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Pearls and myths in pleural fluid analysis

JOSÉ M. PORCEL

Pleural Diseases Unit, Department of Internal Medicine, Arnau de Vilanova University Hospital, Institut de Recerca Biomèdica de Lleida, Lleida, Spain

ABSTRACT

Virtually all patients with a newly discovered pleural effusion should undergo thoracentesis to aid in diagnosis and management. The routine pleural fluid (PF) evaluation usually includes the following: cell count and differential; tests for protein, LDH, glucose, adenosine deaminase, cytology and, if infection is a concern, pH and bacterial and mycobacterial cultures. Distinguishing transudates from exudates with Light's criteria is a pragmatic first step. If the effusion is an exudate, various PF tests have proven diagnostic utility: adenosine deaminase levels >35 IU/L usually indicate tuberculosis in lymphocyte-predominant PF; pH < 7.2 or glucose less than 60 mg/dL allow the clinician to identify complicated parapneumonic effusions; and conventional cytology may reveal malignant cells in 60% of the patients with malignant effusions. A number of optional PF tests may complement the diagnostic approach to an undiagnosed pleural effusion. For example, natriuretic peptide assays significantly improve the accuracy of a diagnosis of cardiac pleural effusion, whereas PF mesothelin levels greater than 20 nmol/L are highly suggestive of mesothelioma.

Key words: pleural effusion, pleural fluid, thoracentesis, tuberculosis.

The Authors: Dr Porcel received his medical degree at Universidad Autónoma of Barcelona in 1985 and trained in Internal Medicine at Vall d'Hebron University Hospital in Barcelona from 1987 to 1991. He subsequently undertook a Fellowship in Immunology at King's College Hospital in London (1991–1992). In 1993 he was appointed to his current post at Arnau de Vilanova University Hospital in Lleida, where he has held different clinical and academic appointments before taking up his current positions as Professor of Medicine and also Chairman of the Department of Internal Medicine. Dr Porcel is ACCP Regent for Spain and, currently, Associate Editor for Respirology and Co-editor of the International Pleural Newsletter. His clinical and research interests focus primarily on pleural effusions.

Correspondence: José M. Porcel, Department of Internal Medicine, Arnau de Vilanova University Hospital, Avda Alcalde Rovira Roure 80, 25198 Lleida, Spain. Email: jporcelp@yahoo.es

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INTRODUCTION

Pleural fluid (PF) analysis, in combination with a targeted history, complete physical examination, and chest imaging, allows the physician to make a definitive or confident clinical diagnosis in the majority of patients with pleural effusion.[1] This review is a succinct compilation of the pieces of wisdom (pearls) gathered from clinical experience in the management of pleural effusions and the myths that may have influenced everyday practice, but have been proven false. The discussion will focus on the tests available in most clinical laboratories and avoid those limited to investigational settings.

PLEURAL FLUID APPEARANCE

Myth: transudates have a clear appearance upon gross inspection

Reality

In a prospective study of 766 consecutive patients with pleural effusion, only 11 of 82 (13%) transudates had a watery appearance.[2] Most transudative effusions were straw-coloured (67%) or even bloody (11%) or turbid (9%), qualities that are also found in exudates.

Pearl: malignancy is one of the most common causes of frankly bloody pleural fluid

Comment

In the above-mentioned study, malignancy was the leading cause of grossly bloody PF (47%), although only 11% of malignant effusions were bloody.[2] Less common causes of bloody effusions are trauma, pneumonia, post-cardiac injury syndrome and pulmonary embolism.

Myth: chylothorax usually has a milky appearance.

Reality

In a study of 61 patients with chylothorax determined by lipoprotein analysis, the gross appearance of the PF was described as non-milky in more than half of the cases (serous in 26%, serosanguineous in 26% and bloody in 3%).[3] Non-milky fluids may be particularly prevalent during periods of fasting.

PLEURAL FLUID PROCESSING

Myth: cytological diagnosis of malignant effusions requires a large volume (hundreds of millilitres) of pleural fluid

Reality

The minimum volume of PF required to diagnose a malignant effusion has not been established. An initial retrospective review of 374 thoracentesis samples, including 132 from malignancies, showed that diagnostic sensitivity did not depend on the volume of PF analysed.[4] In that study, patients were classified into quartiles based on the quantity of PF submitted. The first and fourth quartiles contained mean PF volumes of 6.2 and 1130 mL, respectively. There were no significant differences in sensitivity for predicting pleural malignancy between both quartiles (53.9% and 63.3%, respectively). Recently, the same authors prospectively compared three different fluid volumes (10 mL, 60 mL and 150 mL or more) from 102 patients with suspected malignant effusions.[5] The findings did not support the authors' previous results; they found an incremental yield with increasing volumes of fluid analysis (respective sensitivities were 49%, 63% and 69%). Another prospective analysis of 44 patients concluded that PF samples greater than 50 mL did not increase diagnostic yield.[6] Cytological tests were positive for malignancy in 55% of the cases, regardless of whether the sample analysed was 50 mL or a larger volume.[6] In our experience, the sensitivity of as little as 4 mL of PF for diagnosing 466 pleural malignancies was 56.4% (95% CI: 52–61%),[7] which is in accordance with the figures reported in other studies.[4,6] In this author's opinion, the available data reasonably support the notion that smaller PF volumes (less than 50 mL; probably as little as 4 mL) are diagnostically as valuable as larger volumes.

Pearl: cell blocks can be used as an adjunct to increase the yield of smear preparations for diagnosing malignancy

Comment

Whether using a cell block from the sediment of the PF sample significantly increases the percentage of positive diagnoses compared with using smears alone is somewhat controversial.[5,8,9] Cell blocks are recommended when the cytological smears are negative or misleading and, particularly, when cells need to be evaluated with immunocytochemistry.[10] Likewise, if the PF sample is collected into tubes without additives, examining the fibrinous clot that may form after overnight storage of the specimen at 2–8°C further increases the accuracy of the cytological diagnosis and also allows for immunocytochemical staining.[11]

Myth: pleural fluid should always be sent for Gram stain and microbiological culture

Reality

Microbiological testing of PF should be ordered selectively because the frequency of positive results in non-specific populations is very low. In a retrospective survey, Barnes *et al*. found only 15 true-positive PF cultures (3.2%) among 476 patients with varied causes of pleural effusion whose PF were sent for microbiological investigations.[12] The percentage of positive microbiological smears was even lower (1.3%). Therefore, PF cultures should be ordered only when an infection is suspected (e.g. associated pneumonia, fever, sepsis or loculated effusion), in which case it is best to inoculate PF directly into blood culture bottles at bedside.[13] Cultures are expected to be positive in about 25% of patients with non-purulent complicated parapneumonic effusions and 70% of patients with empyemas.[14]

Myth: a short delay in performing biochemical or cytological pleural fluid analysis critically affects the results

Reality

No doubt the sooner the PF sample is analysed, the better. The analysis should be done within 4 h after the sample is obtained, if possible. However, if a delay is anticipated, a specimen collected in tubes containing an anticoagulant (heparin or ethylenediaminetetraacetic acid (EDTA)) can be refrigerated at 4°C for up to 1 day with no significant effects on white blood cell count and differential.[15] Adenosine deaminase (ADA) measurements[16] and cytological interpretation[17] remain reliable after a longer refrigeration time (up to 28 and 14 days, respectively). Finally, one study demonstrated that in samples kept at room temperature, an analysis delay of up to 4 h did not cause a significant change in PF pH.[18] The increase in PF pH over time could be considered clinically significant at 24 h (mean 0.05, 95% CI: 0.03–0.08). An earlier study showed similar results for refrigerated specimens.[19] In contrast, PF glucose measurement is not significantly affected by a 24-h analysis delay.[18]

Table 1 Measures of diagnostic accuracy for tests that identify an exudative pleural effusion[†]

	N	Sensitivity, % (95% CI)	Specificity, % (95% CI)	LR+	LR–
PF protein >3 g/dL	2283	86.4 (84.7–88)	83.2 (79.8–86)	5.14 (4.26–6.2)	0.16 (0.14–0.19)
PF/S protein >0.5	2027	86.2 (84.4–88)	88.3 (85.2–90.8)	7.35 (5.78–9.35)	0.16 (0.14–0.18)
PF LDH LP > 312 U/L[‡]	2260	78 (76–80)	95.2 (93–96.7)	16.22 (11.14–23.62)	0.23 (0.21–0.25)
PF/S LDH > 0.6	1714	90.1 (88.4–91.6)	83.1 (79.2–86.4)	5.33 (4.31–6.59)	0.12 (0.10–0.14)
Cholesterol >45 mg/dL	517	87.2 (83.3–90.3)	83.2 (77–88)	5.20 (3.72–7.27)	0.15 (0.12–0.20)
S-PF albumin ≤1.2 g/dL	209	51.3 (40.3–62.2)	94.7 (89.5–97.4)	9.75 (4.59–20.71)	0.51 (0.41–0.65)
S-PF protein ≤3.1 g/dL	2027	85 (83.2–86.7)	84.3 (81–87.2)	5.42 (4.42–6.64)	0.18 (0.16–0.20)
PF protein or PF LDH	2277	95.1 (94–96)	80.4 (76.8–83.5)	4.85 (4.09–5.76)	0.06 (0.05–0.08)
PF/S protein or PF LDH	2207	95 (94–96)	84.7 (81.4–87.6)	6.24 (5.08–7.66)	0.06 (0.05–0.07)
Light's criteria	2115	97.5 (96.7–98.2)	73.8 (69.4–77.7)	3.72 (3.17–4.36)	0.03 (0.02–0.05)

[†] This is an unpublished update from our database.
[‡] This figure represents more than two-thirds the upper limits of our laboratory's normal serum LDH.
CI, confidence interval; LR, likelihood ratio; PF, pleural fluid; PF/S, pleural fluid to serum ratio; S-PF, gradient between the serum and the pleural fluid.

DIFFERENTIATING TRANSUDATES FROM EXUDATES

Pearl: Light's criteria remain the best method for separating transudates from exudates

Comment

Determining whether a patient has a transudate or an exudate is an important diagnostic starting point in PF analysis because it dictates further investigations and management. Transudates are more commonly caused by heart failure (80%), whereas malignancy, pneumonia and tuberculosis (TB) account for three-quarters of all exudative effusions.[20] According to Light's criteria, if at least one of the following three criteria is present, the fluid is an exudate:[21] (i) the ratio of PF protein to serum protein is greater than 0.5; (ii) the ratio of PF LDH to serum LDH is greater than 0.6; and (iii) the PF LDH is greater than two-thirds of the upper limit of normal for serum LDH. Light's criteria have proven robust in separating exudates from transudates, with an overall diagnostic accuracy of 95% (Table 1). Notably, the omission of a blood sample does not significantly affect the categorization of PF in routine clinical practice (e.g. the combination of PF protein and PF LDH in an 'either/or' rule has a diagnostic accuracy similar to the traditional Light's criteria).[20]

Myth: the time interval between pleural fluid and serum sample collection may affect fluid categorization after Light's criteria is applied

Reality

One study applied Light's criteria to two separate fluid/serum pairing samples.[22] The first pairing involved serum samples sent within 2 h of the PF collection (mean time interval 1 h 12 min; range 0 min to 2 h), whereas the second pairing involved serum samples not formally paired with the fluid samples (mean time interval 28 h 30 min; range 7 h 25 min to 5.3 days). Discrepant categorization (exudate vs transudate) was observed only in two PF out of the 77 analysed (2.5%).

Pearl: Light's criteria are superior to clinical judgement for discriminating transudates from exudates

Comment

A prospective evaluation of 249 consecutive patients referred for diagnostic thoracentesis compared clinical presumption with the use of Light's criteria to categorize effusions as transudates or exudates.[23] The former was significantly less accurate than the latter (84% vs 93%, $P < 0.01$). Clinical judgement mislabelled 44% of the transudates, whereas only 25% were mislabelled using Light's criteria.

Pearl: measurement of the natriuretic peptide NT-proBNP, either in the blood or pleural fluid, best identifies transudates misclassified by Light's criteria

Comment

Light's criteria erroneously identify approximately 25% of transudates as exudates (Table 1). These misclassifications are most likely in patients with heart failure treated with diuretics or in patients with a PF erythrocyte count greater than 10 000/mm^3.[24] Traditionally, it has been proposed that an albumin gradient (serum albumin minus PF albumin) greater than 1.2 g/dL or a protein gradient greater than 3.1 g/dL be used to identify these misclassified transudates. In the last few years, it has become apparent that measuring natriuretic peptides, either NT-proBNP or BNP, helps diagnose heart failure.[25] One recent study evaluated whether BNP or NT-proBNP levels in PF can be used to

Table 2 Pleural fluid biochemistries for different aetiologies of pleural effusion[†]

	Transudates (%)	Parapneumonics (%)	Tuberculosis (%)	Malignant (%)
RBC count $\geq 10 \times 10^9$/L	93/540 (17.2)	151/427 (35.4)	32/227 (14.1)	261/624 (41.8)
WBC count $\geq 10 \times 10^9$/L	0/538 (0)	134/434 (30.9)	11/230 (4.8)	16/623 (2.6)
Neutrophils ≥50%	60/409 (14.7)	331/403 (82.1)	20/222 (9)	114/576 (19.8)
Lymphocytes ≥50%	349/409 (85.3)	69/403 (17.1)	201/222 (90.5)	458/575 (79.7)
Protein ≥5 g/dL	0/541 (0)	118/440 (26.8)	168/231 (72.7)	130/629 (20.7)
Glucose ≤60 mg/dL	3/538 (0.6)	214/440 (48.6)	64/231 (27.7)	51/625 (8.2)
pH ≤7.20	1/478 (0.2)	185/376 (49.2)	20/208 (9.6)	31/554 (5.6)
LDH ≥1000 U/L	0/541 (0)	253/430 (58.8)	92/227 (40.5)	138/624 (22.1)
ADA ≥40 U/L	0/529 (0)	151/425 (35.5)	217/230 (94.3)	41/615 (6.7)
Amilasa ≥100 U/L	4/71 (5.6)	10/71 (14.1)	8/48 (16.7)	17/124 (13.7)

[†] This is an unpublished update from our database.
ADA, adenosine deaminase; RBC, red blood cell count; WBC, white blood cell count.

diagnose heart failure[26] and whether these natriuretic peptides categorize cardiac effusions misclassified by Light's criteria better than albumin or protein gradients. NT-proBNP levels of >1300 pg/mL discriminated 90 cardiac effusions from 91 non-cardiac effusions more accurately than BNP levels of >115 pg/mL (area under the curve 0.96 vs 0.90). In addition, NT-proBNP levels correctly classified 90% of mislabelled cardiac effusions, more than levels of BNP (70%) or the protein (50%) or albumin (75%) gradients.[26] Thus, NT-proBNP should be measured whenever a suspected cardiac effusion meets the exudative criteria. Interestingly, a comparable diagnostic utility has been demonstrated at the same cut-off values for serum and pleural NT-proBNP.[27] This result means that serum, rather than pleural NT-proBNP, measurement may be preferable, provided thoracentesis is not planned.

Myth: pleural effusion secondary to pulmonary embolism may be transudative

Reality

This misconception is based on a single, old publication that reported that six of 26 (23%) pulmonary embolism-associated effusions were transudates.[28] However, the study had significant methodological limitations: PF LDH was not requested for more than half the patients, and a PF protein level lower than 3 g/dL was the main criteria for identifying transudates. Two subsequent studies analysed 60 and 26 PF from patients with pulmonary embolism and found that all fell into the exudative category when Light's criteria were applied.[29,30]

DIFFERENTIAL WCC

Pearl: in patients with exudates, the pleural fluid differential cell count provides a clue to the origin of the pleural effusion

Comment

If the PF differential cell count shows a predominance of neutrophils (>50%), the most likely diagnosis is parapneumonic effusion. Neutrophils, which indicate acute injury to the pleural surface, are also seen in effusions caused by pulmonary embolism, subphrenic abscess, pancreatitis and, less commonly, malignancy (20%) or TB (<10%) (Table 2). In contrast, when a pleural injury becomes longstanding, the fluid tends to be populated by lymphocytes (>50% of the leukocytes). More than two-thirds of lymphocytic pleural effusions are the result of malignancy or TB. Finally, PF eosinophilia (>10% eosinophils in the total PF nucleated cell count) is virtually never diagnostic because it can be due to idiopathic (25%), malignant (20%), infectious (20%) or miscellaneous benign conditions (35%).[31] It should be stressed that the higher the percentage of pleural eosinophils (e.g. >40%), the lower the likelihood of malignancy and the greater the likelihood of an unknown aetiology.[31]

Myth: repeated thoracentesis is a common cause of eosinophilic effusion

Reality

It is generally accepted that the introduction of air into the pleural space during a prior thoracentesis is a frequent cause of PF eosinophilia. However, the evidence does not support this conclusion. An early study of 130 patients undergoing a single repeated thoracentesis showed that only three (2.3%) cases became eosinophilic on the second pleural tap.[32] This percentage rose to 11% (four of 36 patients) for patients who underwent multiple thoracenteses. Of note, the percentage of eosinophils decreased in seven (5.3%) patients.[32] In another study, 120 patients with pleural effusion who required two or more diagnostic thoracenteses exhibited no significant changes in eosinophil percentage, regardless of the time between procedures.[33] The number of PF eosinophils increased in 16 patients (13%), 10 of whom fulfilled the criteria for eosinophilic pleural effusion, while eosinophils actually decreased in 21 patients (17.5%) upon subsequent aspirations. Finally, the incidence of new eosinophilic effusions reported in a recent

study of patients undergoing a second thoracentesis was also low (14 of 249, 5.6%).[31]

pH AND GLUCOSE MEASUREMENT

Pearl: pleural fluid pH and glucose measurements may aid in decisions related to drainage in non-purulent parapneumonic effusions

Comment

Pleural fluid should be obtained from all patients with parapneumonic effusion to determine pH, glucose and LDH as well as to culture for bacteria. These analyses have implications on whether the patient will eventually need pleural space drainage in addition to antibiotics. Biochemical parameters should not be requested for frankly purulent fluids, as empyema is already an indication for chest tube drainage. Low pleural pH and glucose usually occur together. A PF pH below 7.20 or a glucose level below 60 mg/dL generally indicates a need for drainage of a parapneumonic effusion, although these thresholds should not be utilized rigidly.[14] Of note, PF pH and glucose may be reduced in conditions other than bacterial infections of the pleural space, such as rheumatoid pleurisy, TB and malignancy (Table 2). In these conditions, neither PF acidosis nor low glucose levels are indicators of pleural drainage.

Myth: chest-drain insertion is unnecessary in patients with non-purulent parapneumonic effusions and normal pleural fluid pH levels

Reality

In patients with pleural infection, the PF findings that argue most strongly for a complicated effusion (i.e. one requiring chest tube drainage) are PF pH lower than 7.20 (positive likelihood ratio (LR) = 8.2) and PF glucose levels lower than 60 mg/dL (positive LR = 5.92).[14] However, no single absence of a PF finding argues convincingly against a complicated parapneumonic effusion (i.e. no LR has a value less than 0.30).[14] Decisions regarding pleural drainage should consider additional criteria, namely the presence of positive fluid cultures, loculated pleural collections or large effusions (half or more of the hemithorax).[34]

ADENOSINE DEAMINASE

Pearl: testing for pleural fluid adenosine deaminase is an easy and inexpensive method for diagnosing tuberculosis pleurisy, regardless of the patient's immune status

Comment

A recent single-centre retrospective study including 2104 consecutive patients with pleural effusions, 221 (10.5%) of whom had TB, reported the discriminative properties of PF ADA.[35] In patients with lymphocytic exudates, the typical clinical scenario of TB, an ADA level above 35 IU/L had a sensitivity of 93% (95% CI: 86–99%), a specificity of 90% (95% CI: 88–93%), a positive LR of 10.05 (95% CI: 7.57–13.35), a negative LR of 0.07 (95% CI: 0.03–0.18) and an area under the receiver operating characteristic curve of 0.94 (95% CI: 0.91–0.97) for identifying a TB effusion.[35] ADA analysis is a sensitive marker of TB effusions, even in patients infected with the HIV and very low CD4 cell counts.[36] For instance, in a study of 58 confirmed cases of TB pleuritis, there was no significant difference between the mean PF ADA values for 25 patients with CD4 less than 50 cell/μL and 33 patients with more than 50 CD4 cells/μL (81 IU/L vs 70 IU/L, $P > 0.5$).[36]

Myth: adenosine deaminase testing is not diagnostically useful in countries with a low prevalence of tuberculosis

Reality

In geographic areas with a high or moderate incidence of TB, a TB pleurisy diagnosis can be confidently based on ADA measurement.[37,38] In a country with a low prevalence of pleural TB (e.g. 1% of all exudative effusions), the positive predictive value of an ADA test would be 7.2%, but the negative predictive value would remain at 99.9%.[35] In such epidemiological circumstances, the test can still be used to exclude the diagnosis of TB pleuritis.

Pearl: extremely high adenosine deaminase activity in pleural fluid should raise suspicion of a non-tuberculous effusion

Comment

In a large retrospective study of 221 patients with pleural TB, none exhibited ADA levels higher than 250 IU/L.[35] Such levels were found only in patients with empyema or lymphoid malignancies.

Myth: pleural fluid adenosine deaminase levels are low in tuberculosis effusions with predominantly polymorphonuclear leukocytes

Reality

Adenosine deaminase is a predominant T-lymphocyte enzyme and its activity is the sum of two isoenzymes (ADA1 and ADA2). While many different cell types may produce ADA1, ADA2 is secreted only by monocytes and macrophages. ADA2 is the primary isoenzyme in TB effusions, regardless of whether lymphocytes or neutrophils predominate in the PF (unpubl. obs., Porcel *et al*, 2010). In fact, one study found that the median PF ADA activity was

significantly more elevated in 21 patients with neutrophil-rich TB effusions (77.4 IU/L) than in 191 patients with lymphocytic TB effusions (62.5 IU/L, $P < 0.05$).[35]

TUMOUR MARKERS

Pearl: classic tumour markers have limited usefulness for the routine workup of suspected malignant effusions

Comment

For tumour markers to be clinically useful, the selected cut-off points have to be 100% specific. A cut-off level that is not exceeded by any of the benign effusions renders tumour marker tests very insensitive. One study measured a panel of PF tumour markers, including CEA, CA 125, CA 15–3 and CYFRA 21–1, in 243 patients with malignant and 173 with benign effusions.[39] At cut-off values that achieved 100% specificity for diagnosing malignancy (i.e. none of the benign effusions had levels above these thresholds), 54% of the malignant effusions were correctly classified, and one-third of the cytology-negative malignant effusions could be identified by at least one marker. The discriminating PF cut-off values were considerably higher than those commonly adopted for serum (e.g. CEA > 50 ng/mL, CA 15–3 > 75 IU/mL, CA 125 > 2800 IU/mL, CYFRA 21–1 > 175 ng/mL). These findings probably do not justify the routine measurement of traditional tumour markers in patients with pleural effusion of undetermined aetiology.

Pearl: mesothelin measurement should be considered in all patients with an undiagnosed pleural effusion, particularly if mesothelioma is a concern

Comment

In the past several years, researchers have enthusiastically sought a reliable biomarker for mesothelioma, as this malignancy is difficult to diagnose. The marker that has received the most attention is soluble mesothelin. Davies *et al.* measured PF mesothelin concentrations in 24 patients with mesothelioma, 67 with pleural metastases and 75 with benign pleural conditions, using a commercially available ELISA.[40] Values exceeding 20 nmol/L detected mesothelioma with a positive LR of 7.1 and a negative LR of 0.32. Mesothelin measurement diagnosed mesothelioma more reliably than cytological examination (71% vs 35%). All eight cases of mesothelioma with positive PF cytology exhibited mesothelin levels above the established threshold. In 105 patients with cytology-negative effusions, PF mesothelin levels less than 20 nmol/L offered support for excluding an underlying mesothelioma (negative predictive value of 94%). These results demonstrate that PF mesothelin provides valuable information in addition to PF cytology.

CYTOLOGICAL EXAMINATION

Pearl: a negative pleural fluid cytological examination does not rule out malignancy

Comment

Although cytological examination of the PF is an easy way to diagnose a pleural malignancy, a false-negative rate of about 40% has been reported. For example, in a thoracoscopic series of 556 malignant pleural effusions, the overall sensitivity of PF cytology was 60%.[41] However, the diagnostic yield for malignancy depended on the tumour type: 83% for ovarian cancer, 78% for breast cancer, 71% for unknown primary, 57% for lung cancer, 41% for mesothelioma and just 18% for lymphoma.[41] Among patients with mesothelioma, the lowest sensitivity rate was reported for the sarcomatoid histological category (20%).[42]

Pearl: the yield from sending more than two separate pleural fluid specimens is very low and should be discouraged

Comment

In our unpublished study of 603 patients with malignant pleural effusions, PF cytological analyses had an overall sensitivity of 50% (95% CI: 46–54%) for the first specimen and 57% (95% CI: 53–61%) and 58.2% (95% CI: 54.2–62%) for the second and third separate examinations, respectively. The time interval between the submission of two separate specimens for cytological examination does not influence the results.[7]

Pearl: immunocytochemical evaluation of pleural fluid specimens is helpful in labelling different tumour types

Comment

Immunocytochemistry can be performed on conventional cytological specimens, cell blocks or clots. Its main purposes are to distinguish benign from malignant mesothelial proliferations, differentiate epithelial mesothelioma from adenocarcinoma and reveal a primary site in patients with pleural metastases of an unclear nature. To separate epithelial mesothelioma from adenocarcinoma, at least two mesothelial (e.g. calretinin, keratin 5/6, WT-1 protein) and two carcinoma (e.g. thyroid transcription factor-1-TTF-1-, CEA, B72.3) markers should be used.[43] In a malignant pleural effusion of unknown origin, a positive TTF-1 stain strongly suggests a primary non-small-cell lung cancer.[44] Approximately 80% of lung adenocarcinomas exhibit TTF-1 positivity.[43] Finally, examining cells in the PF for the expression of oestrogen/progestogen receptors or Her-2-neu may have both diagnostic (breast cancer) and therapeutic implications (indicating the benefit of hormone therapy or trastuzumab).

MICROBIOLOGICAL STUDIES

Pearl: pleural fluid cultures for mycobacteria have higher yields in HIV-positive patients than in immunocompetent subjects

Comment

Mycobacterial cultures of PF in the Löwenstein-Jensen medium were positive in 42 (20.5%) of 205 patients with tuberculous pleurisy from our unpublished database. The use of a BACTEC system with bedside inoculation of the PF provides higher yields and faster results than conventional methods. In one study of 109 HIV-positive and 33 HIV-negative patients with TB pleurisy, the BACTEC system provided positive cultures in 24% of HIV-negative and 75% of HIV-positive individuals, while the Löwenstein-Jensen medium provided positive cultures in 12% of HIV-negative patients and 43% of HIV-positive patients.[45] The mean time between collection and the identification of positive cultures was significantly lower using the former than the latter medium (3.5 vs 4.7 weeks).[45]

Myth: the detection of pneumococcal antigen in pleural fluid adds nothing to the urinary antigen assay

Reality

Patients with pneumonia, with or without an associated pleural effusion, usually undergo urine antigen tests to detect *Streptococcus pneumoniae*. One study evaluated a rapid immunochromatographic test (Binax NOW) to detect *S. pneumoniae* in the urine and PF samples of 140 patients with pleural effusion.[46] The PF test was positive in 24 of 34 (70.6%) patients with pneumococcal pneumonia and negative in 83 of 89 (93.3%) patients without pneumococcal pneumonia. Interestingly, three patients with parapneumonic effusion due to *S. pneumoniae* had positive test results in their PF and negative results in their urine samples. Therefore, pneumococcal antigen testing of PF should be considered in patients with parapneumonic effusion and negative urine antigen tests.

MISCELLANEOUS TESTS

Myth: a pleural fluid triglyceride level lower than 110 mg/dL rules out chylothorax

Reality

A triglyceride level in the PF higher than 110 mg/dL is currently being used to establish the diagnosis of chylothorax. In a recent retrospective series, 10 (14%) of 74 pleural effusions from patients with chylothorax (defined by the presence of chylomicrons) had triglyceride levels lower than 110 mg/dL and two exhibited values lower than 50 mg/dL.[3] It should be noted that the lipid content of these effusions varied according to the patient's nutritional status.

Pearl: pleural fluid amylase should be requested only if pancreatic disease or oesophageal rupture is suspected

Comment

The most common causes of amylase-rich effusions (i.e. PF amylase levels higher than the upper limit of normal for serum) are neoplasm (55%) and TB (13%).[47,48] However, the diagnostic utility of the amylase measurement in PF is restricted to pancreatic- and esophageal rupture-associated effusions. In the former condition, the PF contains mainly pancreatic amylase; in the latter (as in malignancy), the salivary isoenzyme predominates.

Pearl: it is not worth performing a pleural fluid antinuclear antibody test because serum antinuclear antibody provides the same key information

Comment

Antinuclear antibody (ANA) testing is usually the first step in establishing immunological support for the clinical diagnosis of systemic lupus erythematosus (SLE). In one study of 266 patients with pleural effusion, including 17 with SLE, PF ANA titres ≥1:160 had a sensitivity of 100% (95% CI: 97–100%), a specificity of 94% (95% CI: 91–97%) and a positive LR of 16.7 (95% CI: 10.4–26.8) for identifying lupus pleuritis.[49] In two SLE patients with effusions from causes other than lupus, the PF ANA was low-positive (1:40 to 1:80) or negative. PF antibodies against dsDNA and extractable nuclear antigens were found only in lupus-related effusions, and all SLE patients had positive serum ANAs. There is no additional value in measuring PF ANA beyond the serum test to diagnose lupus pleuritis. However, in this author's opinion, PF ANA testing can be helpful in selected SLE patients with pleural effusion of unknown origin. Negative or low titres argue strongly against lupus as the aetiology of the effusion and other causes should be sought. In contrast, a positive ANA test or the presence of PF anti-dsDNA greatly supports the diagnosis of lupus-related effusion.

Myth: gamma interferon release assays may have a role in identifying patients with pleural tuberculosis

Reality

T-cell gamma interferon release assays (IGRA) have emerged as attractive alternatives to the tuberculin skin test for the diagnosis of latent TB, although their contribution to the diagnosis of TB pleurisy is less

clear. One recent prospective study of 63 patients from a high TB/HIV-burden setting compared the diagnostic performance of four different IGRA using PF mononuclear cells with that of unstimulated interferon-γ concentrations in PF.[50] All IGRA, including the commercially available QuantiFERON-TB Gold in-tube and T-SPOT-TB, performed poorly because, at best, they missed 15% of TB cases and incorrectly diagnosed a further 20%. In contrast, unstimulated interferon-γ levels above 0.31 IU/mL had 97% sensitivity and 100% specificity for identifying TB pleuritis. Currently, there is little convincing evidence to support the use of IGRA instead of other available markers of TB such as interferon-γ or, even better, ADA.

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