Pleural effusions: the role of biochemical analysis

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Biochemical analysis of pleural fluid may provide answers to important clinical questions. This review summarizes these questions and outlines the value and limitations of pleural fluid analysis.

Pleural fluid is the fluid found in the pleural cavities between the visceral and parietal pleura. In healthy individuals there is a small amount of pleural fluid in each cavity (less than 10 ml). Larger amounts (pleural effusions) can usually be detected by clinical examination of the chest (e.g. by dullness to percussion) or on chest X-ray. The main causes of pleural effusions are congestive cardiac failure, pneumonia and cancer (Light, 2002). The composition varies according to the cause, and this provides the basis for the role of biochemical analysis in the differential diagnosis of pleural effusions. This review summarizes the main clinical questions posed and the role of the laboratory in answering these questions.

TRANSUDATE OR EXUDATE?

Clinicians usually request pleural fluid analysis because they want to know what is causing an effusion. In some cases, a specific cause is suspected, but much more frequently the question is posed in more general terms, by asking if the effusion is a transudate or an exudate. Transudates have less protein than exudates (30 g/litre is the cut off most widely used).

The underlying assumption is that fluid formed by 'exudation' from inflamed or tumour-infiltrated pleura is likely to be high in protein, whereas fluid formed by 'transudation' from normal pleura as a result of an imbalance in hydrostatic and oncotic forces is likely to be low in protein; in general terms, exudates are more likely to reflect local pathology and to warrant further investigation. However, using the pleural fluid total protein concentration on its own to identify exudates results in variable rates of misclassification. Various combinations of biochemical analyses have been used in an attempt to optimize the classification, and these are summarized below.

Light's criteria

The 1972 article by Richard Light and colleagues occupies a seminal position in the literature on pleural fluid analysis (Light et al, 1972). In this prospective study of 150 pleural effusions, protein and lactate dehydrogenase (LDH) were measured in pleural fluid and serum, and red and white blood cell counts were measured in pleural fluid alone. These authors found that the blood cell counts had limited value in discriminating transudates from exudates. By contrast, they found that a combination of characteristics, known collectively as Light's criteria (*Table 1*), gave excellent sensitivity and specificity in the diagnosis of an exudate (99% and 98% respectively).

Subsequent studies using Light's criteria have matched the sensitivity quoted above but not the specificity, i.e. more transudates have been misclassified as exudates. There are two likely explanations for this (Tarn and Lapworth, 2001).

TABLE 1.
Light's criteria for identification of an exudate

Pleural fluid is classified as an exudate if any of the following criteria are met:
Ratio of total protein measured in pleural fluid to total protein measured in serum is greater than 0.5
Pleural fluid lactate dehydrogenase (LDH) activity is greater than 200 U/litre*
Ratio of LDH measured in pleural fluid to LDH measured in serum is greater than 0.6

*Subsequently modified to greater than two thirds of the upper limit of the serum reference interval. From Light et al (1972)

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First, the population studied by Light and colleagues was rigorously selected (33 patients were excluded because diagnostic criteria were not met); this may have enhanced the specificity. Second, the absolute pleural fluid LDH cut-off does not take account of different LDH assays or reference ranges and is inappropriate; this criterion was subsequently modified to two-thirds of the upper limit of the appropriate serum reference interval.

Alternative criteria

Alternative approaches to pleural fluid analysis have focused on ways of improving upon the specificity (somewhere in the region of 70–80%) to be expected when Light's criteria are applied in unselected populations. Additional or alternative markers have included cholesterol, bilirubin and measurement of the serum effusion albumin gradient.

Cholesterol concentrations are higher in exudates than in transudates, although the reasons for this are unclear. Since it was first proposed as a way to increase diagnostic accuracy of pleural fluid analysis, cholesterol measurement has been used in several studies, with diagnostic cut offs varying from 1.17 mmol/litre (Costa et al, 1995) to 1.55 mmol/litre (Hamm et al, 1987). In general it improves the specificity, i.e. reduces misclassification of transudates, but at the expense of sensitivity.

The use of the serum effusion albumin gradient (serum albumin minus effusion albumin) has a similar effect, particularly in patients on diuretic therapy; in one study, all effusions with an albumin gradient of more than 12 g/litre were correctly identified as transudates (specificity 100%, compared with 72% for Light's criteria) (Roth et al, 1990). By contrast, measurement of bilirubin in pleural fluid does not improve diagnostic accuracy.

Which criteria to use, and when?

There is no single test, or combination of tests, which is clearly superior at discriminating exudates from transudates. This is the conclusion of a formal meta-analysis of studies (Heffner et al, 1997). The pleural fluid total protein is at least as good as any other test; this, and practical considerations such as availability, make it the single test of choice. Combinations of more than one test improve sensitivity but at the expense of specificity. Interestingly, combinations of pleural fluid measurements that include total protein, LDH and cholesterol perform as well as modified Light's criteria, challenging the diagnostic superiority of pleural fluid:serum ratios, and thus the need for blood samples (Heffner et al, 1997). However, most of the original studies from which this observation derives allowed 24 hours or more between collection of the pleural fluid and blood samples. It is not clear whether this may have compromised the diagnostic performance of the fluid:serum ratios.

CHEST TUBE DRAINAGE

Is it empyema?

Infection of the pleural space usually occurs in association with bacterial pneumonia, and manifests initially as an exudative pleural effusion. If this does not resolve, it can become fibrotic, loculated and purulent (at which stage it is referred to as empyema). Empyema is resistant to antibiotic therapy and often only amenable to surgical drainage. When pleural fluid is frankly purulent or turbid on sampling, insertion of a chest tube is clearly indicated.

Often it is not clear that an empyema is developing, and here biochemical analysis of pleural fluid may be helpful. Bacteria and neutrophils consume glucose, and anaerobic metabolism increases with heavier bacterial loads, resulting in the production of lactate, which correlates inversely with pH. In a meta-analysis, Heffner et al (1995) found a pleural fluid pH of less than 7.2 to be the most useful predictor of empyema; pleural fluid glucose and LDH did not improve diagnostic accuracy. This finding has been incorporated in the British Thoracic Society guidelines on the use of chest tube drainage in pleural infection (Davies et al, 2003).

TABLE 2.
Protocol for anaerobic collection of pleural fluid for pH analysis

Disconnect the heparinized (i.e. blood–gas) syringe from its needle and discard the needle safely
Transfer the syringe to a larger needle (that can be used to aspirate pleural fluid)
Collect the initial specimen of pleural fluid into the heparinized syringe
With the needle still in place within the pleural cavity, disconnect and remove the heparinized syringe*
Connect a larger syringe (e.g. 50 ml) to the needle and finish the fluid aspiration

*Arrange for prompt analysis of heparinized syringe on blood gas analyzer unless fluid is frankly purulent or turbid (cloudy)

OTHER QUESTIONS

Is it malignant?

Much of the literature in this area has focused on the utility of measuring tumour markers in pleural fluid in the diagnosis of malignancy. Cytological examination on its own has a sensitivity of no more than 60–70%. Greater sensitivity of pleural fluid measurement over serum measurement has been claimed for various tumour markers (Menard et al, 1993; Romero et al, 1996).

In the largest and most comprehensive study to date, Miédougé et al (1999) measured seven tumour markers in 336 pleural effusions. Following discriminant analysis, they claimed optimal sensitivity of 94.4% for a panel of carcinoembryonic antigen (CEA), carbohydrate antigen 15-3 (CA15-3), cytokeratin fragments and neuron-specific enolase, and an overall specificity of 95%. However, Garcia-Pachon (2002) has rightly highlighted the critical bearing on test performance of prevalence, and has suggested that the positive predictive value of tumour markers in pleural fluid may be modest in unselected populations.

Is it tuberculosis?

Tuberculous involvement of the pleural space usually arises from rupture of sub-pleural foci of caseation. The resulting release of tubercle stimulates a delayed hypersensitivity reaction that involves lymphocytes and subsequently macrophages. Pleural fluid lymphocytosis is nearly always associated with either cancer or tuberculosis, prompting the recommendation to test for tuberculosis in the presence of this finding (Light, 2002).

Furthermore, since less than 40% of patients with tuberculous pleurisy have positive pleural fluid cultures, alternative laboratory pointers have been sought. The most useful marker appears to be adenosine deaminase (ADA), an enzyme involved in purine catabolism, a high activity of which is associated with lymphocyte activation; diagnostic sensitivity approaches 100% in areas of high prevalence of tuberculosis (Valdés et al, 1998). Crucially, ADA activity rarely exceeds the diagnostic cut-off (for tuberculosis) in non-tuberculous lymphocytic pleural effusions (Lee et al, 2001). Other parameters, including lysozyme, pH and interferon-γ have been evaluated but do not perform as well as ADA.

Is it rheumatoid?

Pleural effusions occur in only 2–3% of patients with rheumatoid arthritis (RA), and usually in the context of previously diagnosed RA. Rheumatoid effusions characteristically have very low concentrations of glucose (Lillington et al, 1971); the explanations usually advanced – consumption of glucose by inflammatory and other cells, or altered pleural permeability to glucose – are not wholly convincing, given that concentrations are comparatively normal in other connective tissue disorders such as systemic lupus erythematosus (Carr et al, 1970). Glucose concentrations are sometimes low in other conditions such as empyema, malignancy and tuberculosis, but are sufficiently variable that they are not diagnostically useful in these contexts.

Is it chyle?

Chyle is the fluid found in the intestinal lymphatics during absorption of food postprandially, and chylothorax may be defined as the presence of lymphatic fluid (chyle or lymph) in the pleural space. It usually results from the leak or rupture of the thoracic duct or one of its major divisions. There is no unique marker for chyle, although chylomicrons are relatively specific, except postprandially (Murphy, 1999). Triglycerides are more readily measured than chylomicrons and are widely used instead. Based on their series of 141 patients, Staats et al (1980) estimated that pleural fluid with a triglyceride concentration of greater than 1.24 mmol/litre had a 99% chance of being chylous, and fluid with a triglyceride concentration of less than 0.56 mmol/litre had no more than a 5% chance.

SAMPLING REQUIREMENTS

Biochemistry

Most of the biochemical analyses outlined above can be performed on pleural fluid collected into a plain universal container (i.e. no preservative) unless the specimen is bloody or grossly turbid. The current British Thoracic Society guidelines specify that pleural fluid for pH should be collected anaerobically with heparin (Davies et al, 2003). In practice this may be easiest to achieve by adopting the protocol outlined in *Table 2*. However, it must be noted that the majority of manufacturers of blood gas analysers specifically state that their instruments are only intended for use in the analysis of whole blood, serum or plasma. Their use for any other purpose is therefore the responsibility of the user.

Haematology

A full blood count and differential can usually be performed on pleural fluid collected into a universal container. However, for bloody speci-

mens, it is advisable to collect an aliquot into a standard full blood count bottle in order to prevent clotting.

Bacteriology

Conventional culture techniques require only a universal container. The yield with culture may be increased if blood culture bottles are inoculated at the bedside with the pleural fluid. If this is done, an additional specimen should be collected into a universal container so that a Gram stain can be performed.

Cytology

A universal container is adequate for routine cytological analysis. Although blood in a pleural fluid specimen does not preclude cytology, clotting does. For this reason, it may be advisable to collect into an anticoagulant, e.g. a full blood count bottle.

CONCLUSION

Analysis of pleural fluid has an important contribution to make to the investigation and management of patients with pleural effusions, although test requesting patterns do not always make optimal use of laboratory investigations (Jenkinson et al, 2004). No single test or combination of tests has emerged as clearly superior to any other in the differentiation of exudates from transudates, and Light's criteria continue to be widely applied. Specific biochemical analyses may be useful in addressing other questions. HM

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Carr DT, Lillington GA, Mayne JG (1970) Pleural-fluid glucose in systemic lupus erythematosus. *Mayo Clin Proc* **45:** 409–12
Costa M, Quiroga T, Cruz E (1995) Measurement of pleural fluid cholesterol and lactate dehydrogenase. A simple and accurate set of indicators for separating exudates and transudates. *Chest* **108:** 1260–3
Davies CWH, Gleeson FV, Davies RJO (2003) BTS guidelines for the management of pleural infection. *Thorax* **58**(suppl 2): ii18–28
Garcia-Pachon E (2002) Tumor markers for diagnosing malignant pleural effusion? *Chest* **121:** 302–3
Hamm H, Brohan U, Bohmer R, Missmahl H-P (1987) Cholesterol in pleural effusions. A diagnostic aid. *Chest* **92:** 296–302
Heffner JE, Brown LK, Barbieri CA, DeLeo JM (1995) Pleural fluid chemical analysis in parapneumonic effusions. A meta-analysis. *Am J Respir Crit Care Med* **151:** 1700–8
Heffner JE, Brown LK, Barbieri CA (1997) Diagnostic value of tests that discriminate between exudative and transudative pleural effusions. *Chest* **111:** 970–80
Jenkinson F, Kennedy I, Murphy MJ (2004) Pleural and ascitic fluid: how logical is test requesting? In: *Proceedings of Association of Clinical Biochemists National Meeting, Birmingham, UK*: 18–20 May. Association of Clinical Biochemists, London
Lee YCG, Rogers JT, Rodriguez RM, Miller KD, Light RW (2001) Adenosine deaminase levels in nontuberculous lymphocytic pleural effusions. *Chest* **120:** 356–61
Light RW (2002) Pleural effusion. *N Engl J Med* **346:** 1971–7
Light RW, MacGregor MI, Luchsinger PC, Ball WC (1972) Pleural effusions: the diagnostic separation of transudates and exudates. *Ann Intern Med* **77:** 507–13
Lillington GA, Carr DT, Mayne JG (1971) Rheumatoid pleurisy with effusion. *Arch Intern Med* **128:** 7648
Menard O, Dousset B, Jacob C, Martinet Y (1993) Improvement of the diagnosis of the cause of pleural effusion in patients with lung cancer by simultaneous quantification of carcinoembryonic antigen (CEA) and neuron-specific enolase (NSE). *Eur J Cancer* **29A:** 1806–9
Miédougé M, Rouzaud P, Salama G et al (1999) Evaluation of seven tumour markers in pleural fluid for the diagnosis of malignant effusions. *Br J Cancer* **81:** 1059–65
Murphy M (1999) The role of laboratory investigations in identifying body fluids. *Hosp Med* **60:** 281–4
Romero S, Fernandez C, Arriero JM et al (1996) CEA, CA15-3 and CYFRA 21-1 in serum and pleural fluid of patients with pleural effusions. *Eur Respir J* **9:** 17–23
Roth BJ, O'Meara TF, Cragun WH (1990) The serum-effusion albumin gradient in the evaluation of pleural effusions. *Chest* **98:** 546–9
Staats BA, Ellefson RD, Budahn LL, Dines DE, Prakash UB, Offord K (1980) The lipoprotein profile of chylous and nonchylous pleural effusions. *Mayo Clin Proc* **55:** 700–4
Tarn AC, Lapworth R (2001) Biochemical analysis of pleural fluid: what should we measure? *Ann Clin Biochem* **38:** 311–22
Valdés L, Alvarez D, San Jose E et al (1998) Tuberculous pleurisy. *Arch Intern Med* **158:** 2017–21

KEY POINTS

- Light's criteria identify pleural fluid exudates with nearly 100% sensitivity; specificity is variable.
- The best alternative markers, e.g. cholesterol, improve specificity but at the expense of sensitivity.
- Pleural fluid pH is the most accurate predictor of developing empyema.
- Pleural fluid specimens for pH analysis should be collected anaerobically into a heparinized syringe.
- Pleural fluid lymphocytosis is nearly always associated with either cancer or tuberculosis.
- A high activity of adenosine deaminase in pleural fluid is almost 100% sensitive for tuberculous pleurisy in areas where tuberculosis is prevalent.
- Elevated triglycerides in pleural fluid increase the likelihood of a chylous pleural effusion.
- Pleural fluid glucose is very low in effusions resulting from rheumatoid arthritis.
- Elevated tumour markers in pleural fluid make malignancy more likely, but most assays are validated for measurement in plasma or serum only, and positive predictive values depend critically on prevalence.