Pleural fluid investigations for pleural infections

Bapti Roy[1,2], Hui Juin Shak[3,4], Y. C. Gary Lee[2,3,4,5]

[1]Respiratory Medicine, Sir Charles Gairdner Hospital, Western Australia, Perth, WA, Australia; [2]School of Medical & Health Sciences, Edith Cowan University, Western Australia, Perth, WA, Australia; [3]Institute for Respiratory Health, Western Australia, Perth, WA, Australia; [4]School of Medicine, University of Western Australia, Perth, WA, Australia; [5]Department of Medicine, University of Hong Kong, Hong Kong, China

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Correspondence to: Prof Y. C. Gary Lee, MBChB, PhD, FRACP, FRCP, FCCP. Medical School, University of Western Australia, 533 Harry Perkins Building, QE II Medical Centre, Perth, WA 6009, Australia. Email: gary.lee@uwa.edu.au.

Abstract: Over 1.5 million patients are admitted to hospital with pneumonia in the United States each year. Up to 50% of them can develop a parapneumonic effusion which is associated with higher mortality. The incidence of pleural infection continues to rise, particularly in the elderly and those with comorbidities. Parapneumonic effusions cover a spectrum of presentations from a free-flowing 'simple parapneumonic effusion' to a septated 'complicated parapneumonic effusion' (CPE) (usually from bacterial pleural invasion) and 'empyema' (presence of pleural pus). Pleural infection is defined as either CPE or empyema, and usually requires evacuation of the infected fluid. Laboratory investigations play an essential part of the diagnosis and management of pleural infection. A parapneumonic effusion is typically a neutrophil-rich exudate. Presence of bacteria from culture of the fluid defines pleural infection but conventional culture methods have a low yield. Surrogate pleural fluid markers are often employed to confirm a CPE, including low pleural fluid pH (<7.2) or glucose (<3.3 mmol/L) and elevated lactate dehydrogenase (LDH). Measurement of pleural fluid triglyceride and chylomicrons (for chylothorax) and cholesterol (for pseudochylothorax) may be needed to separate lipid pleural effusions from empyema. Tuberculous pleural effusions usually result from a hypersensitivity pleuritis and are lymphocyte predominant with elevated pleural fluid adenosine deaminase (ADA) and interferon gamma levels. Culture yield of mycobacteria is typically low. Caseating granulomas on pleural tissue biopsy is often considered diagnostic. Common organisms for community-acquired pleural infection include *Streptococcus pneumoniae*, *Streptococcus anginosus* group bacteria and *Staphylococcus aureus*. Hospital-acquired pleural infections have higher mortality and are often polymicrobial which can include *S. aureus*, *Enterobacteriaceae* and anaerobes. Antibiotics and evacuation of the infected fluid, usually by chest tube drainage, remain the mainstay of treatment. Intrapleural fibrinolytic and deoxyribonuclease therapy, or occasionally surgical drainage, may be required.

Keywords: Pleural effusion; pleural infection; tuberculosis; chylothorax

Introduction

Pleural infection is not a new disease. Hippocrates (460–377 BC) described pleurisy and its progression to empyema if left untreated and pioneered one of the first methods of drainage of the intercostal space. Pliny the Elder (23–79 AD) described an interesting success story of pleural drainage. When Publius Cornelius Rufus was convinced that he was dying from an empyema and decided to charge into battle, he was fortuitously struck by an arrow in the chest which helped drain his effusion and improved his

condition. Proactive management and drainage of pus with dedicated instruments progressed in the 16th century, predominantly lead by Ambrosie Paré (1510–1590 AD). Further emphasis on early diagnosis of pleural infection began in the 19th century, aided by the invention of the stethoscope (1).

Centuries later, pleural infection continues to be a common problem worldwide, accounting for significant morbidity and mortality. The incidence is rising; particularly vulnerable are the elderly, immunocompromised and hospitalised patients. Despite advances in the diagnosis of pleural infection and paradigm shifts in its management, the disease is still associated with alarming short- and intermediate-term mortality rates.

Terminology

Multiple terms exist to describe the spectrum of pleural cavity infection. A parapneumonic effusion is one that develops following a pneumonic process, usually adjacent to an area of pneumonia.

A simple or uncomplicated parapneumonic effusion is one with free flowing and non-infected fluid. A complicated parapneumonic effusion (CPE) can develop when microbes translocate from the lung parenchyma to infect the pleural tissue/fluid, and is often defined by surrogate biochemical criteria [such as low pleural fluid pH and/glucose, elevated lactate dehydrogenase (LDH)] as well as neutrophilia. CPEs are often characterized by adhesions or septations and resultant loculated fluid collections. Empyema is conventionally referred to as frank pus in the pleural cavity (2,3) but contemporary definition sometimes includes non-purulent effusions where bacteria are identified by culture or gram-staining.

'Pleural infection' is a term used in most multicentre clinical trials and incorporates both 'complicated parapneumonic effusion' and 'empyema', on the basis that both subtypes typically require pleural fluid drainage for patients to improve.

Pathogenesis

The pathogenesis of pleural infection in humans remain unclear. In a murine model of *Streptococcus pneumoniae*, development of empyema involves bacterial invasion of the pleura, the release of proinflammatory cytokines [e.g., interleukin (IL)-6, IL-8 and tumour necrosis factor (TNF)-α] by pleural mesothelial cells, and subsequent pleural influx of neutrophils, the predominant leukocyte subtype seen within the fluids (4). This separates pleural infection from lymphocyte-predominant effusions, such as tuberculous, malignant or cardiac failure related pleural effusions, and are useful in their differentiation (4,5). The rate of progression through the stages of pleural infection vary among patients and may be influenced by the inciting organism, host immunity and comorbidities.

The initial 'exudative phase' of the parapneumonic effusion is characterised by an 'uncomplicated' parapneumonic effusion with a pH >7.2, glucose >3.3 mmol/L and LDH less than 3 times upper limit of its normal serum concentration. Bacteria is not usually identified in the pleural fluid at this stage. Management with antibiotic therapy is sufficient without evacuation of the effusion (5).

Left untreated, bacteria can translocate into the pleural space and the fluid becomes progressively loculated on imaging. Biological activities of bacteria and neutrophils result in increased glucose consumption and production of lactic acid and carbon dioxide. The fluid will therefore become increasingly infected (pH <7.2, glucose <3.3 mmol/L) and bacteria may be detected by culture. The inflammation also leads to complex alteration of the balance between pro- and anti-fibrotic states within the pleura and reduced fibrinolysis (related to elevations in plasminogen activator inhibitors). A more complex parapneumonic effusion eventually forms, with septations and loculations, termed the 'fibrinopurulent phase'.

An 'organising stage' follows, characterised by fibroblast growth into the pleura (likely mediated by growth factors such as platelet-derived growth factors and transforming growth factor-β), producing a thick pleural rind, which prevents lung expansion (2,6).

Bacteria can also invade the pleural cavity through other routes. Haematogenous spread during septicaemia, translocation of skin organisms after penetrating injury or iatrogenic (during pleural procedures), and transdiaphragmatic migration of intraabdominal infections are known to occur (7).

Epidemiology

An estimated 4 million patients are admitted worldwide with pneumonia each year. Up to 57% develop a parapneumonic effusion, with higher incidences in males than females (3,8). Approximately 10% will progress to CPEs or empyema (5,9). In a study of 1269 patients with pneumonia, the

development of pleural infection was associated with alcoholism, intravenous drug use and several biochemical abnormalities on admission including hypoalbuminaemia (<30 g/L), hyponatraemia (<130 mmol/L), thrombocytosis (>400×10^9/L) and high inflammatory markers [C-reactive protein (CRP) >100 mg/L] (9).

Pleural infection is estimated to occur in over 80,000 patients per year in the USA and UK with an estimated annual cost of $US500 million in the USA alone (8,10).

The incidences of pleural infection continue to increase worldwide in all (but particularly the over 65 years old) age groups. In population-based studies, the incidence of pleural infection increases with age and are higher in those with pre-existing comorbid conditions and in hospitalised patients with pneumonia, where it occurs in up to 20–40%, with 5–10% progressing to empyema (2,11-13). A recent systematic review found that 72% (median, IQR 58–83%) of patients with pleural infection had comorbid conditions especially respiratory (~20%) and cardiac (~19%) diseases in addition to diabetes (~17%) (14). The effect of multivalent pneumococcal vaccines on incidence of pleural infection has been a subject of many reports with some suggesting a phenomenon of replacement of serotypes covered by the vaccines with more invasive serotypes (13,15-17) thus causing a rise in empyema.

The presence of pleural effusions in patients presenting to hospital with pneumonia significantly increased their likelihood of needing hospitalization and their length of stay. Importantly those who presented with a pleural effusion had a mortality 2.6 times higher than pneumonia patients who did not have an effusion at presentation, even adjusting for known mortality prediction factors (e.g., CURB-65) (18). Patients with pleural infection often require protracted hospitalization with a median stay of 19 (IQR 13–27) days as found in a recent systematic review of worldwide studies (14).

In hospital or 30-day mortality has been reported to be 4–8% following pleural infection, but is as high as 20% in those with confirmed empyema, particularly in the elderly (11,12,14,19,20). Mortality rates are high in patients with positive bacterial isolates cultured from pleural fluid; a large Australian study (n=601) reported an in-hospital mortality of 17% and a one-year mortality of 32% (21). Patients with bilateral effusions had a 7-fold (and unilateral effusion a 3-fold) higher 30-day mortality than those without an effusion (22). Mortality from pleural infections tends to be higher in the elderly, immunocompromised individuals and those with comorbid conditions (22).

Conventional mortality prediction scores for pneumonia (e.g., CURB-65, pneumonia severity index and SMART-COP) cannot be reliably extended to patients with pleural infection (9). The RAPID (Renal, Age, Purulence of fluid, Infection source, Dietary factors) score is a new clinical risk score specifically derived for pleural infection using data from the MIST trial-1 and has been validated using the MIST 2 cohort and in a multinational (PILOT) study (23). Older age (especially >70 years) was the most powerful predictor of mortality. Higher urea level, hospital-acquired pleural infection and low blood albumin levels were associated with higher mortality risk (23). Interestingly, patients with non-purulent fluid had worse outcome than those with pus. It is unclear at present though what, if any, role these stratification tools will impact care.

Bacteriology of pleural infection

Despite the close relationship with pneumonia, their microbiology differs significantly. *Streptococcus anginosus* group of bacteria were the most common microbial cause of pleural infection in UK series though they are seldom considered as causative agents of pneumonia. Conversely, many common bacterial causes of pneumonia (e.g., *Haemophilus influenzae*) and pathogens in atypical pneumonia (e.g., *Mycoplasma*, *Legionella*, etc.) are seldom reported in pleural infection (24). The ability of bacteria to proliferate in the acidic and hypoxic environment of the infected pleural cavity may contribute to this disparity (25).

The microbial profile of pleural infection has changed significantly since the introduction of antibiotics and vaccines. Before the discovery of antibiotic, *S. pneumoniae* predominated (60–70%) but it now only accounts for ~11% of cases. *Staphylococcus aureus* has been reported as the most common causative agent in some series (8,26). Causative bacteria are different depending on the setting of infection (community-acquired or hospital-acquired), geographical location, and the age group of patients (27,28).

Community- vs. hospital-acquired pleural infection

The microbiology of community- and hospital-acquired pleural infections are distinctively different. A review by Bedawi *et al.* in 2018 summarised data from three studies on pleural infection. Viridans streptococci (including *Streptococcus milleri* and other oral commensals from *Streptococcus* genus) (25%), *S. pneumoniae* (23.8%), *S. aureus* (15.4%), *Enterobacteriaceae* (7.5%) and *Pseudomonas* spp.

(3.2%) were the most common bacteria in community-acquired pleural infection. The most common hospital-acquired aerobic bacteria were *S. aureus* (42%), *Enterobacteriaceae* (13.6%), viridans streptococci (9%), *Pseudomonas* spp. (6.5%) and *Klebsiella* spp. (6%), and were associated with worse outcome in pleural infections (29). Anaerobic bacteria were reported in 18% and 11% of the community- and hospital-acquired pleural infections respectively.

Geographical location

Causative organisms of pleural infection vary among reports from different parts of the world. *S. milleri* have been found as the most common causative bacteria of pleural infection in reports from the UK, Australia, New Zealand and Denmark (8,21,25,30). *S. milleri*, otherwise known as the *S. anginosus* group (SAG), consists of three species, *S. anginosus*, *S. constellatus* and *S. intermedius*. SAG is normally found in the normal flora of the oral cavity, upper respiratory, gastrointestinal and urogenital tracts (31-33).

S. aureus, on the other hand, was the most common bacterial cause in a large report from the USA of 157,094 hospitalized cases of pleural infection (16). SAG was uncommon in that series. Reports from Taiwan and South Korea showed *S. pneumoniae* as the most common causative agent, and also reported higher percentage of *K. pneumoniae* compared to other regions (28,34,35).

A recent systematic review attempted to show the differences in common bacteriology of pleural infection according to the latitude of the regions: *S. pneumoniae* being the most common microorganism in tropical countries (e.g., Singapore, Saudi Arabia and Mexico), *S. aureus* in the subtropics (e.g., South East Asia, Middle East, southern Europe and southern USA) and viridans streptococci (including SAG) in temperate regions (North America and Europe) (26).

Age groups

The microbial profile of pleural infection in paediatric and adult patients vary significantly. *S. pneumoniae* is the predominate cause (85%) of pleural infection in children (8,36-40). *S. pyogenes* was another organism noted in up to 11% of paediatric pleural infections (39). As discussed above, *S. aureus* and viridans streptococci group (including SAG) are the most common aerobic cause of pleural infection in adults. They are followed by *Pseudomonas* spp., *Enterobacteriaceae* group and *S. pneumoniae* (26).

Tuberculous pleural effusion

The World Health Organization estimated that 9.6 million people worldwide suffered from tuberculosis (TB). The incidence of TB effusion varies largely among published series (typically from 5% to 25%), making TB one of the most common causes of pleural effusions in endemic areas (41). TB can cause different types of pleural effusions. The vast majority is a result of hypersensitivity reaction of the pleura to the mycobacterial protein and the actual mycobacterial load in the pleura is very low. It is believed that the mycobacteria may have been present in peripheral lung tissues and ruptured into the pleura inducing an effusion. Histological series from pre-antibiotics era suggested that the effusion will settle spontaneously even without treatment, but more than half the subjects will develop active TB afterwards. Hence treatment is important.

TB empyema is a different and rare entity and should not be confused with the much more common tuberculous pleural effusions. TB empyema is often characterized by bronchopleural fistula and resultant polymicrobial infection including TB and other bacteria (e.g., *S. aureus*). This complex infection results typically in a very thickened pleura (fibrothorax) and multiloculated pleural fluid that pose management challenges with high morbidity.

Other microorganisms causing pleural infection

Fungal pleural infection is uncommon, accounting for 1–3% of total cases worldwide, but is associated with higher mortality and morbidity (26,42). *Candida* spp., especially *Candida albicans*, are the most common causative fungi reported and typically seen in immunosuppressed patients (27,42). *Aspergillus* spp., can also cause pleural infection, particularly in lung transplant patients (42). Examples of other bacteria reported to cause pleural infection include *Mycobacterium abscessus*, *Pasteurella multocida*, non-typhoidal salmonella and *Nocardia* spp. (42).

Initial diagnostic evaluation

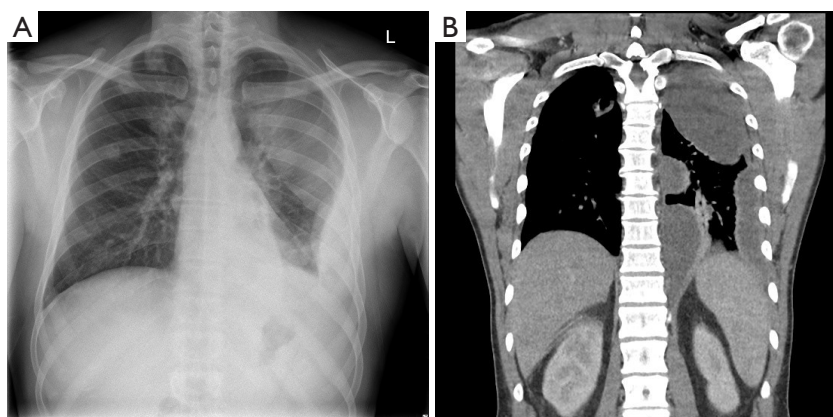The diagnosis of pleural infection may be delayed as

Figure 1 CT imaging can further characterise opacities on CXR in the setting of pleural infection. (A) CXR demonstrates a possible left upper zone pneumonia and left loculated pleural effusion. (B) CT of same patient demonstrates the opacity in fact constitutes a multi-loculated effusion with pleural enhancement suggestive of an empyema. Purulent fluid was drained.

symptoms of pleural infection are not dissimilar to that of the underlying pneumonia. Persistent fevers or raised inflammatory markers in the context of a non-resolving pneumonia may aid the diagnosis; however in many cases, symptoms are more indolent, with fatigue and weight loss predominating, especially in the elderly or those with anaerobic infections (19,43). Better access to imaging modalities, e.g., bedside ultrasound and computed tomography (CT) scanning, have allowed earlier identification of pleural effusions.

Imaging

Chest radiography often provides the first identification of a parapneumonic effusion. When infected, the fluid frequently becomes loculated. Separating pleural fluid from underlying lung consolidation or atelectasis can be difficult. Pleural ultrasound is valuable in detecting pleural fluid with high sensitivity and in separating it from underlying consolidated lung or elevated hemidiaphragm. Pleural ultrasound can detect septations or loculations to guide drainage procedures (27,44,45) and is recommended by clinical guidelines for management of pleural effusions. Patients with septated effusions on ultrasonography have longer hospitalisation and more likely require fibrinolytic therapy or surgery (46). Contrast-enhanced thoracic CT can identify pleural fluid as well as provide valuable information on the underlying pneumonia (± abscess formation), and other potential abnormalities, e.g., subdiaphragmatic

collections (*Figures 1* and *2*) (47). It also helps clarify alternative differentials such as malignancy.

Pleural fluid sampling and analyses

Pleural fluid sampling is an important step in patients with respiratory tract infection and a pleural effusion both to establish a diagnosis and to guide management. Routine workup to establish evidence of systemic infection should include assessment of serum infection markers such as peripheral blood leucocytosis and CRP, as well as blood (and sputum if appropriate) cultures. Elevated serum procalcitonin, which is correlated with increased probability of bacterial infection, is not a reliable marker to rule-in or rule-out pleural infection. Dixon et al found no superior role of procalcitonin over CRP and leukocyte counts in the diagnosis of bacterial pleural infection in a study of 425 patients (48). None of these markers are specific in the diagnosis of pleural infection alone (48).

Presence of microbes from gram-stain and/or culture of pleural fluid defines pleural infection, but such investigations have a poor yield of <50% in most studies. The absence of microbes therefore does not exclude infection. The low culture yield is likely multifactorial. First, patients usually have received broad spectrum antibiotics prior to fluid sampling. Second, bacteria may become unviable from collection or transport issues before reaching the laboratory. Direct inoculation of blood culture bottles at bedside have been shown to increase yield in several series,
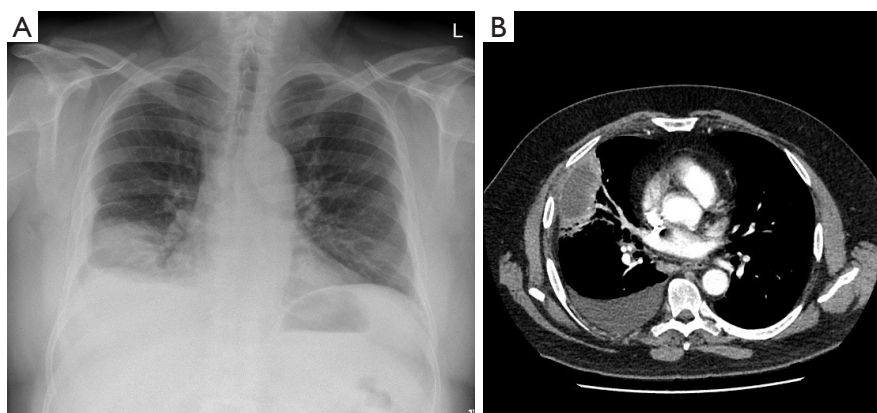
Figure 2 CT is useful in evaluating for other potential complications of pneumonia including abscess formation. (A) CXR demonstrating an apparent right lower lobe pneumonia and small parapneumonic effusion with a 'meniscus sign'. (B) CT of the same patient demonstrating in fact a right lung abscess with rim-enhancement and right pleural effusion without pleural enhancement, suggestive of a parapneumonic effusion.

particularly of anaerobic organisms. In one study use of blood culture bottle increased the proportional of patients with identifiable pathogens from 38% to 59% compared to standard culture alone (49). Third, pleural fluid may not be the most sensitive site for capturing bacteria. Murine experiments of *S. pneumoniae* pleuropulmonary infections showed abundance of bacteria in the pleural tissues (4,50). Ultrasound-guided pleural biopsy before chest drain insertion was performed in 20 patients with pleural infection in the AUDIO study and showed a yield of 45% (versus 20% with pleural fluid culture and 10% by blood culture) (50). Interestingly 75% of patients with positive pleural biopsy cultures had received antibiotics, suggesting the procedure is still worthwhile after commencement of antimicrobial therapy and testify to poor antibiotic penetration of pleural tissues.

Efforts to apply advanced technology to increase culture yield have not been particularly successful to date. Pleural fluid cell free DNA concentration has been shown to be higher in those with exudative over transudative effusions and concentrations are high in patients with parapneumonic effusion compared to those without. One study demonstrated that at the optimal cut-off value of 6,740 ng/mL, the test has a sensitivity of 87.5% and specificity of 80.6% for pleural infection (51). Current data are insufficient to support the use of pleural fluid cell free DNA for clinical use.

Polymerase chain reaction (PCR) to amplify and detect the 16S ribosomal RNA gene (which is present in all bacteria) from pleural fluid samples have shown confusing results (52,53). Maskell *et al.* found that in 34% of samples (107 of 316 patients), conventional cultures were positive but PCR was not (25). Another difficulty with micro-organisms identified with PCR is to determine if the microbe is a bystander or genuine causative agent. PCR, unlike cultures, will also detect non-viable microbes such as those already killed by antibiotics. Multiplex PCR, with results generally available within hours, is able to detect multiple pre-specified pathogens in a single nucleic acid experiment. This has been applied in a study to detect the more common pathogens associated with pneumonia and pleural infection (54). This approach risks missing organisms not included in the panel and thus should only be considered in addition to standard bacterial culture. For these reasons, PCR of pleural fluid remains experimental rather than routine practice.

The high false negative culture rate, and the need to await culture results make the use of surrogate markers for pleural infection necessary (25). Parapneumonic effusions should be exudates by Light's criteria and cell counts should reveal a neutrophil predominance. Lymphocyte-rich fluid should raise concern of alternative diagnoses, including tuberculous pleuritis or non-infective causes. Measures of metabolic activities (e.g., acidic pH, low glucose and high LDH levels) in the fluid are the common surrogate markers used. These metabolic markers progress as fluid transit from simple to complicated parapneumonic phases. A CPE is often defined as presence of any of the followings: low pleural fluid pH (<7.20) or glucose concentration (<3.3 mmol/L), high pleural fluid LDH level (e.g., >1,000 IU/L)
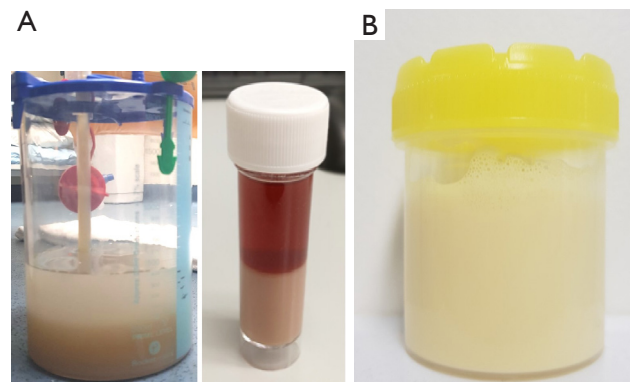
Figure 3 Turbid effusions secondary to empyema can be distinguished from a lipid effusion after either centrifuging or allowing fluid to settle. (A) Two examples of empyema with clear supernatant after the drained effusion was allowed to settle overnight. Empyema classically separates into a clear supernatant (top) and sediment (bottom). (B) Example of chylous effusion. Unlike empyema, lipid effusions, including chyle, do not separate and remain turbid despite centrifuging or after a allowing the fluid to settle.

(2,5,27). An alkaline pleural fluid pH can occur with infection from urease-producing organisms, e.g., *Proteus mirabilis*, which hydrolyses urea to ammonia (55,56).

Accuracy of pleural fluid pH in this context is important, and is dependent on sample collection method, with most reliable results available when collected in a heparinised blood gas syringe. However residual air in collection tube will artificially raise the pH and carried over lignocaine (which is acidic) can reduce pH (57,58). Pleural fluid pH remains relatively stable at room temperature for up to around 4 hours, beyond which the values may not be trustworthy (57,58). Alternatively, a low pleural fluid glucose and high pleural fluid LDH level are useful in supporting the diagnosis of pleural infection. Variations in fluid appearances and biochemistry among different locules have been shown in a small series (59).

Other biomarkers, including pleural fluid procalcitonin and CRP have been investigated for their role in identifying pleural infections. None have been proven to be effective tests and are not recommended for routine use in this context. Pleural fluid procalcitonin has been found to have low sensitivity (62%) and specificity (71%) for differentiating parapneumonic effusion from other causes (60).

Novel pleural fluid diagnostic markers such as presepsin and soluble triggering receptor expressed in myeloid cells-1 (sTREM-1) have shown to be elevated in pleural infection. Presepsin is a protein which is released from the surface of various immune cells in response to pathogens and is reported to be increased specifically in the blood of patients with sepsis. A recent study has demonstrated that at a cut off of 754 pg/mL, presepsin had a diagnostic sensitivity of 90.9% and a specificity of 74.4% for diagnosing empyema (61).

The triggering receptor expressed in myeloid cells-1 (TREM-1) is shed by the membrane of activated phagocytes and is involved in the inflammatory response. A meta-analysis demonstrated sTREM-1 had a sensitivity of 78% and specificity was 84%. For the diagnosis of bacterial pleural effusion, however studies are lacking regarding whether it can differentiate between parapneumonic effusion and empyema (62).

More recently, pleural fluid soluble urokinase-type plasminogen activator receptor (suPAR) has been shown to be associated with loculated pleural effusions and may better predict the need for chest tube drainage, intrapleural fibrinolytic therapy or thoracic surgery compared with conventional pleural fluid biomarkers (63). This promising data however requires further prospective validation which should encompass usual serum and pleural fluid biomarkers to establish the full clinical utility of suPAR (for a detailed discussion, please see recent editorial by Idell and Lee) (64).

Chylous effusions

For macroscopically turbid effusions, differentiation of an empyema from a lipid effusion (chylothorax or pseudochylothorax) is important. After centrifugation of the turbid fluid, empyema typically shows a clear supernatant with dense sediment whereas lipid effusions will remain turbid (*Figure 3*). Empyema fluid are neutrophil

predominant whereas lipid effusions are lymphocyte rich. Chylous effusions are often exudates but transudative chyloascites or chylothorax can occur as a result of concurrent other aetiologies, such as liver cirrhosis or cardiac failure.

Presence of chylomicrons, defines a chylothorax. In their absence, high pleural fluid triglyceride content with a lower cholesterol level (<5.18 mmol/L) is useful. An effusion is definite/highly likely a chylothorax if pleural fluid triglyceride level exceeds 1.24 mmol/L and is suspicious of a chylothorax if triglyceride of 0.6 mmol/L. It is important to remember that the appearance of chylothorax may vary; and may not be turbid in a fasting or malnourished patient (65). Pseudochylothorax on the other hand has a high cholesterol content (66) and contain cholesterol crystals.

Tuberculous effusions

Investigations for tuberculous pleuritis should be initiated when clinically indicated. Pleural fluid is typically serous and contains very few viable mycobacteria, hence <10% will have acid fast bacilli (AFB) on fluid smear and mycobacterial cultures are positive in only a fraction (<50%) of cases (67). Nucleic acid amplification tests allow rapid detection of mycobacterium TB complex in a variety of tissue, however the sensitivity of the test on pleural fluid remains relatively low (68). Pleural tissue biopsy has a significantly higher likelihood of providing a diagnosis (69–97%) through finding AFB on microscopy, culture of the tissue (69) or, more commonly, the presence of caseating granuloma on histological examination. A randomized trial of thoracoscopic versus percutaneous blind pleural biopsies found a yield of 100% *vs.* 79% respectively, suggesting that the latter is a reasonable bedside test and, if it fails to provide an answer, thoracoscopy should be considered (70).

Many surrogate pleural fluid biochemical tests have been tried to increase yield of tuberculous pleuritis in a minimally invasive manner. Adenosine deaminase (ADA) is an enzyme of the lymphocytes and its activity is increased during tuberculous pleuritis. Pleural fluid ADA levels are therefore raised in practically all tuberculous effusions and with a threshold of 40±4 IU/L, it has a sensitivity of 93% and specificity of 90% in a recent meta-analysis (71,72). An elevated ADA is used in some endemic regions as a confirmatory test, in the clinically setting of a patient with lymphocytic pleural effusion and compatible history and chest imaging for TB. ADA is cheap and relatively easy to perform with a fast turnaround time and can be reliably used even in stored samples (73). ADA has been shown to be valid in immunocompromised hosts and paediatric populations (74,75). However, it must be recognized that elevated pleural fluid ADA level also occurs commonly with other (e.g., bacterial) pleural infections, rheumatological disorders and, occasionally, malignant pleural effusions. Diagnosis relying purely on pleural fluid ADA also does not provide information on mycobacterial resistance. Therefore, ADA is best used as a supportive rather than definitive diagnostic test. Restricting testing of ADA to only lymphocytic effusions help to reduce false negative diagnosis from bacterial CPE/empyema (which are neutrophilic). Testing for ADA subsets also serves that purpose but is expensive and not easily available. In non-endemic regions, negative pleural fluid ADA is a very useful rule-out test for TB and other causes for a lymphocytic effusion should be sought (76).

Interferon gamma levels in pleural fluid is raised in tuberculous pleural effusions and many studies have confirmed a similar diagnostic performance (77,78). However, interferon gamma essays are significantly more expensive than ADA and the latter is preferred especially in resource limited regions. It must be emphasized that interferon gamma releasing assays however have no role in the diagnosis of tuberculous effusions (76). Promising early data have found IL-27 as another potential surrogate marker though larger studies are needed (68).

TB empyema has a very different presentation. Imaging will show features of thickened pleura and multiloculated fluid that is difficult to evacuate and is purulent. Culture of TB empyema has a significantly higher yield of mycobacteria as well as other bacteria such as *S. aureus*.

Treatment of pleural infection

The mainstay of treatment for pleural infection is antimicrobial therapy and drainage of pleural fluid as outlined below. Detailed discussion is outside the scope of this review and can be found elsewhere (27,79).

Intravenous antibiotics

Antibiotics are often used empirically given the low yield of cultures. International guidelines recommend the use of penicillins combined with β-lactamase inhibitors,

Metronidazole or Cephalosporins for empiric treatment of pleural infection (27). Clindamycin or Meropenem provide a reasonable alternative and methicillin resistant *S. aureus* (MRSA) cover should be included if clinically appropriate (79,80). It should be emphasized that very limited information exists on pleural pharmacokinetics of commonly used antibiotics after their systemic delivery. Standard practice is largely based on clinicians' 'best guess'.

Dexamethasone hastened clinical recovery in a small randomized controlled trial (RCT) of paediatric parapneumonic effusions (81). Its benefits in adult patients are being examined in a pilot RCT (82).

Fluid evacuation

Chest tube drainage is usually needed for CPE and empyema. A delay to drainage of >2 days correlated with increased 90-day mortality in a large Danish study (83). Imaging-guided drain insertion is valuable especially in loculated effusions. Drain size (larger or smaller than 15 Fr) did not affect outcome in a large but non-randomized observational series (84). Multiple tubes may be required to evacuate non-communicating locules. About 20% of patients failed to improve with antibiotics and chest tube drainage in large RCTs, and necessitated additional interventions to aid fluid removal (19,20).

Combined intrapleural tissue plasminogen activator (tPA) and deoxyribonuclease (DNAse) therapy has revolutionized clinical practice since a landmark RCT and subsequent large open-labelled series demonstrated its efficacy in improving fluid output, radiographic clearance and length of hospitalization, leaving <10% of patients needing surgical referral. It is hypothesized that tPA breaks pleural loculations and DNase reduces fluid viscosity. Fibrinolytics also induces significant pleural fluid formation, via a monocyte chemotactic protein-1 (MCP-1) pathway, which may provide a lavage of the infected pleural cavity (85,86). Complications of tPA/DNase therapy are acceptable (87). Pleural bleeding requiring blood transfusion occur in ~3% of patients and no systemic or fatal bleeding have been reported in most series. Dose de-escalation studies are underway to establish the lowest effective dose of tPA (88,89).

Video-assisted thoracoscopic surgery (VATS) with decortication is needed if the above measures fail (27), although the optimal timing of surgery remains debated (90). Post-operative complications including pain, persistent pleural and/or wound infection, bleeding and prolonged air leak (91-93).

Future directions

Two millennia since Hippocrates' work, pleural infection remains a major illness. Our understanding of how and why some patients with pneumonia develop pleural complications is critical to designing strategies to avoid secondary pleural infections. The parapneumonic fluid encompasses a range of presentations from a simple effusion to complex multiloculated collections. Individualized therapy based on fluid biochemical activities has been postulated.

The RAPID (Renal, Age, Purulence of fluid, Infection source, Dietary factors) score is well validated and permits clinical risk stratification. Increasing age, elevated urea, low albumin, presence of hospital-acquired infection, and non-purulence fluid were associated with poorer outcomes including mortality and increased length of hospital stay (23). Mortality of pleural infection at 3 or 6 months are high, even after the acute infection has resolved. It is increasingly realised that pleural infection reflects advancing age and comorbidity—which are the most predictive risks of mortality in the RAPID score. Studies are urgently needed to establish the cause of death so that targeted prevention can be attempted.

Antibiotics are key to treatment but their pleural penetration has largely unknown, especially in patients with pleural thickening/loculations. Intrapleural antibiotics delivery has attracted much clinical interests but has not been formally studied.

The majority of the literature on pleural infection focussed on community acquired diseases, whereas the latest epidemiological data suggested that hospital acquired cases are several-fold more common and are associated with significantly worse outcome. This 'untapped' area requires urgent attention.

Footnote

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