

## ERS Monograph

# Pleural infection – moving from treatment to prevention

Dr. Eihab O Bedawi MBBS MRCP<sup>1,2</sup>

Professor Najib M Rahman DPhil MSc FRCP<sup>1,2,3</sup>

<sup>1</sup>Oxford Pleural Unit, Oxford University Hospitals NHS Trust, Oxford, United Kingdom

<sup>2</sup>Oxford Respiratory Trials Unit, University of Oxford, Oxford, United Kingdom

<sup>3</sup>NIHR Biomedical Research, University of Oxford, Oxford, United Kingdom

Email: [eihab.bedawi@ndm.ox.ac.uk](mailto:eihab.bedawi@ndm.ox.ac.uk)



@BedawiEihab

Email: [najib.rahman@ndm.ox.ac.uk](mailto:najib.rahman@ndm.ox.ac.uk)



@naj\_rahman

Correspondence to:

Dr Eihab Bedawi  
Oxford Pleural Unit  
Churchill Hospital  
Old Road  
OX3 7LE  
Oxford, United Kingdom  
Tel: 01865 226767



*Pleural infection incidence is on the rise. Many unknowns remain with regard to the etiopathogenesis of this condition. Risk stratification at the front door and early aggressive treatment by specialist teams could help improve outcomes*

## Brief summary (to act as an abstract) (160 words)

The incidence of pleural infection is rising, moreso in the elderly, where it is associated with the highest mortality. Despite notable limitations in animal models replicating the human pleural space, there has been some progress in our understanding of the evolution of pleural infection. Studies continue to demonstrate that the microbiology is inherently different from pneumonia emphasising that this is a distinct disease. Great headway has been made in the last decade with regard to optimising drainage. The place of intrapleural enzyme therapy in the therapeutic armamentarium is growing in importance, with research efforts now focused on optimising dosing, administration and exploring new targets. Surgery continues to play an important role but timing and patient selection remain unclear. An increased awareness of at risk groups, coupled with early aggressive management strategies supported by risk stratification at time of presentation are likely to be essential components in aiding the healthcare community improving outcomes of this morbid condition.

## Introduction

Up to 57% of patients with pneumonia have an associated pleural effusion [1], which varies in size from a tiny effusion, almost not visible on a chest radiograph, to a large effusion contributing to ventilatory compromise. The presence of these effusions is associated with a significant increase in mortality regardless of where it falls in the 'parapneumonic' effusion spectrum [2]. The priority for clinicians is to make the important distinction between the so-simple parapneumonic effusion and pleural infection (approximately 10% of all parapneumonic effusions), which incorporates 'complex' parapneumonic effusions or frank pus in the pleural space (empyema), as this has substantial treatment and prognostic implications. It is important to highlight that these so-called 'simple' parapneumonic effusions are associated with an increased admission rate, longer hospital stays, longer durations of antibiotic therapy and mortality upto 240% higher than pneumonia without effusion [2]. They will usually resolve with antibiotic therapy targeted at the underlying pneumonia, whereas a pleural infection requires prompt drainage and prolonged broad-spectrum antibiotic therapy, targeting a distinct microbiological niche [3, 4]. Importantly, with increasing use of cross-sectional imaging, pleural infection without adjacent consolidation is becoming increasingly recognised, in up to 30% of cases.

Pleural infection is reported to affect about 80,000 patients in the US and UK annually. The incidence of pleural infection is said to be about 8-fold that of cystic fibrosis, five-fold that of idiopathic pulmonary fibrosis [5, 6]. Worryingly, the incidence of pleural infection is steadily increasing world-wide, with a skew towards older patients in population based cohort studies[7, 8]. In parallel, little progress has been made in terms of improving outcomes over the last two decades [9, 10]. Pleural infection is often associated with considerable morbidity and a recent systematic review demonstrated the high prevalence of pre-existing

comorbidity (median 72%) [11]. Mortality exceeds that of myocardial infarction [12]. The largest population-based cohort study of pleural infection recently reported a 30-day mortality of 10% [8] whilst a recent outcome study of >600 patients from Western Australia found a one-year mortality of up to 32% [13]. Worldwide, the average length of hospital stay is 19 days [11] with significant healthcare resource utilization. The financial burden on the National Health Service in the United Kingdom, for inpatient costs alone, is estimated at £90 million per year and reported figures in the United States are close to half a billion dollars annually [9].

## Epidemiology

In the early 21<sup>st</sup> century, a plethora of evidence emerged demonstrating a rise in the rates of pneumococcal disease with resultant increases in the incidence of pneumonia and pleural infection [9, 10, 14]. Studies have suggested that widespread vaccination programmes might have caused a replacement phenomenon with non vaccine serotypes becoming increasingly responsible for disease [15]. The prevalence of non-PVC7 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, which was registered for paediatric vaccination from 2009 and adult vaccination from 2011 [16]. Early studies on the consequent effects on pleural infection incidence have been inconclusive [17, 18] and data from large epidemiological studies are eagerly awaited.

Nonetheless, the change in the epidemiology of pleural infection is not sufficiently explained by non vaccine serotypes alone, and does not adequately cover non-pneumococcal pleural infection as well as pleural infection without pneumonia. An ageing population may explain the increasing incidence of pleural infection in older patients with comorbidities living longer with an increased risk of aspiration of oropharyngeal commensals, previously under-recognised. The use of more specific imaging such as CT and ultrasound is likely to have contributed to more accurate diagnoses, as well as increased use of improved microbiological diagnostics (PCR and blood culture bottles vs standard culture alone). 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.

## Current treatment of pleural infection including diagnosis, drainage, intrapleural agents and surgery

## Clinical presentation and assessment

The diagnosis of pleural infection can often be delayed and challenging; with clinician awareness being key. Classical biochemical parameters are not absolute. Fever and rigours in the presence of an effusion in the context of a non-resolving pneumonia makes matters more straightforward. However, there is 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, mistakenly enrolled primarily onto a pathway of investigation of suspected malignancy. The delayed recognition of pleural infection in this often frail and co-morbid cohort of patients, inevitably carries a negative effect on treatment success and subsequent recovery [19]. It is also important to identify younger patients who are at greater risk of developing complex parapneumonic effusion from pneumonia, even if an effusion is not initially present (or does not meet diagnostic criteria for pleural infection), as these groups require close monitoring. Risk factors independently predictive of an increased likelihood of pneumonia progressing to pleural infection include diabetes, immunosuppression, gastroesophageal reflux disease (GERD), alcohol excess, intravenous drug use and poor oral hygiene [4, 20, 21]

Thoracic ultrasound (TUS) is not only vital for guiding the safe sampling of the pleural fluid [22] but can also help to assess for features that are known to be associated with complicated parapneumonic effusions and empyema, such as echogenic swirling (in the presence of pus), septations and loculations [23]. Despite widespread use of TUS, there are limited data regarding its predictive potentials in diagnosing pleural infection [24]. The significance of septations, and locules in particular, was demonstrated in a small case series demonstrating diagnostically significant variations in pH values within the same pleural effusion depending on which locule was sampled [25]. Whilst Computed Tomography (CT) offers a variety of useful information regarding pleural pathology in general, TUS appears to be a superior modality to rule in a CPPE when compared with chest CT and CXR [24], and whether or not CT has a routine place in the imaging of pleural infection, is debatable. However, in cases of persistent pleural sepsis beyond the initial 48 hours, evaluation with a contrast-enhanced CT scan (in the venous 'pleural' phase) can be invaluable in revealing malpositioned chest tubes in complex pleural collections, parenchymal lung abscesses, an adjacent subdiaphragmatic abscess as well as bronchopleural fistulas.

## Pleural fluid analysis

To date, the optimal recommended sampling for microbiology continues to be obtaining pleural fluid for standard culture (30-40% yield), and inoculating into blood culture bottles increasing yield by upto a further 20% [26]. It is also noteworthy that whilst routine blood sampling for culture is often overlooked, analysis of the MIST-1 study found blood cultures

yielded the only positive microbiology in 12% of pleural infection cases, and hence guidelines recommend that these are routinely performed [27].

Nucleic acid amplification testing (NAAT) can amplify and detect DNA (or RNA) present in clinical samples. NAAT has significant theoretical advantages including organism detection being less susceptible to prior antibiotic use, overcoming the technical difficulties of culturing the more fastidious organisms as well as multiplex polymerase chain reaction (PCR) being able to test for multiple pathogens in a single NAAT experiment [28]. A common sequencing target used for bacterial identification is the 16S ribosomal RNA gene, present in all bacteria [29]. In the past this technique was methodologically restricted to identifying only one pathogen per clinical sample, unless expensive cloning techniques were used. These limitations have been overcome with Next generation sequencing (NGS), capable of sequencing an entire human genome within a single day [30]. However, until these techniques become more cost-effective, technically less complex (or expertise becomes more widespread), they are yet to be incorporated into routine clinical practice. Whilst large retrospective studies have demonstrated frank pus to be associated with the greatest chance of a positive microbiological yield [31], the current reality in clinical practice is that in approximately 2 out of 5 pleural infection cases, the micro-organism remains unknown and antibiotic therapy is completely empirical.

When one considers the reasons for this low microbiology yield, it is logical to ask the question 'are we looking in the right place?' An infected pleural space contains pleural fluid that is acidic, hypoxic and lacking in nutrition. It would seem reasonable that bacteria would find the pleural lining to be a more favourable environment, due to its richer blood supply. To this effect, the AUDIO feasibility study demonstrated that ultrasound-guided pleural biopsies performed at time of chest drain insertion provided a greater microbiological yield (approximately 60%) independent of the presence of pleural thickening [32]. A large prospective multicentre trial is planned before this procedure can be embedded into standard practice. What is of particular interest is that, in the AUDIO study, 75% of culture positive pleural biopsy had prior antibiotic administration. This may simply reflect the limited antibiotic penetration into the pleural space or emphasize the importance of other features of the pathogenesis such as biofilm formation in this condition.

As microbiology culture results pose an unacceptable delay, biochemical surrogates of bacterial infection are often more helpful in the initial diagnosis. Remarkably, pleural fluid pH has stood the test of time as the single most useful index for predicting the need for drainage of a parapneumonic effusion[33]. While guidelines suggest a binary cut-off of 7.2 to simplify everyday practice [27], it is important to recognise that such a criterion cannot be 100% sensitive and studies have shown that patients with a pH of up to 7.37 may require chest tube drainage to be adequately treated [34]. It is noteworthy that pleural fluid pH can be prone to instability and contamination[35]. In this regard, recent multicentre data from just fewer than 3000 patients, has demonstrated that concordance rates of pleural fluid

glucose with pH are high, and therefore in cases of uncertainty of the reliability of a pH reading, a pleural fluid glucose measurement ( $<2.2\text{mmol}$ ) is likely to perform just as well. Along with LDH ( $>1000$ ), clinical and radiological evaluation, in combination, are necessary to making a correct and timely diagnosis. Biochemical parameters in the blood, such as serum procalcitonin, have not yet been proven to be superior to white cell count ( $>15$ ) and C-reactive protein ( $>100$ )[21, 36]. Additionally, in a prospective observational study of 1269 patients presenting to hospital with pneumonia, a high platelet count ( $>400$ ) and low albumin ( $<30$ ) were found to be significant predictors of pleural infection [20].

## Overview of Bacteriology

The bacteriology of pleural infection has been clearly shown to be distinct from that of pneumonia [4]. A recent systematic review additionally demonstrated that this has evolved over time. There has been an increase in the role played by gram-positive bacteria in recent years [3, 9]. Staph aureus appears to have overtaken Streptococcus viridans group (the most common subgroup of the latter being the S. Milleri group) as the most common isolate across all settings. Worryingly, the proportion of methicillin resistant staph aureus (MRSA) has increased to almost 60% of all staph aureus infections, particularly in hospital-acquired infections. The incidence of polymicrobial pleural space infections in the current literature is estimated at approximately 23% [3], but as described in earlier sections, this is likely to be underestimated by standard culture techniques. Recent metagenomic studies using DNA sequencing techniques have interestingly described polymicrobiality to be significantly higher in primary pleural infection (without pneumonia) with figures closer to 60%, compared to 25% in empyema as a complication of bacterial pneumonia [37].

The setting and geographical location of infection have a considerable bearing on the expected causative organism and hence an awareness of the local microbiology should always be reflected in the choice of empirical antimicrobial treatment [3]. Gram-positive aerobes form the majority of community acquired pleural infection, while gram-negative aerobes have the largest share of hospital-acquired cases. Anaerobic organisms are notoriously difficult to culture using standard methods, and hence are often under-represented in the literature (13% of all culture-positive cases in a recent systematic review) [3]. However, it is important to note that these were traditionally the most common causative organisms of empyema [38] and indeed this would seem logical given the favourable hypoxic environment of the pleural space, as opposed to the oxygen rich lung parenchyma. Previous studies addressing anaerobes specifically identified them in up to 74% of culture-positive cases [39]. This highlights the importance of anaerobic cover in empirical regimens. Despite the high frequency with which atypical organisms cause pneumonia, these organisms are rarely identified in pleural infection, suggesting an absence

of tropism for the pleural space and also that routine atypical antibiotic coverage is not necessary in pleural infection [40].

The significance of the setting and causative organism is not only in relation to antibiotic cover but has also been shown to predict clinical outcomes. One large multicentre epidemiological study demonstrated hospital acquired pleural infection to be associated with a significantly worse 30-day mortality (45% vs 32%)[41]. Analysis of the bacteriology of participants of the MIST-1 study (n=454) found that non-streptococcal pleural sepsis was associated with longer durations of hospitalisation and one-year mortality was significantly worse in staph aureus and mixed (polymicrobial) aerobic infections [4]. Interestingly, this may be supported by the recent finding that Plasminogen Activator Inhibitor-1 (PAI-1) levels are higher in pleural effusions from gram-positive bacteria, compared to gram-negative and culture-negative parapneumonic effusions[42]. This could suggest that in the future, if more rapid techniques of analysing microbiological samples become mainstream, this could potentially be a method of risk stratification.

### **Antibiotics in Pleural Infection**

Pleural pharmacokinetics in pleural infection are complex, not least due to the heterogeneity of patients' presentation; with variable degrees of pleural thickening, pleural fluid characteristics and levels of inflammation, all of which are likely to influence penetration of antibiotics in the pleural space [43]. In terms of antibiotic choices, a study specifically addressing antibiotic levels in empyemic pleural fluid [44] found that equilibrium of serum and pleural fluid levels occurred most rapidly with penicillin and metronidazole and guidelines recommend these as appropriate initial therapy [27]. Ceftriaxone and clindamycin followed in efficacy, and these would be reasonable choices in cases of penicillin allergy. It is noteworthy that such studies, as well as being historic, were based on rabbit models of empyema, which would have obvious limitations, the most obvious being the thinner pleura in rabbits. Studies of antibiotic penetration in human pleural fluid are lacking and based on small numbers [43]. As recently reviewed, animal model data would suggest that most antibiotics exceed the minimum inhibitory concentration (MIC) for the bacteria for which it would normally be used [45]. The notable exception is gentamicin and therefore guidelines recommend against the use of aminoglycosides in pleural infection [27]. As alluded to earlier, in the hospital acquired infection setting, e.g. in post-surgical pleural infection, agents such as vancomycin and piperacillin-tazobactam should be used to cover the added risk of MRSA and pseudomonas. Recent clinical pharmacokinetic studies using specific assays that can help quantify concentrations of commonly used antibiotics in human plasma and pleural fluid have suggested that it is still not clear how effective these are at inhibiting bacterial proliferation within the pleural space [46] and further studies in this area are needed. Duration and optimal route are also areas where the evidence base is lacking. The current recommendation is that the switch from intravenous to oral therapy be

guided by cessation of pyrexia, clinical improvement and resolution of inflammatory markers, aiming for a total duration of 4 weeks as a minimum [27]. As recently reviewed, there is currently no role for intrapleural antibiotics in the routine treatment of pleural infection [45].

## Chest tube drainage

In a recent study of clinical outcomes from a large Danish cohort, drainage delay was found to correlate independently to 90-day mortality [19] and hence the adage ‘the sun should never set on an empyema’ holds strong to this day, thirty years on [47]. The everlasting chest tube size debate has not been settled due to the absence of prospective randomised trials to address it specifically in pleural infection. However, retrospective analysis of the MIST-1 data [48] by chest tube size demonstrated that smaller bore (<15-French) drains did not negatively impact clinical outcomes in any way as well as being significantly more comfortable for patients [49]. It is noteworthy that this prospectively recruited cohort (n=405) included patients with septated collections as well as frankly purulent effusions [48]. The findings would seem logical when one considers the associated physiology. 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.

## Intrapleural Enzyme Therapy

Of greater interest to pleural physicians, has been the use of intrapleural enzyme therapy (IET) to aid drainage and improve outcomes. Research in this area actually began approximately 70 years ago [50], and has been predominantly driven by the recognition of the physiological changes that occur in an infected pleural space, notably the depression of intrinsic fibrinolytic activity and consequent increase in fibrin load [51]. It has been assumed that reversal of this process could prevent, or at least reduce the burden of, organised fibrous thickening that would otherwise result in chronic lung restriction. Despite studies in the paediatric population being more favourable, the use of ‘lone’ intrapleural fibrinolytic therapy (IPFT) (urokinase or streptokinase) in adult pleural infection could never be convincingly proven to have any positive impact on meaningful clinical outcomes [52]. This was subsequently confirmed in the two largest, adequately powered randomised trials in pleural infection to date (MIST-1 and MIST-2)[5, 48]. It is important to note that pleural infection in children is a different clinical entity with different diagnostic criteria, different

comorbidities and different outcomes, so comparisons of efficacy of interventions between the two populations are probably not valid. Where some of the controversy regarding the negative results for lone IPFT in the aforementioned trials is possibly due to the fact that the study population encompassed patients at variable stages of their infection and did not stratify for presence or absence of sonographic septations. Consequently, there is likely to have been heterogeneity in the intrapleural activity of endogenous PAI-1, which may be of relevance considering what we now understand about the role of this mediator, not only in directly inhibiting streptokinase, but also contributing to severity of loculation [53]. In-vivo studies in animal models have suggested that direct inhibition of PAI-1 may be a plausible therapeutic target [54] but is presently deterred by the absence of widely available means of monitoring or measuring baseline PAI-1 activity in humans with pleural infection.

Nonetheless, streptokinase has no effect on fluid viscosity, which is frequently increased in infection as a consequence of extracellular uncoiled DNA liberated from dead leukocytes and other bacterial components, representing another barrier to successful drainage. Following the positive results of an two in-vitro studies of purulent human pleural fluid samples incubated with combination streptokinase and streptodornase or rhDNase in combination achieving superior liquefaction (versus streptokinase and urokinase in isolation) [55, 56], it became clear that combination therapy was likely to provide superior results in terms of clearing the infected pleural space. This was then proven in a rabbit model of empyema using combination of intrapleural direct tissue plasminogen activator (tPA), which has the advantage of avoiding the plasminogen complex step needed by streptokinase, in addition to deoxyribonuclease (DNase) [57]. Translating this to a human population then came with the MIST-2 study, which showed that the combination of tPA, as a direct fibrinolytic to disrupt septations, and DNase to reduce viscosity, enhanced drainage of infected fluid in patients with pleural infection [5]. The other important consideration is the formation of biofilms, incorporating fibrin as well as DNA, which has been described in many common respiratory bacteria. DNase has been shown to have the ability to interfere with this matrix and potentially enhance the effect of antibiotics [58, 59], which whilst not proven, may be an important component of its action in the infected pleural space.

The challenge remains that IET is subject to relatively rapid inactivation by inhibitors including PAI-1, the major plasminogen activator inhibitor within pleural fluid. High pleural fluid PAI-1 activity levels increase pleural organisation and strongly influence the outcomes of IET [60, 61]. As previously mentioned, it is also now understood that adult patients with pleural infection have a wide range of pleural fluid PAI-1 as was demonstrated in the variability in fibrinolysin inhibitor profile in the MIST-2 population. This becomes relevant when we consider that currently IET dosing is chosen empirically as a 'one size fits all' when it is likely that an effective dose in one patient may be insufficient in another and vice versa. Personalised dosing regimes may be more beneficial, safer and cost-effective and this is an area of ongoing exploration [62]. Since publication of MIST-2, there are now approximately 400 patients in the literature who have been safely and effectively treated with IET,

including large case series [59] as well as studies involving dose de-escalation [63], once daily administration [64], and modification of the course duration [65]. Despite this, the routine widespread use of IET has met some resistance, probably due to financial reasons pertaining to the high cost of the tPA component. Although the MIST-2 study itself was not designed to detect meaningful differences in healthcare costs, a recently published secondary cost analysis study has shown that the use of IET (even in the likely over-estimated empirical doses used in the original study) is likely to be highly cost-effective [66]. Future confirmatory trials are awaited.

More recently, IET research has taken novel directions. PAI-1 neutralising antibodies have been shown to enhance the effectiveness of low doses of IET [54]. LTI-01, a new form of single-chain urokinase plasminogen activator (scuPA), is relatively resistant to PAI-1 inhibition and has shown durable, low grade bioavailability beyond 24 hours with promising results in a recent phase I study [67]. Over the coming years, it could represent a paradigm shift in IET use.

Besides mechanical disruption of septations, data from human and animal studies of fibrinolytics [57, 68] have shown that the IPFT component of IET (tPA) is associated with an upto ten-fold increase in pleural fluid output. Studies using an in-vivo mouse model of pleural infection have demonstrated this to be a class effect (with streptokinase, urokinase and tPA all stimulating excess pleural fluid formation) and suggested that this is mediated via MCP-1 expression and protein release by mesothelial cells, with pleural fluid levels of this cytokine directly correlating with volume of fluid produced. Conversely, blockade of MCP-1 activity resulted in loss of the fluid stimulating effect of tPA [69]. However, when this hypothesis was tested in a human pleural fluid samples from the MIST-2 dataset, whilst intrapleural tPA stimulated a statistically significant rise in volume of drained pleural fluid and increased MCP-1 pleural fluid levels, pleural fluid MCP-1 expression did not correlate with drainage volume, suggesting there are likely to be additional pathways at play [70]. What is clear is that intrapleural fibrinolytics result in an additional therapeutic lavage effect in the pleural space, which is likely to aid clearance of infected material. This was demonstrated in a pilot study showing potential benefit of saline irrigation via chest tube, as an alternative therapeutic option in pleural infection[71].

## **Surgery**

Despite randomised trial data [5, 48] having shown that around 80% of patients can be managed medically, surgery continues to play an important role in the management of pleural infection. While there is significant inter-patient variability, patients presenting with later stages of empyema with an organised pleural cortex, are less likely to achieve a full recovery and lung re-expansion without surgical intervention. There has been a significant advance in minimally invasive techniques, potentially widening the population who might be suitable for surgical intervention, although large case series [10, 72] continue to show a preference for operating on younger and less comorbid individuals than those seen in

unselected populations of pleural infection [5, 48]. VATS is accepted as an alternative treatment option in earlier stages of fibrinopurulent empyema, with no statistically significant difference in mortality [73].

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 [27]. 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, with significant conversion rates up to 61% [74]. 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 [75]. The reported complication rate after VATS decortication varies from 9% to 40% [76], the most frequent complications being prolonged air leak, bleeding, recurrence or persistence of the disease, surgical wound infection and a residual pleural space [75]. The 30-day post-operative mortality ranges from 2-6% [77].

Studies have addressed whether any preoperative radiological features, intraoperative findings or pleural fluid microbiology can predict operative success or risk of conversion to thoracotomy, but these have had conflicting results and therefore this domain remains inconclusive [77–81]. Delays in surgical intervention have been shown to be the most common predictor of conversion [77, 78]. It is noteworthy that the guidelines 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. There may be some patients in whom the mortality risk at time of diagnosis outweighs the risk of an operation and general anaesthetic, and perhaps earlier surgical intervention in this cohort maybe beneficial. The question that follows on from this is whether or not surgery can truly improve key clinical outcomes. In terms of current support for its use first line, data from paediatric studies would suggest that it has no clinical benefit and incurs a greater financial cost. A multi-centre trial comparing early VATS with IET, assessing key clinical outcomes, is underway and likely to add valuable insights into the place of surgery in the management of adult pleural infection.

### **3. Development of pleural infection – transition from simple to complex 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. The direct invasion of

microorganisms in the lung parenchyma leads to breakdown of host defences and provocation of intra-alveolar exudates. The resultant inflammation of the parenchyma causes an increased permeability of the visceral pleural membranes and resultant leakage of interstitial fluid. The mesothelial cell lining is further disrupted, as a result of neutrophil migration and the release of pleural pro-inflammatory cytokines into the pleural space by pleural mesothelial cells occurs, including tumour necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6) and interleukin-8 (IL-8), particularly higher in effusions of an infectious aetiology compared to tuberculosis, malignancy and heart failure [82]. This results in an anatomical distortion of the visceral pleural mesothelial lining, creating intercellular 'gaps' and increasing permeability across the membrane, which allows accumulation of additional fluid [83–85]. This initial *exudative phase* is consistent with simple parapneumonic effusions, where the fluid will not have any 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 [86].

If inflammation persists, depression of the, normally high, levels of fibrinolytic ensues through a rise in PAI-1 and, to a lesser extent, PAI-2 [51]. Along with other mediators including TNF-alpha, these are directly released from pleural mesothelial cells; particularly seen in infection, compared with inflammatory effusions of other aetiologies [51, 87]. As a consequence of the reduced fibrinolytic activity, fibrin deposition occurs over the visceral and parietal pleura, with strands of fibrin dividing the pleural space by septations, forming adhesions and localising 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 [88]. This may explain why patients who enter *this fibrinopurulent phase*, and subsequently diagnosed with complex parapneumonic effusion, require urgent drainage to prevent detrimental effects on lung function. The bacterial metabolism and neutrophil phagocytic activity that occurs in this phase leads to increased lactic acid production, reflected in a drop in pleural fluid pH and glucose; the biochemical hallmarks of pleural infection [89, 90]. The lactate dehydrogenase levels rise and this seems to be due to release of these enzymes by the polymorphs and mononucleate cells involved in pleural inflammation [91]. 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 macroscopic visualisation of pus on aspiration is diagnostic of an empyema mandating immediate drainage.

Throughout the transition from simple to complex parapneumonic effusion, so far the lung typically remains expandable. The final stage, known as *the 'organising' stage* is characterised by proliferation of fibroblasts and pleural scarring, where lung entrapment may ensue due to visceral pleural fibrosis, likely to result in significant lung function impairment. Platelet-derived growth factor (PDGF) [92] and transforming growth factor beta (TGF-beta) [93] have been found to be the mediators most likely to be responsible for this process, based on animal model data. The clinical significance of this phase has also been thought to be important in marking the point at which surgical intervention (VATS) becomes

inevitable to successfully treat the patient. The rationale for this being that IET is 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 [94]. This is of particular importance in the elderly whom, as mentioned above, may present with a more indolent 'slow burning' infection, and implies that a trial of medical treatment may still be worthwhile. The timeframes over which patients progress through these stages of pleural infection are likely to depend on several factors, including the patient's own immunity and the virulence of the infecting organism. Whether or not progression is truly linear, is also unclear, as not all patients will develop pus but rather some patients end up with heavily loculated collections. It is plausible that a combination of bacterial factors and host fibrinolytic response 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 in the formation of an empyema, or whether or not a certain degree of septation prevents 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 spontaneous resolution of pleural thickening, recovering fully at 12 weeks [95], others may develop a chronic sepsis state and longstanding lung function deficits [96].

#### 4. Etiology of the infected pleural space – where does the infection arise?

As alluded to in the previous section, most cases of pneumonia-associated pleural infection are typically broken down into 3 phases with rates of transition, and time spent in each stage, highly variable between patients. The trigger is usually aspiration of oropharyngeal bacteria with development 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. These are likely to be more complex than simply a delay in administration of antibiotics or suboptimal treatment of the initial pneumonia, as historically assumed. An understanding of the mechanisms that contribute to pleural injury has been largely impeded by the lack of a survivable murine model resembling human disease that permits investigation of the pathogenesis of pleural infection. Moreso, the majority of animal model studies have been produced using intrapleural inoculation, which bypasses how bacteria managed to infiltrate to reach the pleural space in the first place. In one study, *intranasal* inoculation of a mouse with streptococcus pneumoniae (*S. pneumoniae*) followed by pleural lavage at different timepoints showed that even as early as 4 hours, the bacteria had managed to rapidly penetrate into the pleural space as evidenced by bacterial concentration in the lavage fluid [97]. Within 24 hours, there was evidence of bacteria and necrosis within the mesothelial cell layer with formation of adhesions at 48 hours. Whilst this progression is far quicker than what we see in humans, in

particular the commonly seen indolent presentations, it does suggest that translocation of bacteria through mesothelial cells could be an important route, at least for *S. pneumoniae* specifically. In the same study, direct *intrapleural* inoculation of *S. pneumoniae* into the pleural space resulted in a rapid septicaemia, insinuating that the pleural space itself is permissive for bacterial replication capable of overwhelming local immune defences. This was observed to a much lesser extent when the bacteria was injected *intravenously* suggesting that indirect haematogenous spread of bacteria into the pleural space appears less likely, but again, this could be organism specific. Whilst this study demonstrated *S. pneumoniae* to be effective at causing pneumonia and inevitable empyema in a mouse model, it is obvious that this is not always the case with humans, as not every episode of pneumonia results in pleural infection. However, it may suggest that humans have efficient mechanisms that prevent pleural infection possibly relating to pre-existing immunity from previous colonisation, or early use of antibiotics.

A study that aimed to improve on this mouse model survival demonstrated that it was not possible to induce an empyema in a mouse with the maximal, survivable intranasal dose of the organism [98]. The authors managed to demonstrate that antibiotic treatment improves survival of mice intrapleurally infected with *S. pneumoniae* (maintaining them up to 14 days) and demonstrated that PAI-1 deficiency is likely to play an important role in worsening local inflammation in the pleura, resulting in the earlier development of neutrophilia and purulent pleural effusion independent of bacterial load in mice that were PAI-1 deficient [98].

The study that demonstrated human pleural fluid to be a potent growth medium for *S. pneumoniae* [99], suggested that higher levels of pneumococcal surface adhesion A (PsaA), a manganese uptake transporter common to all pneumococci, was associated with increased bacterial proliferation. In mutant pneumococci without PsaA, bacterial proliferation in the pleural fluid was significantly deterred, with subsequent manganese supplementation of the pleural fluid returning proliferation to normal rates. This suggests that there are factors that affect bacterial proliferation that if identified, could provide novel therapeutic targets in the future. Importantly, it is also unclear why some bacteria such as *S. pneumoniae*, have been shown to proliferate rapidly in pleural fluid [99] whilst isolates of otherwise common respiratory pathogens in empyema, do not.

The streptococcus milleri group of bacteria are facultatively anaerobic commensals of the oropharynx. They are amongst the most frequent cause of community-acquired pleural infection and yet, as described above, they rarely cause 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 and MIST-2 cohorts respectively[100](Franklin et al, in press). 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. The frequent role and polymicrobiality of oropharyngeal

bacteria in pleural infection adds to the evidence that aspiration plays a key part in the development of pleural infection. It is important to note that whilst aspiration is often associated with elderly patients and hospital acquired infections, our recent systematic review found anaerobic isolates to be relatively more common in community acquired infections and in younger patients, which may be related to poor dental hygiene as an under-recognised risk factor for pleural infection [101], with spread to the pleura likely to be via the haematogenous route.

Spontaneous bacterial empyema is increasingly recognised as a complication of hepatic hydrothorax in patients with cirrhosis [102]. Transient bacteraemia together with the impaired reticuloendothelial phagocytic activity associated with cirrhosis, are likely to be important factors to the development of SBEM with transcolonic translocation followed by transdiaphragmatic translocation of bacteria into the pleural space as an alternative route of entry.

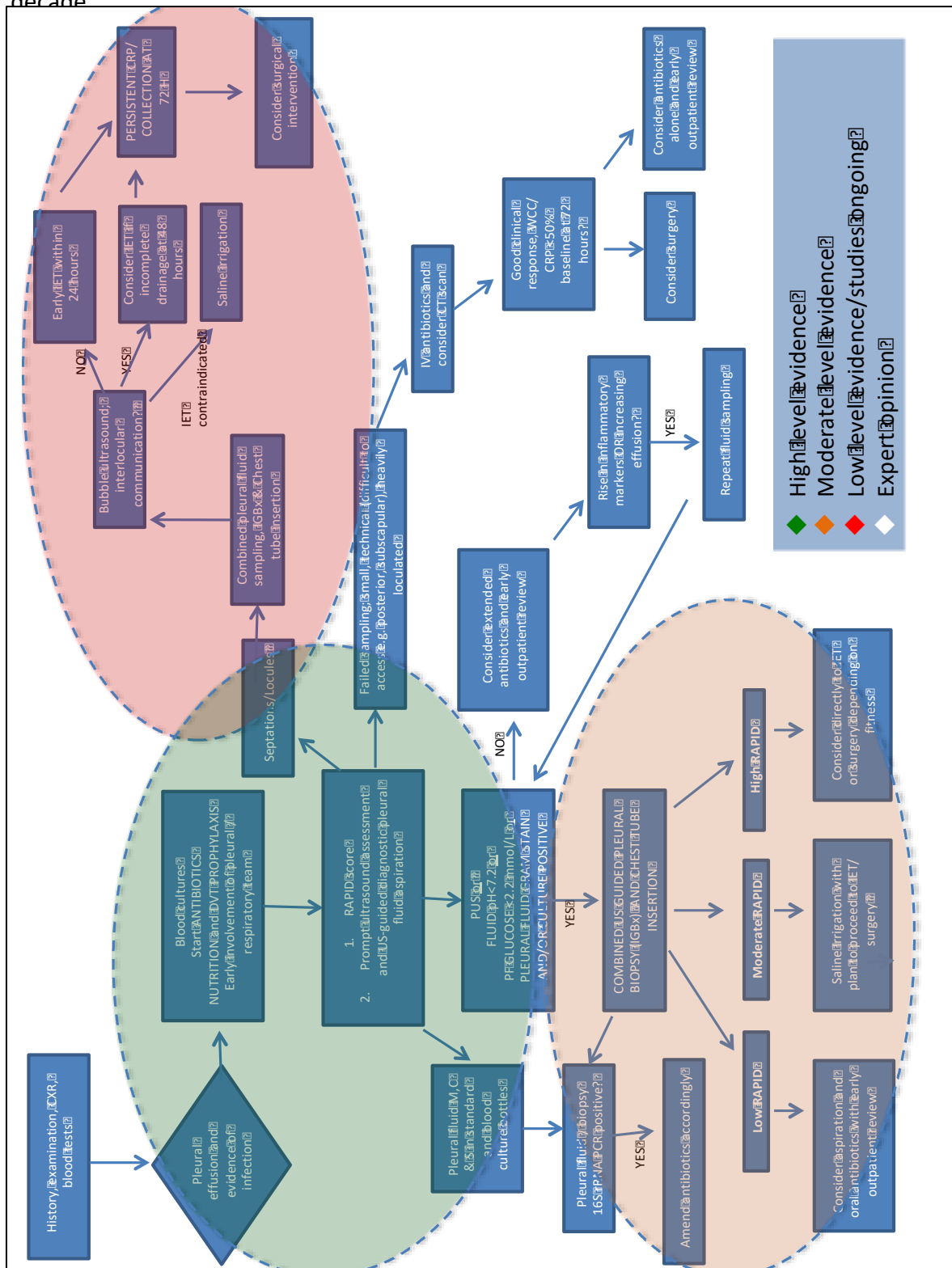
Other possible routes for the development of pleural infection include translocation through visceral pleural defects or fistulae in the context of lung cancer, post radiotherapy or postoperatively. Penetrating injury through the parietal pleural (akin to intrapleural inoculation of bacteria) occurring in the context of trauma or chest tube insertion is likely to be of significance, as well as spread from the mediastinum in cases of oesophageal rupture and transdiaphragmatic spread in the context of intra-abdominal infection [101, 103, 104].

## **5. Risk scoring and altering the treatment pathway**

Generic sepsis and pneumonia scores have been shown to perform poorly in predicting the development and course of pleural infection[20]. Historically, studies have suggested factors, including purulence [105] and septations [106], that could be implicated in the potential poor outcome of patients with pleural infection. However, these have not been prospectively validated in large clinical studies. The widespread uptake and accessibility of thoracic ultrasound means that sonographic surrogates of poor outcome would certainly be of interest to pleural physicians and easily applicable in everyday clinical assessment. However, our review of the literature on predictive ability of septations has found the studies to include small numbers with weak results due to their retrospective and un-blinded designs[107]. There is also likely to be a distinction required between septations and non-communicating locules, which are likely to have a greater impact on drainage. Consequently, this is an area that requires further prospective study.

Due to the increased risk associated with surgery in the elderly and patients with comorbidity, they are often denied this intervention. This inherent selection bias is nowhere clearer than when one considers the median age in large, unselective randomised controlled

Figure 1 – Possible developments in the pleural infection pathway re-imagined over the next decade



trials (61 years) [5] compared with large surgical series (52 years) [10]. The same studies have shown that patients undergoing operations had a much lower initial risk of early death [10], leading us to question how much they needed it in the first place. One would therefore envisage that it would be advantageous to have a tool that would allow risk stratification of patients according to their estimated mortality at time of diagnosis of pleural infection. Depending on their 'score', higher risk patients could be triaged to more aggressive interventions earlier in the course of the illness, while lower risk patients to earlier discharge or even outpatient treatment.

The RAPID score was reported in 2014 [108] as the first published prognostic risk model specifically for patients with pleural infection. This was derived from the MIST-1 cohort, and validated in the MIST-2 cohort, two large prospectively collected datasets [5, 48]. Baseline serum urea (Renal), patient age (Age), pleural fluid purulence (Purulence), infection source (hospital versus community - Infection) and serum albumin (Dietary) were independently associated with mortality at 3 months. Categorisation of patients in to low risk (RAPID score 0-2), medium risk (RAPID score 3-4) and high risk (RAPID score 5-7) groups was associated with mortality at 3 months of 3%, 9% and 31%, respectively [108]. Since its publication, positive correlation of clinical outcomes with the RAPID score has been demonstrated [109] and prospective assessment has recently been completed in an international, multicentre study [110] due to publish later this year. This should add greater insight into its potential utility in the clinical setting.

It is not yet clear how risk stratification could alter the treatment pathway in current practice. The growing expertise of specialist pleural multidisciplinary teams may mean that some of the steps in the current pathway can be refined (figure 1). It may be possible to manage low risk patients safely on an ambulatory pathway, with higher risk patients receiving upfront IET or surgery. In addition, advances in TUS may allow clinicians to alter treatment approach based on bedside sonographic appearances and escalate accordingly.

The recurring message of outcome studies has been that delays in treatment negatively impact outcomes [19]. The currently recruiting MIST-3 study is a feasibility study of randomising patients to early surgery vs early IET within 24 hours of presentation. As well as aiming to see whether this is feasible in the current clinical setting, it will be interesting to see whether this pathway is acceptable to participants and their carers who carry the burden of this morbid condition.

## Conclusion

Pleural infection was described over five thousand years ago. During this time, it has claimed many lives, the most high profile belonging to the medical profession, Guillain Dupuytren (1835) and William Osler (1919). These two esteemed clinicians of their eras had opposing views of whether empyema was best managed by a surgeon or a physician. It is striking, and somewhat comical, that to this day, more than a century later, this debate has

still not been settled. With multicentre, randomised head to head trials of IET versus VATS finally on the horizon, one hopes that we are getting close.

Despite the advances in medical and surgical drainage made in the last decade and the best efforts of treating clinicians, outcomes in pleural infection remain unacceptably poor. The traditional treatment pathway recommended by international guidelines, based largely on expert opinion, of sequential escalation of therapy, based on failure of conservative approaches, has proven ineffective. Whilst ongoing research into early risk stratification and personalised approaches to treatment are likely to result in further advances over the coming years, the healthcare and research communities may need to invest equal efforts into prevention strategies in pleural infection that have proven fruitful in other specialities, even within respiratory medicine.

Increased awareness in primary care settings and the hospital 'front door' is urgently needed to identify patients with features of infection and pleural effusions, those at increased risk of developing pleural infection and early involvement of specialist pleural services to prevent late presentations. As well as the established risk factors of older age and immunosuppression, poor dental hygiene and aspiration need to be addressed more proactively to prevent bacterial penetration into the pleural space. Translational research efforts into prevention of bacterial proliferation within the pleural space are ongoing and urgently needed to understand the virulence factors of certain organisms, and their complex interactions with host defence mechanisms within the human pleural space. In addition, preventing complexity of the pleural fluid by targeting cytokine mediators and reducing inflammation within the pleural space is likely to be of key importance. Finally, once pleural infection has been diagnosed, early, aggressive therapy with upfront IET or surgery may help prevent progression to more complex, organised stages, with inevitable long term sequelae and adverse outcomes.

## References

1. Sahn SA (2007) Diagnosis and management of parapneumonic effusions and empyema. *Clin Infect Dis* 45:1480–1486
2. Dean NC, Griffith PP, Sorensen JS, McCauley L, Jones BE, Lee YCG (2016) Pleural Effusions at First ED Encounter Predict Worse Clinical Outcomes in Patients With Pneumonia. *Chest* 149:1509–1515
3. Hassan M, Cargill T, Harriss E, Asciak R, Mercer RM, Bedawi EO, McCracken DJ, Psallidas I, Corcoran JP, Rahman NM (2019) The microbiology of pleural infection in adults: a systematic review. *Eur Respir J*. <https://doi.org/10.1183/13993003.00542-2019>

4. Maskell NA, Batt S, Hedley EL, Davies CWH, Gillespie SH, Davies RJO (2006) The bacteriology of pleural infection by genetic and standard methods and its mortality significance. *Am J Respir Crit Care Med* 174:817–823
5. Rahman NM, Maskell NA, West A, et al (2011) Intrapleural Use of Tissue Plasminogen Activator and DNase in Pleural Infection. *New England Journal of Medicine* 365:518–526
6. Corcoran JP, Hallifax R, Rahman NM (2013) New therapeutic approaches to pleural infection. *Curr Opin Infect Dis* 26:196–202
7. Finley C, Clifton J, Fitzgerald JM, Yee J (2008) Empyema: an increasing concern in Canada. *Can Respir J* 15:85–89
8. Søgaaard M, Nielsen RB, Nørgaard M, Kornum JB, Schønheyder HC, Thomsen RW (2014) Incidence, length of stay, and prognosis of hospitalized patients with pleural empyema: a 15-year Danish nationwide cohort study. *Chest* 145:189–192
9. Grijalva CG, Zhu Y, Nuorti JP, Griffin MR (2011) Emergence of parapneumonic empyema in the USA. *Thorax* 66:663–668
10. Farjah F, Symons RG, Krishnadasan B, Wood DE, Flum DR (2007) Management of pleural space infections: a population-based analysis. *J Thorac Cardiovasc Surg* 133:346–351
11. Cargill TN, Hassan M, Corcoran JP, Harriss E, Asciak R, Mercer RM, McCracken DJ, Bedawi EO, Rahman NM (2019) A systematic review of comorbidities and outcomes of adult patients with pleural infection. *Eur Respir J*. <https://doi.org/10.1183/13993003.00541-2019>
12. Smolderen KG, Buchanan DM, Gosch K, Whooley M, Chan PS, Vaccarino V, Parashar S, Shah AJ, Ho PM, Spertus JA (2017) Depression Treatment and 1-Year Mortality Following Acute Myocardial Infarction: Insights from the TRIUMPH Registry. *Circulation* 135:1681–1689
13. Brims F, Popowicz N, Rosenstengel A, et al (2018) Bacteriology and clinical outcomes of patients with culture-positive pleural infection in Western Australia: A 6-year analysis. *Respirology*. <https://doi.org/10.1111/resp.13395>
14. Burgos J, Lujan M, Falcó V, Sánchez A, Puig M, Borrego A, Fontanals D, Planes AM, Pahissa A, Rello J (2011) The spectrum of pneumococcal empyema in adults in the early 21st century. *Clin Infect Dis* 53:254–261
15. Byington CL, Hulten KG, Ampofo K, Sheng X, Pavia AT, Blaschke AJ, Pettigrew M, Korgenski K, Daly J, Mason EO (2010) Molecular Epidemiology of Pediatric Pneumococcal Empyema from 2001 to 2007 in Utah. *J Clin Microbiol* 48:520–525
16. Fletcher MA, Schmitt H-J, Syrochkina M, Sylvester G (2014) Pneumococcal empyema and complicated pneumonias: global trends in incidence, prevalence, and serotype epidemiology. *Eur J Clin Microbiol Infect Dis* 33:879–910
17. Chacon-Cruz E, Lopatynsky-Reyes EZ, Rivas-Landeros RM, Volker-Soberanes ML, Alvelais-Palacios JA (2016) Trends in Pediatric Pneumococcal Pleural Empyema Following Pneumococcal Conjugate 13-Valent Vaccination: 10 Years of Active Surveillance in a Mexican Hospital. *Open Forum Infectious Diseases*. <https://doi.org/10.1093/ofid/ofw172.637>

18. Thomas M, Sheppard C, Guiver M, Simmister C, Elemraid M, Clark J, Rushton S, Paton J, Spencer D (2013) S72 Paediatric pneumococcal empyema serotypes have not changed following introduction of the 13 valent pneumococcal vaccine. *Thorax* 68:A39
19. Meyer CN, Armbruster K, Kemp M, Thomsen TR, Dessau RB, Danish Pleural Empyema group (2018) Pleural infection: a retrospective study of clinical outcome and the correlation to known etiology, co-morbidity and treatment factors. *BMC Pulm Med* 18:160
20. Chalmers JD, Singanayagam A, Murray MP, Scally C, Fawzi A, Hill AT (2009) Risk factors for complicated parapneumonic effusion and empyema on presentation to hospital with community-acquired pneumonia. *Thorax* 64:592
21. Falguera M, Carratalà J, Bielsa S, García-Vidal C, Ruiz-González A, Chica I, Gudíol F, Porcel JM (2011) Predictive factors, microbiology and outcome of patients with parapneumonic effusion. *Eur Respir J* 38:1173–1179
22. Diacon AH, Brutsche MH, Solèr M (2003) Accuracy of pleural puncture sites: a prospective comparison of clinical examination with ultrasound. *Chest* 123:436–441
23. Marchetti G, Arondi S, Baglivo F, Lonni S, Quadri F, Valsecchi A, Venturoli N, Ceruti P (2018) New insights in the use of pleural ultrasonography for diagnosis and treatment of pleural disease. *Clin Respir J* 12:1993–2005
24. Svigals PZ, Chopra A, Ravenel JG, Nietert PJ, Huggins JT (2017) The accuracy of pleural ultrasonography in diagnosing complicated parapneumonic pleural effusions. *Thorax* 72:94
25. Maskell NA, Gleeson FV, Darby M, Davies RJO (2004) Diagnostically significant variations in pleural fluid pH in loculated parapneumonic effusions. *Chest* 126:2022–2024
26. Menzies SM, Rahman NM, Wrightson JM, et al (2011) Blood culture bottle culture of pleural fluid in pleural infection. *Thorax* 66:658–662
27. Davies HE, Davies RJO, Davies CWH (2010) Management of pleural infection in adults: British Thoracic Society pleural disease guideline 2010. *Thorax* 65:ii41–ii53
28. Wrightson JM, Rahman NM, Crook DWM, Wray JA (2012) Improving Pathogen Identification In Pleural Infection - Application Of Molecular Techniques. In: C107. PROGRESS IN BIOMARKERS AND DIAGNOSTICS FOR RESPIRATORY INFECTIONS. American Thoracic Society, pp A5244–A5244
29. Insa R, Marín M, Martín A, Martín-Rabadán P, Alcalá L, Cercenado E, Calatayud L, Liñares J, Bouza E (2012) Systematic use of universal 16S rRNA gene polymerase chain reaction (PCR) and sequencing for processing pleural effusions improves conventional culture techniques. *Medicine (Baltimore)* 91:103–110
30. Behjati S, Tarpey PS (2013) What is next generation sequencing? *Arch Dis Child Educ Pract Ed* 98:236–238
31. Porcel JM, Esquerda A, Vives M, Bielsa S (2014) Etiology of Pleural Effusions: Analysis of More Than 3,000 Consecutive Thoracenteses. *Arch Bronconeumol* 50:161–165

32. Psallidas I, Kanellakis NI, Bhatnagar R, et al (2018) A Pilot Feasibility Study in Establishing the Role of Ultrasound-Guided Pleural Biopsies in Pleural Infection (The AUDIO Study). *Chest*. <https://doi.org/10.1016/j.chest.2018.02.031>
33. Heffner JE, Brown LK, Barbieri C, DeLeo JM (1995) Pleural fluid chemical analysis in parapneumonic effusions. A meta-analysis. *Am J Respir Crit Care Med* 151:1700–1708
34. Jiménez Castro D, Díaz Nuevo G, Sueiro A, Muriel A, Pérez-Rodríguez E, Light RW (2005) Pleural fluid parameters identifying complicated parapneumonic effusions. *Respiration* 72:357–364
35. Rahman NM, Mishra EK, Davies HE, Davies RJO, Lee YCG (2008) Clinically important factors influencing the diagnostic measurement of pleural fluid pH and glucose. *Am J Respir Crit Care Med* 178:483–490
36. Dixon G, Lama-Lopez A, Bintcliffe OJ, Morley AJ, Hooper CE, Maskell NA (2017) The role of serum procalcitonin in establishing the diagnosis and prognosis of pleural infection. *Respir Res*. <https://doi.org/10.1186/s12931-017-0501-5>
37. Dyrhovden R, Nygaard RM, Patel R, Ulvestad E, Kommedal Ø (2019) The bacterial aetiology of pleural empyema. A descriptive and comparative metagenomic study. *Clin Microbiol Infect* 25:981–986
38. Bartlett JG, Gorbach SL, Thadepalli H, Finegold SM (1974) Bacteriology of empyema. *Lancet* 1:338–340
39. Boyanova L, Vladimir Djambazov null, Gergova G, Dragomir Iotov null, Petrov D, Osmanliev D, Minchev Z, Mitov I (2004) Anaerobic microbiology in 198 cases of pleural empyema: a Bulgarian study. *Anaerobe* 10:261–267
40. Wrightson JM, Wray JA, Street TL, Chapman SJ, Gleeson FV, Maskell NA, Peto TEA, Rahman NM, Crook DWM (2015) Absence of Atypical Pathogens in Pleural Infection. *Chest* 148:e102–e103
41. Park C-K, Oh H-J, Choi H-Y, Shin H-J, Lim JH, Oh I-J, Kim Y-I, Lim S-C, Kim Y-C, Kwon Y-S (2016) Microbiological Characteristics and Predictive Factors for Mortality in Pleural Infection: A Single-Center Cohort Study in Korea. *PLoS ONE* 11:e0161280
42. Lee K-L, Chen W-L, Chen R-J, Lai KS, Chung C-L (2018) Lipoteichoic acid upregulates plasminogen activator inhibitor-1 expression in parapneumonic effusions. *Respirology* 23:89–95
43. Popowicz N, Sparling B Pleural pharmacokinetics. *Textbook of Pleural Disease*
44. Teixeira LR, Sasse SA, Villarino MA, Nguyen T, Mulligan ME, Light RW (2000) Antibiotic levels in empyemic pleural fluid. *Chest* 117:1734–1739
45. Bedawi EO, Hassan M, McCracken D, Rahman NM (2019) Pleural infection: a closer look at the etiopathogenesis, microbiology and role of antibiotics. *Expert Rev Respir Med* 13:337–347
46. Popowicz ND, O'Halloran SJ, Fitzgerald D, Lee YCG, Joyce DA (2018) A rapid, LC-MS/MS assay for quantification of piperacillin and tazobactam in human plasma and pleural fluid; application to a clinical pharmacokinetic study. *J Chromatogr B Analyt Technol Biomed Life Sci* 1081–1082:58–66

47. Sahn SA, Light RW (1989) The sun should never set on a parapneumonic effusion. *Chest* 95:945–947
48. Maskell NA, Davies CWH, Nunn AJ, et al (2005) U.K. Controlled Trial of Intrapleural Streptokinase for Pleural Infection. *New England Journal of Medicine* 352:865–874
49. Rahman NM, Maskell NA, Davies CWH, Hedley EL, Nunn AJ, Gleeson FV, Davies RJO (2010) The relationship between chest tube size and clinical outcome in pleural infection. *Chest* 137:536–543
50. Tillett WS, Sherry S (1949) THE EFFECT IN PATIENTS OF STREPTOCOCCAL FIBRINOLYSIN (STREPTOKINASE) AND STREPTOCOCCAL DESOXYRIBONUCLEASE ON FIBRINOUS, PURULENT, AND SANGUINOUS PLEURAL EXUDATIONS. *J Clin Invest* 28:173–190
51. Idell S, Girard W, Koenig KB, McLarty J, Fair DS (1991) Abnormalities of pathways of fibrin turnover in the human pleural space. *Am Rev Respir Dis* 144:187–194
52. Cameron R, Davies HR (2004) Intra-pleural fibrinolytic therapy versus conservative management in the treatment of parapneumonic effusions and empyema. *Cochrane Database Syst Rev* CD002312
53. Komissarov AA, Rahman N, Lee YCG, Florova G, Shetty S, Idell R, Ikebe M, Das K, Tucker TA, Idell S (2018) Fibrin turnover and pleural organization: bench to bedside. *Am J Physiol Lung Cell Mol Physiol* 314:L757–L768
54. Florova G, Azghani A, Karandashova S, Schaefer C, Koenig K, Stewart-Evans K, Declerck PJ, Idell S, Komissarov AA (2015) Targeting of plasminogen activator inhibitor 1 improves fibrinolytic therapy for tetracycline-induced pleural injury in rabbits. *Am J Respir Cell Mol Biol* 52:429–437
55. Simpson G, Roomes D, Heron M (2000) Effects of streptokinase and deoxyribonuclease on viscosity of human surgical and empyema pus. *Chest* 117:1728–1733
56. Light RW, Nguyen T, Mulligan ME, Sasse SA (2000) The In Vitro Efficacy of Varidase Versus Streptokinase or Urokinase for Liquefying Thick Purulent Exudative Material from Loculated Empyema. *Lung* 178:13–18
57. Zhu Z, Hawthorne ML, Guo Y, Drake W, Bilaceroglu S, Misra HL, Light RW (2006) Tissue plasminogen activator combined with human recombinant deoxyribonuclease is effective therapy for empyema in a rabbit model. *Chest* 129:1577–1583
58. Hall-Stoodley L, Nistico L, Sambanthamoorthy K, et al (2008) Characterization of biofilm matrix, degradation by DNase treatment and evidence of capsule downregulation in *Streptococcus pneumoniae* clinical isolates. *BMC Microbiol* 8:173
59. Piccolo F, Popowicz N, Wong D, Lee YCG (2015) Intrapleural tissue plasminogen activator and deoxyribonuclease therapy for pleural infection. *J Thorac Dis* 7:999–1008
60. Komissarov AA, Florova G, Azghani AO, et al (2016) Dose dependency of outcomes of intrapleural fibrinolytic therapy in new rabbit empyema models. *Am J Physiol Lung Cell Mol Physiol* 311:L389–399
61. Karandashova S, Florova G, Azghani AO, Komissarov AA, Koenig K, Tucker TA, Allen TC, Stewart K, Tvinnereim A, Idell S (2013) Intrapleural adenoviral delivery of human plasminogen activator

inhibitor-1 exacerbates tetracycline-induced pleural injury in rabbits. *Am J Respir Cell Mol Biol* 48:44–52

62. Idell S, Florova G, Shetty S, Tucker T, Idell R, Koenig K, Azghani A, Rahman NM, Komissarov A (2017) Precision-guided, Personalized Intrapleural Fibrinolytic Therapy for Empyema and Complicated Parapneumonic Pleural Effusions: The Case for the Fibrinolytic Potential. *Clin Pulm Med* 24:163–169
63. Popowicz N, Bintcliffe O, De Fonseka D, et al (2017) Dose De-escalation of Intrapleural Tissue Plasminogen Activator Therapy for Pleural Infection. The Alteplase Dose Assessment for Pleural Infection Therapy Project. *Ann Am Thorac Soc* 14:929–936
64. Mehta HJ, Biswas A, Penley AM, Cope J, Barnes M, Jantz MA (2016) Management of Intrapleural Sepsis with Once Daily Use of Tissue Plasminogen Activator and Deoxyribonuclease. *RES* 91:101–106
65. McClune JR, Wilshire CL, Gorden JA, Louie BE, Farviar AS, Stefanski MJ, Vallieres E, Aye RW, Gilbert CR (2016) Safety and Efficacy of Intrapleural Tissue Plasminogen Activator and DNase during Extended Use in Complicated Pleural Space Infections. *Can Respir J* 2016:9796768–9796768
66. Luengo-Fernandez R, Penz E, Dobson M, Psallidas I, Nunn AJ, Maskell NA, Rahman NM (2019) Cost-effectiveness of intrapleural use of tissue plasminogen activator and DNase in pleural infection: Evidence from the MIST2 randomised controlled trial. *Eur Respir J*. <https://doi.org/10.1183/13993003.01550-2018>
67. Beckert L, Brockway B, Simpson G, et al Phase I trial of the single-chain urokinase intrapleural LTI-01 in complicated parapneumonic effusions or empyema. *JCI Insight*. <https://doi.org/10.1172/jci.insight.127470>
68. Piccolo F, Pitman N, Bhatnagar R, et al (2014) Intrapleural tissue plasminogen activator and deoxyribonuclease for pleural infection. An effective and safe alternative to surgery. *Ann Am Thorac Soc* 11:1419–1425
69. Lansley SM, Cheah HM, Varano Della Vergiliana JF, Chakera A, Lee YCG (2015) Tissue plasminogen activator potentially stimulates pleural effusion via a monocyte chemotactic protein-1-dependent mechanism. *Am J Respir Cell Mol Biol* 53:105–112
70. Kanellakis NI, Wrightson JM, Hallifax R, et al (2019) Biological effect of tissue plasminogen activator (t-PA) and DNase intrapleural delivery in pleural infection patients. *BMJ Open Resp Res* 6:e000440
71. Hooper CE, Edey AJ, Wallis A, et al (2015) Pleural irrigation trial (PIT): a randomised controlled trial of pleural irrigation with normal saline versus standard care in patients with pleural infection. *Eur Respir J* 46:456–463
72. Marks DJB, Fisk MD, Koo CY, et al (2012) Thoracic empyema: a 12-year study from a UK tertiary cardiothoracic referral centre. *PLoS ONE* 7:e30074
73. Redden MD, Chin TY, van Driel ML (2017) Surgical versus non-surgical management for pleural empyema. *Cochrane Database Syst Rev* 3:CD010651

74. Divisi D, Gabriele F, Barone M, Zaccagna G, Di Francescantonio W, Crisci R (2017) Clinical history and surgical management of parapneumonic empyema what is the role of video-assisted thoracoscopic surgery (VATS)? Video-Assisted Thoracic Surgery 2:
75. Subotic D, Lardinois D, Hojski A (2018) Minimally invasive thoracic surgery for empyema. *Breathe* 14:302
76. Jagelavicius Z, Jovaisas V, Mataciunas M, Samalavicius NE, Janilionis R (2017) Preoperative predictors of conversion in thoracoscopic surgery for pleural empyema. *Eur J Cardiothorac Surg* 52:70–75
77. Lardinois D, Gock M, Pezzetta E, Buchli C, Rousson V, Furrer M, Ris H-B (2005) Delayed referral and gram-negative organisms increase the conversion thoracotomy rate in patients undergoing video-assisted thoracoscopic surgery for empyema. *Ann Thorac Surg* 79:1851–1856
78. Stefani A, Aramini B, della Casa G, Ligabue G, Kaleci S, Casali C, Morandi U (2013) Preoperative predictors of successful surgical treatment in the management of parapneumonic empyema. *Ann Thorac Surg* 96:1812–1819
79. Roberts JR (2003) Minimally invasive surgery in the treatment of empyema: intraoperative decision making. *Ann Thorac Surg* 76:225–230; discussion 229-230
80. Cassina PC, Hauser M, Hillejan L, Greschuchna D, Stamatis G (1999) Video-assisted thoracoscopy in the treatment of pleural empyema: stage-based management and outcome. *J Thorac Cardiovasc Surg* 117:234–238
81. Striffeler H, Gugger M, Im Hof V, Cerny A, Furrer M, Ris HB (1998) Video-assisted thoracoscopic surgery for fibrinopurulent pleural empyema in 67 patients. *Ann Thorac Surg* 65:319–323
82. Strieter RM, Koch AE, Antony VB, Fick RB, Standiford TJ, Kunkel SL (1994) The immunopathology of chemotactic cytokines: the role of interleukin-8 and monocyte chemoattractant protein-1. *J Lab Clin Med* 123:183–197
83. Broaddus VC, Boylan AM, Hoeffel JM, Kim KJ, Sadick M, Chuntharapai A, Hébert CA (1994) Neutralization of IL-8 inhibits neutrophil influx in a rabbit model of endotoxin-induced pleurisy. *J Immunol* 152:2960–2967
84. Broaddus VC, Hébert CA, Vitangcol RV, Hoeffel JM, Bernstein MS, Boylan AM (1992) Interleukin-8 is a major neutrophil chemotactic factor in pleural liquid of patients with empyema. *Am Rev Respir Dis* 146:825–830
85. Kroegel C, Antony VB (1997) Immunobiology of pleural inflammation: potential implications for pathogenesis, diagnosis and therapy. *Eur Respir J* 10:2411–2418
86. Light RW, Girard WM, Jenkinson SG, George RB (1980) Parapneumonic effusions. *Am J Med* 69:507–512
87. Alemán C, Alegre J, Monasterio J, Segura RM, Armadans L, Anglés A, Varela E, Ruiz E, Fernández de Sevilla T (2003) Association between inflammatory mediators and the fibrinolysis system in infectious pleural effusions. *Clin Sci* 105:601–607

88. Chung C-L, Hsiao S-H, Hsiao G, Sheu J-R, Chen W-L, Chang S-C (2013) Clinical importance of angiogenic cytokines, fibrinolytic activity and effusion size in parapneumonic effusions. *PLoS ONE* 8:e53169
89. Sahn SA, Reller LB, Taryle DA, Antony VB, Good JT (1983) The contribution of leukocytes and bacteria to the low pH of empyema fluid. *Am Rev Respir Dis* 128:811–815
90. Light RW, MacGregor MI, Ball WC, Luchsinger PC (1973) Diagnostic significance of pleural fluid pH and PCO<sub>2</sub>. *Chest* 64:591–596
91. Saint-Rémy P, Buret J, Radermecker M (1986) [Significance of lactate dehydrogenases in pleural effusions]. *Rev Pneumol Clin* 42:74–81
92. Mutsaers SE, Kalomenidis I, Wilson NA, Lee YC (2006) Growth factors in pleural fibrosis. *Curr Opin Pulm Med* 12:251–8
93. Kunz CR, Jadus MR, Kukes GD, Kramer F, Nguyen VN, Sasse SA (2004) Intrapleural injection of transforming growth factor-beta antibody inhibits pleural fibrosis in empyema. *Chest* 126:1636–1644
94. Landreneau RJ, Keenan RJ, Hazelrigg SR, Mack MJ, Naunheim KS (1996) Thoracoscopy for empyema and hemothorax. *Chest* 109:18–24
95. Neff CC, vanSonnenberg E, Lawson DW, Patton AS (1990) CT follow-up of empyemas: pleural peels resolve after percutaneous catheter drainage. *Radiology* 176:195–197
96. Hamm H, Light RW (1997) Parapneumonic effusion and empyema. *Eur Respir J* 10:1150–1156
97. Wilkosz S, Edwards LA, Bielsa S, Hyams C, Taylor A, Davies RJO, Laurent GJ, Chambers RC, Brown JS, Lee YCG (2012) Characterization of a new mouse model of empyema and the mechanisms of pleural invasion by *Streptococcus pneumoniae*. *Am J Respir Cell Mol Biol* 46:180–187
98. Tucker TA, Jeffers A, Boren J, et al (2016) Organizing empyema induced in mice by *Streptococcus pneumoniae*: effects of plasminogen activator inhibitor-1 deficiency. *Clin Transl Med* 5:17
99. Popowicz ND, Lansley SM, Cheah HM, Kay ID, Carson CF, Waterer GW, Paton JC, Brown JS, Lee YCG (2017) Human pleural fluid is a potent growth medium for *Streptococcus pneumoniae*. *PLoS ONE* 12:e0188833
100. Jaffe A, Calder AD, Owens CM, Stanojevic S, Sonnappa S (2008) Role of routine computed tomography in paediatric pleural empyema. *Thorax* 63:897–902
101. Corcoran JP, Wrightson JM, Belcher E, DeCamp MM, Feller-Kopman D, Rahman NM (2015) Pleural infection: past, present, and future directions. *The Lancet Respiratory Medicine* 3:563–577
102. Tu C-Y, Chen C-H (2012) Spontaneous bacterial empyema. *Curr Opin Pulm Med* 18:355–358
103. Smith JA, Mullerworth MH, Westlake GW, Tatoulis J (1991) Empyema thoracis: 14-year experience in a teaching center. *Ann Thorac Surg* 51:39–42

104. McCauley L, Dean N (2015) Pneumonia and empyema: causal, casual or unknown. *J Thorac Dis* 7:992–998
105. Davies CW, Kearney SE, Gleeson FV, Davies RJ (1999) Predictors of outcome and long-term survival in patients with pleural infection. *Am J Respir Crit Care Med* 160:1682–1687
106. Chen KY, Liaw YS, Wang HC, Luh KT, Yang PC (2000) Sonographic septation: a useful prognostic indicator of acute thoracic empyema. *J Ultrasound Med* 19:837–843
107. Bedawi EO, Hassan M, Harriss E, McCracken D, Asciak R, Mercer R, Wrightson JM, Rahman NM (2018) S57 Sonographic septations in pleural infection – what do they actually mean? *Thorax* 73:A35–A35
108. Rahman NM, Kahan BC, Miller RF, Gleeson FV, Nunn AJ, Maskell NA (2014) A clinical score (RAPID) to identify those at risk for poor outcome at presentation in patients with pleural infection. *Chest* 145:848–855
109. Touray S, Sood RN, Lindstrom D, Holdorf J, Ahmad S, Knox DB, Sosa AF (2018) Risk Stratification in Patients with Complicated Parapneumonic Effusions and Empyema Using the RAPID Score. *Lung* 196:623–629
110. Corcoran J p., Psallidas I, Gerry S, et al (2018) Prospective Validation of The RAPID Clinical Risk Prediction Score in Patients with Pleural Infection: The Pleural Infection Longitudinal Outcome Study (PILOT). In: C43. TOPICS IN INTERVENTIONAL PULMONARY AND PLEURAL DISEASES. American Thoracic Society, pp A7767–A7767