Interpretation of pleural fluid biochemistry

A pleural effusion refers to an accumulation of fluid in the pleural cavity between the visceral and parietal pleurae that occurs as a result of an imbalance between the formation and absorption of fluid by the pleura. This imbalance could be secondary to an alteration in the pleural surface and vascular permeability or the result of a change in the hydrostatic pressures. Causes of pleural effusions vary from pleuro-parenchymal pathologies to more generalized systemic causes and organ dysfunction (Hooper et al, 2010).

A diagnostic thoracentesis is an easy and relatively safe procedure in experienced hands, with the use of thoracic ultrasound. Determining the cause of an effusion is greatly facilitated by the analysis of the offending pleural fluid.

This article looks at which biochemical tests, specific biomarkers and other characteristics of pleural fluid can be used to investigate the cause of an effusion.

Appearance

The appearance of fluid in itself can provide important information as to the aetiology of the effusion as shown in *Table 1*. Straw-coloured fluid could be secondary to either a transudative or an exudative effusion. Red or orange discolouration of the fluid is usually caused by the presence of blood in the fluid.

Transudate and exudates

Traditionally pleural effusions have been classified as transudates or exudates using Light's criteria (*Table 2*), by comparing the protein and lactate dehydrogenase content of the pleural fluid and serum (Light, 2013). This differentiation provides important information as causes of transudates are far fewer than those of exudates and rigorous further investigations can be avoided in most patients with transudative effusions.

The effusion is an exudate if one or more of the criteria in *Table 2* are met. A transudate would not meet any of the criteria.

Using Light's criteria 99% of exudates can be identified correctly. However, when applied to transudates the same criteria have a lower sensitivity, in one series mislabelling 25% of transudates as exudates (Porcel, 2011a). This was particularly common in patients with cardiac failure who were treated with diuretics (Porcel, 2011a).

Transudates are formed as a result of systemic factors affecting oncotic and hydrostatic pressures. The pleural surface and capillary permeability tend to be normal in transudative processes. In contrast, exudative effusions are the result of alteration of the pleural surface and vascular permeability in the areas where the fluid is produced and inefficient lymphatic drainage of absorbed fluid, which leads to the accumulation of fluid (*Table 3*).

Pleural fluid pH

Pleural fluid pH is an important parameter that could influence management in patients with pleural infection. Normal pleural fluid pH measures approximately 7.6. A low pleural fluid pH is secondary to increased metabolic activity in the pleural fluid and is often seen in conditions such as pleural infection, rheumatoid-associated effusions and advanced malignancy.

An effusion with a pleural fluid pH of <7.2 in the context of clinically suspected pleural infection is considered to be a 'complicated' parapneumonic effusion, often necessitating invasive management of the effusion with tube drainage (Light et al, 1973; Hooper et al, 2010).

A low pH of <7.3 in the context of pleural malignancy is associated with a worse

Table 1. Appearance of pleural fluid and possible causes

Appearance of fluid	Possible cause of the effusion
Grossly bloody	Haemothorax (check haematocrit to confirm haemothorax*)
Creamy	Empyema, chylothorax
Black	Infections such as *Aspergillus niger* or *Rhizopus oryzae*. Metastatic melanoma
Chocolate coloured	Amoebiasis or hepato-pleural fistula
Offensive smell	Anaerobic infection
Presence of food particles	Ruptured oesophagus

** Consider haemothorax if pleural fluid haematocrit is ≥ 50% of peripheral blood haematocrit*

Table 2. Light's criteria

Pleural fluid protein divided by serum protein >0.5
Pleural fluid lactate dehydrogenase divided by serum lactate dehydrogenase >0.6
Pleural fluid lactate dehydrogenase level is more than two thirds the upper limit of normal serum lactate dehydrogenase level

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Table 3. Causes of transudates and exudates

Exudates	Common causes	Malignancy Parapneumonic effusions Tuberculosis
	Less common causes	Pulmonary embolus Rheumatoid arthritis and other autoimmune pleuritis Benign asbestos effusion Pancreatitis Post myocardial infarction Post coronary artery bypass graft surgery
	Rare causes	Yellow nail syndrome (and other lymphatic disorders) Drugs Fungal infections
Transudates	Common causes	Left ventricular failure Liver failure
	Less common causes	Hypoalbuminaemia Peritoneal dialysis Hypothyroidism Nephrotic syndrome Mitral stenosis
	Rare causes	Constrictive pericarditis Urinothorax Meig's syndrome

prognosis (less than 30-day prognosis) and less success with chemical pleurodesis of the pleural space (Light, 2013).

A very low pH of <7.0 is rare and is found in empyema, collagen vascular disease and oesophageal perforation (Good et al, 1980).

When measuring pleural fluid pH, care should be taken to ensure pH is measured using a blood gas analyser or equivalent. Measurements using pH meters and strips can be inaccurate. Efforts must be made to ensure fluid is collected anaerobically, placed in ice and analysed within 1 hour to minimize alteration of the pH by external factors such as air and pCO_2. Similarly lidocaine can falsely reduce the pleural pH, even with volumes as small as 0.2 ml (Rahman et al, 2008). Heparin was also found to lower pleural fluid pH.

Glucose

Pleural fluid pH and glucose are closely related, reflecting their relationship with metabolic activity in the pleural fluid (Potts et al, 1978). The level of glucose in the effusion is independent of the serum glucose level. A glucose level of <3.4 mmol/litre is sufficiently low to raise concerns of conditions such as complicated parapneumonic effusions, rheumatoid effusions, malignancy and tuberculous effusions (Hooper et al, 2010). Glucose measurements are less vulnerable to the effects of air, lignocaine and delayed processing (Rahman et al, 2008).

The features in *Table 4* are not specific for pleural infection alone and should be considered in conjunction with the patient's overall clinical condition. However, where the clinical suspicion of pleural infection is high and if one or more of the above criteria is met, tube drainage of the effusion should be considered, provided the collection is large enough for drainage. It is not unusual to find low glucose and an elevated lactate dehydrogenase level in the pleural fluid of advanced malignant effusions (Light, 2013).

Table 4. Criteria that would support a diagnosis of pleural infection

Macroscopic appearance of pus
Pleural fluid pH <7.2
Pleural fluid glucose <3.4 mmol/litre
Bacterial growth on culture
Pleural fluid lactate dehydrogenase >1000 IU/litre

Other useful biochemical tests

Amylase and lipase

The utility of measurement of pleural fluid amylase levels is limited to conditions where the suspected underlying diagnosis is pancreatic disease or occasionally ruptured oesophagus (Light, 2002).

Lipid studies

Cholesterol and triglyceride levels in pleural fluid are useful where an effusion is milky in appearance and a diagnosis of chylothorax is suspected. A triglyceride level >110 mg/dl (1.24 mmol/litre), cholesterol to triglyceride level of more than 1 and a fluid to serum cholesterol level of less than 1 is considered a chylothorax.

Adenosine deaminase

Adenosine deaminase is an enzyme that catalyses the conversion of adenosine to inosine and is found in high concentration in a number of cells including lymphocytes and neutrophils. It acts as a marker of inflammation and is usually elevated in tuberculous pleuritis and bacterial empyema. In a low tuberculous incidence setting such as in the UK, a lymphocytic effusion and a raised level of adenosine deaminase in the pleural fluid has a sensitivity of 85.7% and specificity of 98.9% for a diagnosis of tuberculous pleuritis, at a cut off of 35 IU/litre whereby it has an important potential role as a rule-out test (Arnold et al, 2015).

NT-proBNP

Natriuretic hormones are neurohormones secreted by the cardiac myocytes in response to increased pressure and stretch of the cardiac chambers. The N-terminal of the pro-brain natriuretic peptide (NT-proBNP) is an easily measurable marker in both serum and pleural fluid. As the pleural fluid NT-proBNP level is adequately reflected in the serum there is no additional value in performing routine measurement of pleural fluid levels of NT-proBNP.

At a cut-off value of 1300 pg/ml pleural fluid NT-proBNP has a sensitivity of 93% and a specificity of 89.9% (Porcel, 2011b). The authors find this a useful test in excluding heart failure as a cause of an undiagnosed unilateral effusion if the NT-proBNP level is normal, but would exercise caution in concluding that a raised level denotes cardiac disease as the only cause for the unilateral effusion, as a result of the high level of concurrent occult heart disease in this patient population where the average age at presentation is the sixth or seventh decade of life.

C-reactive protein and procalcitonin

Both pleural fluid C-reactive protein and procalcitonin have been studied at length to evaluate their role in the diagnostic arena of pleural effusions. Their role is somewhat limited as these markers of infection can also be raised in other conditions, specifically malignancy, thereby limiting their role as a diagnostic marker in infection.

In isolation the level of C-reactive protein in pleural fluid has limited value, but it can help in the differentiation of complicated from uncomplicated pleural effusions where there is clinical evidence of systemic infection. Uncomplicated parapneumonic effusions may resolve with antibiotics alone without the necessity for tube drainage but complicated parapneumonic effusions require either tube drainage or surgery. Currently accepted criteria for a complicated parapneumonic effusion are pleural fluid pH <7.20, pleural fluid glucose <3.3 mmol/litre or the finding of septations on thoracic ultrasound within the right constellation of clinical features suspicious of pleural infection. A large cohort study has shown that a pleural fluid level of C-reactive protein >100 mg/litre is as effective as the above measures in correctly identifying complicated parapneumonic effusions (Porcel et al, 2012). Furthermore if the effusion is neutrophilic, a much lower C-reactive protein level of 45 mg/litre or more can identify complicated parapneumonic effusions quite reliably (Porcel et al, 2012).

Measuring pleural fluid procalcitonin level adds no further information and several studies have shown mixed and conflicting results as to the utility of pleural fluid procalcitonin measurement in addition to other inflammatory markers as mentioned above (Porcel et al, 2012; Zou et al, 2012; Lee et al, 2013).

Other tests

Cell differentials

Cytological analysis of the pleural fluid can sometimes yield a definitive diagnosis where the malignant cells are easily identified from the pleural fluid. Even if no malignant cells are seen, the differential cell count of the pleural fluid can point towards the aetiology of the effusion. The differential cell count reflects the percentage composite of cells in the fluid, for example neutrophils, eosinophils, lymphocytes, mesothelial cells and macrophages. The type of the predominant cells can narrow the differential causes of the effusion as certain types of cells tend to be more prominent in certain diseases.

Neutrophils

These tend to be predominant in effusions that are the result of an underlying inflammatory pathology such as pneumonia, pancreatitis, sub-phrenic abscesses, pulmonary emboli and early tuberculosis.

Eosinophils

If eosinophils make up >10% of the cells in the fluid, the effusion is termed eosinophilic. These are rare – approximately 7% of all effusions are eosinophilic and most often the eosinophilia is secondary to the presence of air or blood in the pleural space. Other rarer causes include benign asbestos-related pleural effusions, eosinophilic granulomatosis with polyangiitis, eosinophilic pneumonia, drugs and parasitic disease (Light, 2013).

Lymphocytes

Small lymphocytes are often found in chronic effusions. The commonest causes for lymphocytic effusions are malignancy, post coronary artery bypass graft surgery and tuberculous pleuritis. Where an effusion is lymphocytic but the first cytology sample is negative for malignancy, lymphocyte subset analysis at the time of the second cytology sample may help to exclude a haematological malignancy (Bhatnagar et al, 2013).

Mesothelial cells

These cells line the pleural cavity and can become dislodged at times where they are found in the pleural fluid. Any condition that coats the pleura such as fibrosis of the pleura following a sclerosing agent (as in pleurodesis) or significant inflammation of the pleural surface in conditions such as pleural tuberculosis can lead to an increased number of mesothelial cells found in the pleural fluid. Generally where the pleura is not significantly affected only sparse or no mesothelial cells are seen on the differential cell count.

Macrophages

These are common in pleural fluid but of limited diagnostic value. Often they can be confused with mesothelial cells and care should be taken by the pathologist when examining these cells.

> **TOP TIPS**
> - Effusions secondary to cardiac failure can sometimes be exudative if the patient is taking diuretics.
> - Avoid checking the pleural fluid pH through a blood gas analyser when frank pus is aspirated, as the debris in the fluid would occlude the channels of the analyser. Frank pus is an indication for tube drainage, if the effusion is large enough.
> - Chylothorax may not always appear milky. If suspected perform lipid studies.

Biomarkers of cancer

Soluble mesothelin level

When a pleural effusion is suspected to be of a malignant aetiology, biomarkers can play an important role in the diagnostic pathway. Soluble mesothelin is a novel biomarker that can be measured in both serum and pleural fluid where a diagnosis of mesothelioma is suspected. The literature shows a correlation between mesothelioma and elevated mesothelin levels, with an exponential relationship between increasing tumour burden and increasing mesothelin levels (Creaney et al, 2011). In the setting where a diagnosis of mesothelioma is suspected an elevated mesothelin level in pleural fluid would strongly support the diagnosis, with a sensitivity of 67% and a specificity of 98% (Creaney et al, 2007).

Serum mesothelin may also have a role in disease monitoring in mesothelioma, Hooper et al (2015) have demonstrated that a falling serum mesothelin level during chemotherapy is associated with a prolonged overall survival. The exact role of serum mesothelin to the oncologist in aiding the decision-making process when managing patients with mesothelioma is yet to be prospectively validated.

Fibulin-3

Fibulin-3 is another serum and pleural fluid marker which has a potential role in the investigation of mesothelioma. Pass et al (2012) demonstrated that fibulin-3 levels in pleural fluid of those who had mesothelioma were significantly higher than those who did not have the disease. They also demonstrated

KEY POINTS

- Differentiating between transudate and exudate can narrow the differential diagnoses and guide further investigations.
- Where 'pus' is aspirated on initial aspiration proceed to tube drainage of the effusion (if large enough) as the patient has empyema.
- If the pleural fluid pH is <7.2 in the clinical context of infection, tube drainage is required (if the effusion is large enough) as this is unlikely to respond to antibiotic treatment alone.
- A low level of adenosine deaminase in the pleural fluid in the context of a lymphocytic effusion virtually excludes tuberculous pleuritis as the underlying cause.
- Testing for lymphocyte subsets on a lymphocytic pleural effusion may help to exclude a haematological malignancy.

that the levels were higher with advanced stage of disease and there remains a significant relationship between fibulin-3 and survival. Further studies have not been able to replicate these results; more recently Creaney et al (2014) demonstrated fibulin-3 to be of low specificity for mesothelioma where a large number of patients with benign aetiology showing elevated levels of fibulin-3.

Conclusions

Pleural effusions are a relatively common pathology seen on the acute medical wards. Evaluation of pleural fluid with simple and rapid diagnostic tests that are routinely performed in hospital laboratories can inform the clinicians as to the underlying cause of the effusion and aid in the subsequent management pathway.

In addition to the investigations mentioned here pleural fluid when aspirated should routinely be sent for microbiological tests (microscopy, culture and sensitivity), including acid-alcohol fast bacilli stain and culture.

Cytological analysis not only provides information as to the cell differential, it can at times confirm the aetiology of the effusion where malignant cells are easily identified from the fluid. **BJHM**

Conflict of interest: none.

Arnold DT, Bhatnagar R, Fairbanks LD et al (2015) Pleural fluid adenosine deaminase (pfADA) in the diagnosis of tuberculous effusions in a low incidence population. *PLoS One* **10**: e0113047 (doi: 10.1371/journal.pone.0113047)

Bhatnagar R, Clive AO, Zahan-Evans N et al (2013) P209 The clinical utility of pleural lymphocyte subset analysis in undiagnosed effusions. *Thorax* **68**: A170–A171 (doi: 10.1136/thoraxjnl-2013-204457.361)

Creaney J, Yeoman D, Naumoff LK et al (2007) Soluble mesothelin in effusions: a useful tool for the diagnosis of malignant mesothelioma. *Thorax* **62**: 569–76 (doi: 10.1136/thx.2006.068114)

Creaney J, Francis RJ, Dick IM, Musk AW, Robinson BW, Byrne MJ, Nowak AK (2011) Serum soluble mesothelin concentrations in malignant pleural mesothelioma: relationship to tumor volume, clinical stage and changes in tumor burden. *Clin Cancer Res* **17**: 1181–9 (doi: 10.1158/1078-0432.CCR-10-1929)

Creaney J, Dick IM, Meniawy TM et al (2014) Comparison of fibulin-3 and mesothelin as markers in malignant mesothelioma. *Thorax* **69**: 895–902 (doi: 10.1136/thoraxjnl-2014-205205)

Good JT Jr, Taryle DA, Maulitz RM, Kaplan RL, Sahn SA (1980) The diagnostic value of pleural fluid pH. *Chest* **78**: 55–9

Hooper C, Lee YC, Maskell N, BTS Pleural Guideline Group (2010) Investigation of a unilateral pleural effusion in adults: British Thoracic Society Pleural Disease Guideline 2010. *Thorax* **65** (Suppl 2): ii4–17 (doi: 10.1136/thx.2010.137042)

Hooper CE, Lyburn ID, Searle J et al (2015) The South West Area Mesothelioma and Pemetrexed trial: a multicentre prospective observational study evaluating novel markers of chemotherapy response and prognostication. *Br J Cancer* **112:** 1175–82 (doi: 10.1038/bjc.2015.62)

Lee SH, Lee EJ, Min KH et al (2013) Procalcitonin as a diagnostic marker in differentiating parapneumonic effusion from tuberculous pleurisy or malignant effusion. *Clin Biochem* **46**: 1484–8 (doi: 10.1016/j.clinbiochem.2013.03.018)

Light RW (2002) Clinical practice. Pleural effusion. *N Engl J Med* **346**: 1971–7 (doi: 10.1056/NEJMcp010731)

Light RW (2013) *Pleural Disease.* Lippincott Williams & Wilkins, New York

Light RW, MacGregor MI, Ball WC Jr, Luchsinger PC (1973) Diagnostic significance of pleural fluid pH and PCO2. *Chest* **64**: 591–6 (doi: 10.1378/chest.64.5.591)

Pass HI, Levin SM, Harbut MR et al (2012) Fibulin-3 as a blood and effusion biomarker for pleural mesothelioma. *N Engl J Med* **367**: 1417–27 (doi: 10.1056/NEJMoa1115050)

Porcel JM (2011a) Pearls and myths in pleural fluid analysis. *Respirology* **16**: 44–52 (doi: 10.1111/j.1440-1843.2010.01794.x)

Porcel JM (2011b) Utilization of B-type natriuretic peptide and NT-proBNP in the diagnosis of pleural effusions due to heart failure. *Curr Opin Pulm Med* **17**: 215–19 (doi: 10.1097/MCP.0b013e3283455cda)

Porcel JM, Bielsa S, Esquerda A, Ruiz-Gonzalez A, Falguera M (2012) Pleural fluid C-reactive protein contributes to the diagnosis and assessment of severity of parapneumonic effusions. *Eur J Intern Med* **23**: 447–50 (doi: 10.1016/j.ejim.2012.03.002)

Potts DE, Taryle DA, Sahn SA (1978) The glucose-pH relationship in parapneumonic effusions. *Arch Intern Med* **138**: 1378–80 (doi: 10.1001/archinte.1978.03630340048016)

Rahman NM, Mishra EK, Davies HE, Davies RJ, Lee YC (2008) Clinically important factors influencing the diagnostic measurement of pleural fluid pH and glucose. *Am J Respir Crit Care Med* **178**: 483–90 (doi: 10.1164/rccm.200801-062OC)

Zou MX, Zhou RR, Wu WJ, Zhang NJ, Liu WE, Fan XG (2012) The use of pleural fluid procalcitonin and C-reactive protein in the diagnosis of parapneumonic pleural effusions: a systemic review and meta-analysis. *Am J Emerg Med* **30**: 1907–14 (doi: 10.1016/j.ajem.2012.04.004)