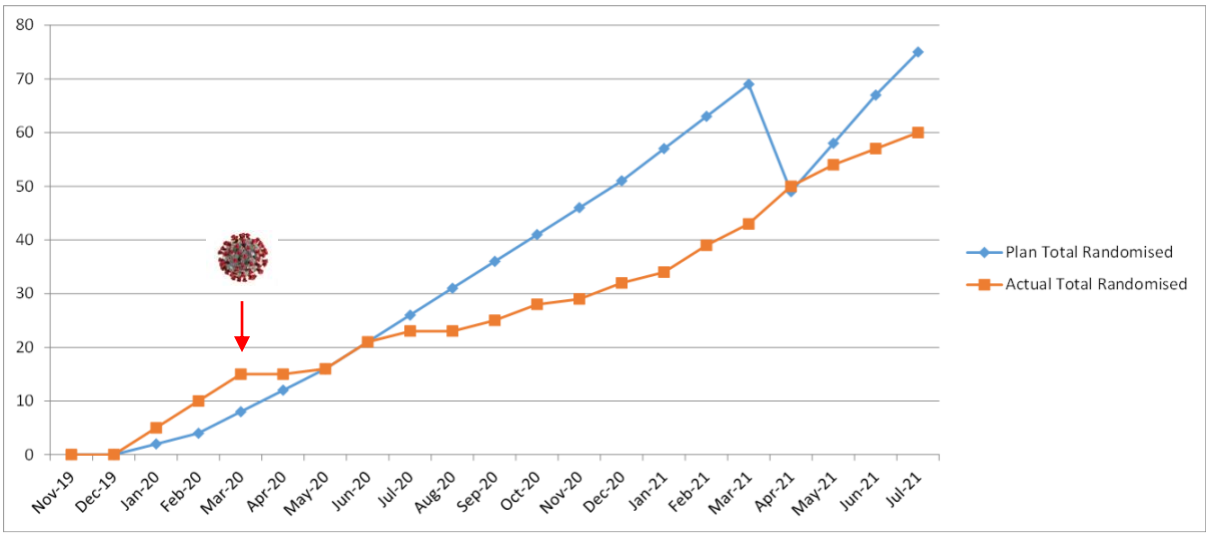
The role of surgery is well established in pleural infection, and some current guidelines advocate for it to be the primary treatment strategy (Shen et al., 2017). Indeed, modern Video Assisted Thoracoscopic Surgery (VATS) outcomes have shown considerable success with fewer complications when compared to open thoracotomy (Towe et al., 2019). However, there remains the requirement for general anaesthetic and a small, but not insignificant rate of conversion to open thoracotomy. In addition, the limitations in the evidence base for early surgery, including selection bias and lack of standardized criteria in the only two small randomized studies of surgery (Bilgin et al., 2006; Wait et al., 1997) and the multiple case series, should not be overlooked. The use of IET as an alternative potential “rescue” treatment has been a much-needed and practice- changing addition to the landscape in the last decade, driven by the MIST-2 study (Rahman et al., 2011). However, IET has not to date been directly compared head-to-head with surgery. As discussed in a recent editorial entitled ‘The Cold Steel of a Surgeon or Some Fool of a Physician? The Debate Continues’ (Bedawi et al., 2022c) with reference to Sir William Osler and Guillain Dupuytren (who both sadly passed away due to empyema and had conflicting views on its management), the reason this question is of

importance is because if early surgery is indeed associated with improved patient outcomes, healthcare systems should be restructured to allow earlier surgical evaluation as the treatment of choice, e.g. by redirecting empyema admissions to surgical centres directly from the emergency department or acute admissions unit to prevent subsequent delays of referral and transfer.

The MIST-3 study (Chapter 3) was the first attempt in the literature at a multicentre head-to-head randomised controlled trial comparing early IET and early surgery against ‘standard care’ with chest tube and antibiotics. This was designed as a feasibility study with the aim of intentionally enrolling and randomising all-comers regardless of fitness for surgery to overcome the selection bias of previous surgical series and RCTs. As a feasibility study, MIST-3 primarily aimed to establish the key outcome measures which are important to participants to allow for relevant outcomes in a subsequent RCT, to collect information on feasibility of recruitment, participant acceptability and our ability to collect outcome data. Importantly, we also wanted to understand whether there was equipoise amongst clinicians (both physicians and surgeons) with regards to the question before we embark on a definitive study, in turn, hugely expensive in terms of cost and resource. MIST-3 included eight geographically diverse UK centres, all of which I set up myself, conducting their site initiation visits in person. The trial started very strongly with recruitment ahead of target in the first 3 months, and we had even started discussing whether we should make a funding application to the NIHR to run it straight into a definitive study (following completion of a 12 month pilot phase). Little did we know that in March 2020, the world would change significantly, with a dramatic impact on MIST-3 recruitment (figure 6.1). The COVID-19 pandemic created significant challenges through redirection of research resources, redeployment of myself to clinical work as well as a reduction in hospitalisations with pleural infection by up to a third compared to the previous year (Bedawi et al., 2022b). Nonetheless, with interventions including persistent and frequent email reminders to centres to look out for patients, as well as simplification of the protocol to ease data collection burden, we managed to complete the study to a revised figure agreed by the trial steering committee (TSC) of 60 participants.

This study has been hugely informative in terms of future trial design and powering, and there are some clear limitations to MIST-3 to be acknowledged. Despite the headline result being positive in favour of feasibility (having met the predefined feasibility criteria), we had a clear protocol compliance issue. The 47.6% compliance

**Figure 6.1 – MIST-3 recruitment graph**




to standard care was somewhat expected given modern practice where clinicians generally would choose to escalate to one of either IET or surgical referral within 3-5 days. Ideally, we had intended for this to be a 2-arm study of IET vs surgery, but the funders mandated an additional standard care (control) arm. Based on our results, I was able to make a strong argument in my NIHR RfPB (Research for Patient Benefit) report at the end of the study that this arm should be removed from a future definitive trial. However, the question then becomes how you can deliver a protocol of IET vs surgery when surgical compliance is only 50%? The COVID-19 pandemic inevitably did play a significant role in shaping this compliance figure. Although not explicitly spelt out in the screening data, the delayed surgical reviews or in some cases, physician reluctance to refer, was influenced by the well-known COVID-Surg data, at the time, citing increased mortality and pulmonary complications in patients undergoing surgery with peri-operative SARS-CoV-2 infection (Nepogodiev et al., 2020), specifically stating “thresholds for surgery during the COVID-19 pandemic should be higher than during normal practice” and “consideration should be given for promoting non-operative treatment to delay or avoid the need for surgery” thereby possibly making it the worst time to conduct a trial involving acute surgical treatment.

The ‘quick responder’ rate of 12% - those patients who make a good response to initial chest tube drainage (within 24 hours) and are thereby unsuitable for randomisation – was a significant (and previously unreported) finding from MIST-3 that will need to be factored into any future sample size calculations for a definitive study.

With reference to the demographics of the study population, the median age of 66 in the VATS arm represents the oldest surgical cohort in a pleural infection study to date, and the oldest patient to be operated on was 74 years of age. The reason this is important is that older patients are often denied surgery due to an assumed risk of surgical complications and general anaesthesia by treating physicians. However, these are also the patients that are often at highest risk of poor outcomes from their pleural infection, as evidenced by ‘Age’ being an independent predictor in the RAPID score (Rahman et al., 2014), and this risk-benefit balance is rarely considered in clinical practice. With increasing surgical experience, modern VATS has become a safe intervention with favourable outcomes and clinician equipoise is going to be key in moving the field in this area. As seen in the recently published VIOLET study (Lim et al., 2022) of VATS versus open lobectomy in early-stage lung cancer, with a median age of 69 in both VATS and open surgery arms, it is possible to randomise these patients in a phase 3 study.

## 6.5 Understanding patient priorities in pleural infection

An important question following MIST-3, particularly given the challenges with protocol compliance, is ‘what is the optimal primary outcome for a definitive early surgery vs IET trial?’ Given that IET can be started within 24 hours of decision whilst the median time to surgery in MIST-3 was 3.5 days, length of hospital stay, whilst being objective, easily measurable and a priority to patients, is problematic and likely to be biased in favour of IET. These factors would need to be taken into consideration in future trial design and in data analysis. The MIST-3 qualitative substudy (Chapter 4) offered, for the first time, an opportunity to hear directly from patients regarding their priorities of care when being treated for pleural infection and, in turn, what outcomes are important to them. We obtained first-hand accounts of the

patient experience of having pleural infection, the treatment pathway comparing the modalities of IET vs surgery and the experience of going through a trial. Length of hospital stay, time to complete recovery and treatment failure rates emerged as important patient centred primary outcomes in pleural infection and are important considerations in a future definitive study. The qualitative data highlighted clearly that pleural infection is a serious and distressing condition requiring support through improved communication in terms of explanation of interventions but also information provided regarding recovery prospects and timelines. From a trial perspective, we also learnt that due to how acutely unwell these patients are, the ability to process and retain information is significantly compromised, thus the incorporation of visual aids and simplified explanations is necessary. In response I plan to design such visual aids and simplified patient information sheets and leaflets, that may be transferrable to general management of pleural infection outwith such future trials. Another factor that emerged from the study was the significant pain experienced by patients throughout their journey. Pain is likely to be underestimated both from the condition itself, and also the associated interventions including the chest tube, the intrapleural therapy and post-operatively. Superior pain management following surgical intervention was noted and should be considered in future trial design but also in our management of pleural disease in the non-surgical setting more widely. This requires the use of adequate analgesic protocols throughout admission, including proactive pain management approaches during therapeutic procedures.

Whilst complications such as pain in the setting of modern VATS, although sparse in the literature specific to pleural infection, can be extrapolated from other conditions where VATS is commonly used (Feray et al., 2022; Tong et al., 2020). IET is a unique treatment to pleural infection and while there have been number of series reporting on its efficacy (Kheir et al., 2018; McClune et al., 2016; Piccolo et al., 2014; Popowicz et al., 2017) since the original MIST-2 trial (Rahman et al., 2011) a recent Cochrane systematic review on the use of intrapleural fibrinolytics in pleural infection with assessment of harm and serious adverse events amongst its main objective, concluded that there was insufficient data to give a precise estimate of the overall risk of significant adverse events (Altmann et al., 2019). This lack of data instigated the RETROLYSIS study (Chapter 5), which represents the largest study of IET use in pleural infection to date. By reaching out to and collaborating with the United

States (US) based Interventional Pulmonary Outcomes Group (IPOG), and their vast network of 25 regional centres, we agreed on a retrospective data collection protocol and invited centres to contribute their previous 6 years' worth of data during an 18 month period. For ease of logistic and research governance processes, the database was based in the US (coordinated by a colleague in North Carolina), which upon completion of the data entry was transferred to me for all analysis and reporting. With a study population 18-times larger than the previous largest series of pleural infection patients treated with IET, the data can now be used to reassure clinicians (within the limitations of retrospective data), regarding safety of IET. The study focused primarily on bleeding complications as this is often the major concern among clinicians and to ensure accuracy of reporting, we intentionally aligned the definition of a 'pleural bleed' with the universally agreed definition of a haemothorax, where clinicians had measured a pleural fluid haematocrit >50% of peripheral blood or there was a significant drop in haemoglobin (>1g/dL) following initiation of IET. By virtue of the size of the dataset, we were able to provide evidence on additional bleeding risk in various scenarios (e.g., anticoagulation) and offer advice on how to mitigate bleeding risk in these scenarios. I also conducted a series of univariate and multivariate regression analyses to identify independent predictors of IET associated pleural bleeding. Through identification of the rate of bleeding and non-bleeding adverse events e.g., significant pain causing interruption/cessation of therapy in 12.2%, this study provides precise information to directly inform shared decision making between clinicians and patients as well as patient consent.

## 6.6 Future work

The work outlined in this thesis is based on patient-derived samples and clinical trials. This has the benefit of clear translational relevance, but lacks the ability to undertake mechanistic evaluations early in disease. As noted, animal models relevant to the establishment of pleural infection, and this is an area of study that needs extensive work to develop. In addition, there is a paucity of normal human mesothelial cell lines available (such lines focus on mesothelioma and hence do not recapitulate normal biology). Recent technological advances such as proteomics and single cell RNAseq are likely to shed further light on the biology of pleural infection,

and indeed my group have recently undertaken an unbiased proteomics study and will be pursuing some of the findings of this major initiative. I hope to work with them and with scientists locally to further such basic mechanistic studies. However, as my studies have been purely clinical and will shape my future research career and directions, I will now focus on the more clinical studies that I plan to undertake in the next few years, funding permitting.

### 6.6.1 Contrast-enhanced ultrasound (CEUS) – SONODRAIN study

As alluded to in section 6.3, with thoracic ultrasound now being at the forefront of pleural disease, I plan to undertake further work evaluating its potential use as a theranostic tool. I am currently in the process of analysing images and videos acquired from a small prospective pilot study of 15 patients with pleural effusions with varying degrees of septation (including 3 non-septated controls), where I explored the novel application of contrast enhanced pleural ultrasound using sulphur hexafluoride microbubbles (a contrast agent known as SonoVue) to assess interlocular communication (Figure 6.2) and corelate this with pleural effusion drainage (the SONODRAIN protocol). CEUS has an established role in the assessment of liver lesions (Ferraioli and Meloni, 2018) but also a number of emerging non-hepatic applications including respiratory and intracavitary uses (Sidhu et al., 2018) e.g. to guide lung biopsy and assessment of vesico-ureteric

**Figure 6.2a – Baseline (pre-contrast) image of a loculated effusion appearing to show 3 separate locules but unclear if communicating.**

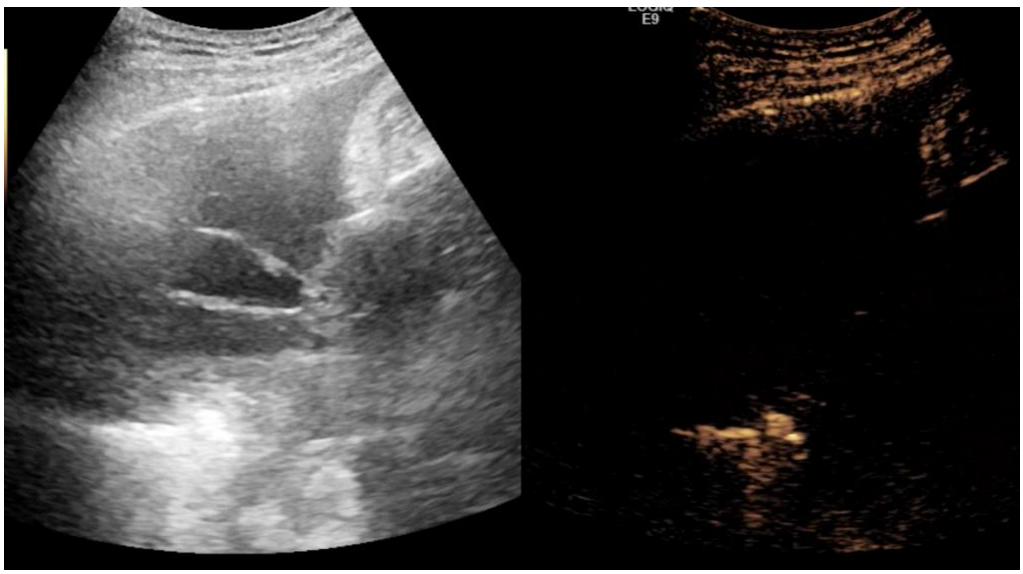


**Fig 6.2b – Mid Contrast filling (+30 seconds) after injecting diluted SonoVue contrast intrapleurally**

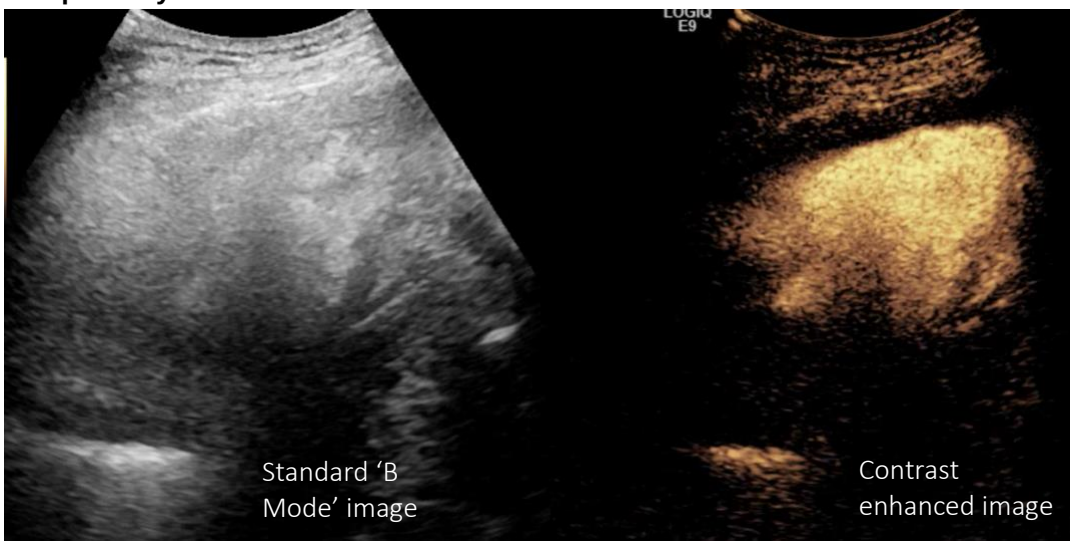
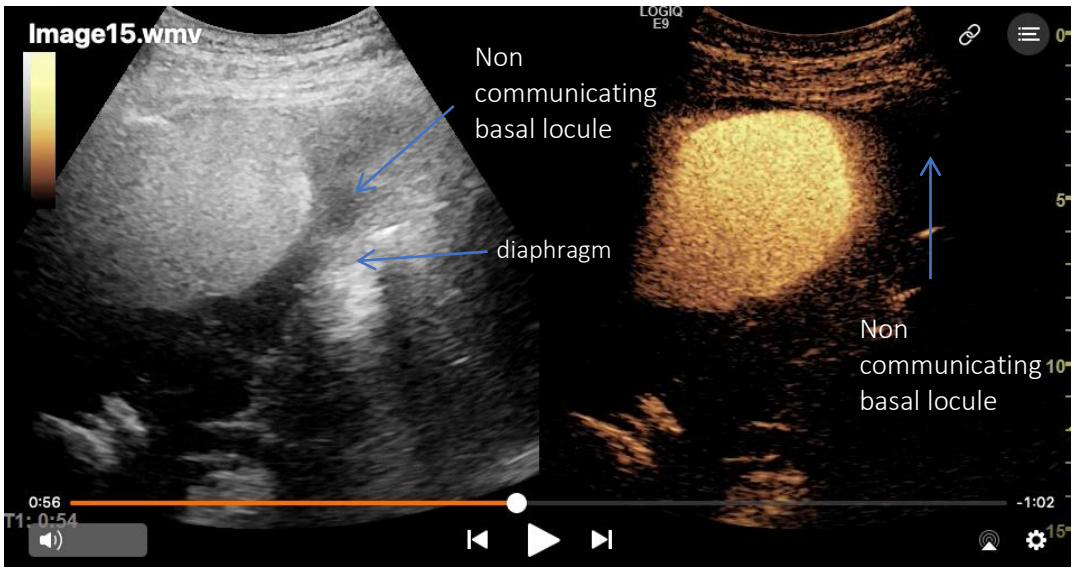



**Fig 6.2c – Post contrast (+1min) – contrast image clearly showing non communicating basal locule and one could predict unlikely to drain.**




reflux, hence I hypothesised that it could potentially be used in the pleural space to predict early drainage failure and allow decisions regarding escalating intervention to begin sooner. The contrast allows for optimised imaging (compared to intrapleural saline bubbles, which are often difficult to visualise in the context of heavily echogenic pleural collections) and SonoVue has an excellent safety profile (Sidhu et al., 2018).

### 6.6.2 MIST-4

Based on the results of the MIST-3 trial, I have co-written (as co-PI) an NIHR HTA application with my co-supervisor, Professor Rahman, for the MIST-4 study which aims to definitively evaluate early surgery versus early IET in 604 patients across 25 centres over 54 months, including a 9 month set up and 9 month recruitment pilot phase. An approximation of 15% 'quick responders' based on MIST-3 has been factored into the sample size calculation hence enriching the study population with those who stand to benefit most. The trial protocol has been modified to incorporate a minimum fitness criterion accepting that the 'all comer' strategy would not be feasible. Compliance and crossover definitions have been refined considering that IET is not a one-off treatment and the one-way nature of crossing over from IET to VATS surgery. A primary outcome of treatment failure rate over 90 days has been chosen based on patient prioritisation of wanting the "best definitive treatment" "as soon as possible", being objectively measurable and less prone to the limitations of length of stay given the differences in time to intervention of IET and surgery. Learnings from the qualitative data will be incorporated into the design of the patient facing documents as well as the study protocol (eg the provision of analgesia and discussions with family as well as patients when appropriate) and a member of the MIST-3 PPI will be invited to lead patient representation in MIST-4.

### 6.6.3 AUDIO-2

Precise antibiotic choice is a significant problem in pleural infection, due to poor diagnostic yields of conventional microbiological tests. This means that a significant number of patients are on prolonged courses of empiric antibiotic therapy as routine care in the NHS. Strategies to increase microbiological yield are desperately needed, to improve bacterial identification, and thereby narrow antibiotic treatment and provide antibiotic stewardship – crucial to the global antimicrobial resistance (AMR) agenda. Furthermore, even if empirical antibiotic therapy covers the causative organism, both clinicians and patients would like certainty that this is the case, and unfortunately our ability to confirm this is poor. On the back of the AUDIO pilot feasibility study (Psallidas et al., 2018), the aims of AUDIO-2 will be to critically assess whether the routine use of pleural biopsy in all adult patients with pleural

infection at time of diagnostic sampling results in a more accurate ‘actionable’ microbiological diagnosis and thereby results in altered clinician practice of focussed and / or more confident use of the correct antibiotics – through either narrowed spectrum agents or a shortened course. A funding application is in preparation with plans to submit in Spring 2024.

## 6.7 Concluding summary

In summary, the body of work presented in this thesis provides original contributions to the literature in terms of novel directions in the biological predictors of pleural infection, and in turn the biological mechanisms underpinning inflammation and fibrinolysis inhibition in pleural infection. It nullifies the simplistic assumption that the presence of sonographic septations predict poor outcomes in pleural infection. It presents the first attempt at a multicentre trial of pleural infection directly comparing surgery and intrapleural therapy, demonstrating feasibility and important insights into trial design. It brings the first qualitative study of pleural infection directly informing patient experience and priorities and the largest series of IET treated pleural infection patients to inform safety and complications. These findings will stimulate further research and trials both undertaken by myself and by the teams I have worked with, and elsewhere.

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