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Interpretation of pleural fluid biochemistry.

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Core training supplement. Top tips from the shop floor

2000 words. 5 key points. Top tips

Background:

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 due to a change in the hydrostatic pressures. Causes of pleural effusions vary from pleuro-parenchymal pathologies to more generalised systemic causes and organ dysfunction(Maskell and British Thoracic Society Pleural Disease Guideline, 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.

In this article we look at what biochemical tests, specific biomarkers and other characteristics of pleural fluid that can be employed 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 due to presence of blood in the fluid.

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

Table 1

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

Transudate and exudates

Traditionally pleural effusions have been classified as transudates or exudates using Light's criteria (Table 2), according to the pleural fluid protein and LDH content in comparison to serum protein and

LDH(Light, 2013). This differentiation provides important information as causes of transudates are far fewer compared to exudates and rigorous further investigations can be avoided in most transudative effusions.

Light's Criteria
Pleural fluid protein divided by serum protein > 0.5
Pleural fluid LDH divided by serum LDH > 0.6
Pleural fluid LDH is more than two thirds the upper limit of normal serum LDH

Table 2

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

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

Transudates are formed due to 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 due to 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.

Causes of transudates/exudates:

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

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 prognosis (less than 30 day prognosis) and less success with chemical pleurodesis of the pleural space (2).

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 minimise 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 0.2ml (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). Level of glucose in the effusion is independent of the serum glucose level. A glucose level of < 3.4mmol/l is sufficiently low to raise concerns of conditions such as complicated parapneumonic effusions, rheumatoid effusions, malignancy and tuberculous effusions (5). Glucose measurements are less vulnerable to the effects of air, Lignocaine and delayed processing (Rahman et al., 2008).

Criteria that would support a diagnosis of pleural infection
Macroscopic appearance of pus
Pleural fluid pH < 7.2
Pleural fluid glucose <3.4 mmol/L
Bacterial growth on culture
Pleural fluid LDH >1000 IU/L

Table 4

The above features are not specific for pleural infection alone and should be used in conjunction with patient's overall clinical condition. However, where the clinical suspicion of pleural infection is high and if one or more of the above criteria are 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 LDH level in the pleural fluid of advanced malignant effusions (2).

Other useful biochemical tests:

Amylase/Lipase:

Role of pleural fluid amylase 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 >110mg/dl (1.24mmol/l), cholesterol to triglyceride level of more than 1 and a fluid to serum cholesterol level of less than 1 is considered a chylothorax.

ADA

Adenosine deaminase (ADA) is an enzyme that catalyses 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 pleural fluid ADA has a sensitivity of 85.7% and specificity of 98.9%, at a cut off of 35 IU/L where by 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 measureable 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 pleural fluid NT-proBNP.

At a cut-off value of 1300pg/ml pleural fluid NT-pro-BNP 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 is normal, but would exercise caution in concluding that a raised level denotes cardiac disease as the only cause for the unilateral effusion, due to the high level of concurrent occult heart disease in this patient population where the average age at presentation is sixth or seventh decade of life.

CRP and Procalcitonin

Both pleural fluid C-reactive protein (CRP) and Procalcitonin (PCT) 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.

The level of CRP in pleural fluid in isolation has limited value, but can aid 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/l 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 a pleural fluid CRP > 100 mg/l is as effective as the above measures when correctly identifying complicated parapneumonic effusions (Porcel et al., 2012). Furthermore if the effusion is neutrophilic, a much lower CRP of 45mg/l or more can identify complicated parapneumonic effusions quite reliably (12).

Measuring pleural fluid PCT adds no further information and several studies have shown mixed and conflicting results as to the utility of pleural fluid Procalcitonin 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: commonly effusions that are due to 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 (BAPE), Churg-Strauss disease, eosinophilic pneumonia, drugs and parasitic disease (Light, 2013).

Lymphocytes: Small lymphocytes are often found in chronic effusions. Commonest causes for lymphocytic effusions are malignancy, post coronary artery by-pass graft (CABG) 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. (Dixon, 2015)

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

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 (Hooper et al., 2015) have demonstrated 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 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 the levels were higher with advanced stage of disease and there remains a significant relationship between Fibulin 3 and survival (Pass et al., 2012). Further studies on Fibulin-3 have not been able to replicate these results, more recently Creaney et al 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 (Creaney et al., 2014).

Conclusion

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.

5 key points:

1. Differentiating between transudate and exudate can narrow the differential diagnoses and guide further investigations
2. Where 'pus' is aspirated on initial aspiration proceed to tube drainage of the effusion (if large enough) as the patient has empyema.
3. If the pleural fluid pH < 7.2 in the clinical context of infection, tube drainage is required (if effusion is large enough) as unlikely to respond to antibiotic treatment alone
4. A low pleural fluid ADA level in the context of a lymphocytic effusion virtually excludes TB pleuritis as the underlying cause
5. Lymphocyte sub sets should be sent in patients with an undiagnosed cytology negative, lymphocytic effusions at the time of sending a second cytology sample.

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