Pleural fluid biochemistry: A first step toward an etiological diagnosis of pleural effusions

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Abstract

The analysis of pleural fluid (PF) is the most important diagnostic element in identifying the cause of pleural effusions. PF biochemistries, in particular, are available immediately and offer relevant clinical information. The measurement of proteins and lactate dehydrogenase (LDH) in PF and blood (Light's criteria) establishes the transudative or exudative nature of effusions. Transudates are commonly caused by heart failure (HF), but diuretic therapy may concentrate PF causing higher levels of protein and/or LDH and, therefore, leading to a misclassification as an exudate. In this scenario, a serum-PF albumin gradient > 1.2 g/dL points to a true transudate. Furthermore, elevated concentrations of the natriuretic peptide N-terminal pro-brain natriuretic peptide are virtually pathognomonic of HF as the primary or, at least, secondary diagnosis. In exudates with predominantly polymorphonuclear leukocytes ($> 50\%$), a bacterial infection of the pleural space should be considered, particularly if PF C-reactive protein levels are high. A pleural pH < 7.15 or a glucose < 40 mg/dL in a parapneumonic effusion indicates the need for a drainage tube. When lymphocytes predominate in an exudate, cancer and tuberculosis are the two main diagnoses to consider; an adenosine deaminase activity > 35 U/L strongly supports the diagnosis of tuberculosis. Measuring tumor markers (e.g., carcinoembryonic antigen, CA15-3) under the premise of using 100% specific cutoff points can increase the diagnostic yield for malignancy.

Key words: Pleural effusion. Pleural fluid. Tumor markers. Tuberculosis. Empyema.

Introduction

Under physiological conditions, pleural fluid (PF) is an ultrafiltrate of plasma with an estimated quantity of 0.26 mL/kg in each hemithorax¹. PF is normally composed of approximately 1-2 g of protein per dL, with levels of lactate dehydrogenase (LDH) $< 1/2$ of those found in serum. Differential cell count yields a predominance of macrophages (75%) and lymphocytes (20%), with a marginal presence of mesothelial cells, neutrophils, and eosinophils¹. Through ultrasonography, a

minimal film of PF, approximately 3 mm, can be observed in 30% of healthy individuals². Any clinically detectable quantity of PF is considered abnormal. A pleural effusion (PE) may indicate the presence of a pleural, pulmonary or extra-pulmonary disease. Some cases of PE have a clear clinical etiology, but in others there may be more than one etiology or else the cause is uncertain. For example, a bilateral PE in the clinical context of heart failure (HF) is generally secondary to this condition, while a unilateral PE in a patient with a medical history of breast cancer is indicative of

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metastasis until proven otherwise. PEs secondary to multiple etiologies are common, and accounted for 30% of 126 unilateral effusions in one prospective study³; HF being a common contributing cause in these cases.

An analysis of PF obtained by thoracentesis allows the physician to establish a definitive or presumptive cause of PE in more than two-thirds of cases and, at minimum, allows for the reliable ruling out of some etiologies⁴. This narrative review focuses on those routine and optional biochemical parameters that can be measured in PF for narrowing the differential diagnosis of PEs.

Macroscopic appearance of PF

The organoleptic characteristics of PF can provide useful diagnostic information. In the majority of cases, PF can be classified into one of the following color categories: watery (light yellow or transparent), serous (yellowish), blood tinged (reddish), bloody (dark red and similar to blood), purulent (pus), turbid (yellowish, but viscous or opalescent), and milky (whitish and thinner than pus). Approximately half of all bloody PFs are due to neoplasms. However, a malignant PE appears serous in 50% of cases, blood tinged in approximately 30% of cases, and bloody in only 10% of cases⁵. The bloody appearance of malignant PEs does not influence the yield of cytology⁶. Patients with trauma, parapneumonic effusions, post-cardiac injury syndrome, and pulmonary emboli may all exhibit a bloody PF. A watery appearance of PF is indicative of a transudate (see definition below), although the majority are serous (67%) and may even have a blood tinged (11%) or turbid (9%) color⁵.

A milky PF is characteristic of chylothorax (though half the cases do not have this appearance) and cholesterol PEs (chyliform PEs or pseudochylothorax). Chylothorax implies a chyle leak that is due to the disruption or blockage of the thoracic duct or its tributaries. Cholesterol PEs have no relationship with lymphatic vessels, but rather are connected with long-standing PEs with or without thickened pleural membranes. The most frequent causes of chylothorax are surgery, lymphoma, and cirrhosis, while cholesterol PEs are usually secondary to tuberculosis and, in fewer instances, to rheumatoid arthritis⁷. The presence of a purulent PF is diagnostic of empyema. Occasionally, the whitish appearance of chylous or chyliform PEs may be difficult to distinguish from the turbidity of empyema. However, after centrifugation, the supernatant in empyema is clear (cells and detritus produce the opalescence),

whereas it remains cloudy or milky in chylous or chyliform effusions, due to high lipid content. In addition, chylous PEs are inodorous, while anaerobic empyemas are foul-smelling in over half the cases. The whitish appearance of PF is rarely due to the leakage of an enteral nutrition formula from a central catheter into the pleural space (nutrithorax)⁸.

Unusual greenish PFs may be observed if the patient has a biliopleural fistula (bilothorax)⁹, while PFs usually appear black if the pleural space is infected by *Aspergillus niger* or *Rhizopus oryzae* or rarely black if the PE is malignant (metastatic melanoma and lung cancer) or there is a pancreaticopleural fistula or a chronic hemothorax¹⁰.

Finally, extremely viscous PFs can be seen in patients with mesothelioma (due to high concentrations of hyaluronic acid) or pleural metastases from mucinous adenocarcinomas.

Processing of PF

The extraction of 20-40 mL of PF is necessary for a complete analysis, which includes biochemical, cytological, and microbiological studies. PF should be divided into sterile tubes that contain an anticoagulant (heparin or EDTA).

To analyze pH, it has traditionally been taught that fluid should be collected under anaerobic conditions in a heparinized syringe, without being mixed with the local anesthetic used for the thoracentesis. Air falsely elevates pH, whereas local anesthetic falsely lowers the pH. More commonly, the sample is obtained using an unheparinized syringe and then transferred to a heparinized tube; a maneuver which does not cause a clinically relevant increase in pH¹¹. The measurement should ideally be obtained with a blood gas machine within 4 h following the extraction of PF, since time delay in processing also falsely elevates pH¹².

PF samples should be transported to the laboratory at room temperature immediately after collection, and processed on receipt. All PF chemistry analytes are stable up to 6 h at room temperature¹³. On storage at -20 °C, stability is maintained for days, with the exception of LDH.

Routine biochemical analysis of PF

Tests routinely performed on PF include cell count and differential, protein, LDH, glucose, pH, adenosine deaminase (ADA), cytology, and microbiological studies¹⁴. Some of them should only be ordered when

specific conditions are suspected, such as tuberculosis (ADA), mycobacterial cultures, nucleic acid amplification tests, pleural infection (bacterial cultures), or malignancy (cytology). Many diagnoses can be established by PF analysis in isolation (Table 1).

Proteins and LDH

The dichotomous classification of PF as a transudate or exudate simplifies diagnostic efforts in determining the cause of PEs. By definition, transudates result from a disequilibrium between the hydrostatic and oncotic forces in the pulmonary or systemic circulation over a structurally intact pleural surface, whereas exudates accumulate because of local factors affecting the pleura, such as increased capillary permeability and/or impaired lymphatic drainage resulting from many inflammatory and malignant causes. If the PE is a transudate, no additional diagnostic procedures are required because they are generally caused by HF (80%) or, to a lesser extent, liver cirrhosis (10%)¹⁵. In these cases, the administration of diuretics, sometimes in combination with a therapeutic thoracentesis, is sufficient to resolve the PE. In contrast, exudates require a more extensive diagnostic evaluation given that they may have numerous etiologies (Table 2)¹⁵. Of note, although previously thought to be transudates on occasion, PEs due to pulmonary embolism are invariably exudates when Light's criteria are used as the reference standard¹⁶.

In clinical practice, exudates are separated from transudates through the simultaneous determination of protein and LDH concentrations in blood and PF (Light's criteria)⁴ (Table 3). It is accepted that the time interval between collection of PF and blood samples can be up to 24 h instead of necessarily having to be formally paired; a circumstance which does not alter the fluid categorization as exudate or transudate in most cases¹⁷. The main limitation of Light's criteria is that, despite correctly identifying nearly all exudates (98%), their application misclassifies approximately 30% of PEs secondary to HF and 18% of hepatic hydrothorax as exudates¹⁸. This misclassification is particularly frequent in patients who have received diuretic treatment or have bloody PFs (> 10,000 erythrocytes/ μ L)¹⁹. If a patient presents clinically with HF, but the PF meets Light's criteria as an exudate (generally by a small margin), a calculation of the gradient (difference) between the serum and PF albumin or protein levels is recommended. If the albumin gradient is greater than 1.2 g/dL or the protein gradient surpasses 2.5 g/dL, which

Table 1. Diagnoses that can be established by pleural fluid analysis

Condition	Pleural fluid test
Malignancy	Positive cytology
Empyema	Pus or positive culture
Tuberculosis	Positive acid-fast bacilli, culture or NAAT
Fungal or parasite effusion	Positive culture or detection of parasites
Hemothorax	Pleural fluid hematocrit divided by serum hematocrit > 0.5
Chylothorax	Triglycerides > 110 mg/dL, chylomicrons present
Cholesterol effusion	Cholesterol crystals on polarized light microscopy
Pancreatic disease	Elevated amylase (pancreatic isoenzyme)
Esophageal rupture	pH < 7 and elevated salivary isoenzyme form of amylase
Lupus pleuritis	High levels of anti-dsDNA
Rheumatoid pleurisy	Tadpole cells in a background of amorphous debris
Peritoneal dialysis	Pleural fluid to serum glucose ratio > 1 ^a
Bilothorax	Pleural fluid to serum bilirubin ratio > 1
Urinorhorrax	Pleural fluid to serum creatinine ratio > 1 ^b with a pH < 7.30
Glycinorhorrax ^c	Transudate with a high concentration of glycine
Ventriculoperitoneal shunting or duropleural fistula	Presence of beta-2 transferrin

^aAnalysis for lactate D-isomer that is only found in the dialysate is an alternative diagnostic method.

^bDiagnostic if ratio > 1.7.

^cOccurs after urological procedures involving bladder irrigation with glycine-containing solutions.

dsDNA: double-stranded DNA; NAAT: nucleic acid amplification test.

occurs in about 80% of patients with these “false exudates,” it should be assumed that such a PF is actually a transudate²⁰. An alternative for the identification of HF-related PEs is the measurement of natriuretic peptides in PF specimens (see below).

When it is not possible to obtain a blood sample (an unusual circumstance), the combination of PF LDH > 67% of the normal upper limit of serum LDH and PF cholesterol > 55 mg/dL, using an “or” rule (wherein positivity of any of these tests represents a positive result) can be used as an alternative criterion for the

Table 2. Causes of pleural effusions

Transudates	Exudates
Common causes (90% overall) Heart failure (80%) Cirrhosis (10%)	Common causes (75% overall) Metastatic malignancy (40%) Parapneumonic (25%) Tuberculosis (10%) Mesothelioma (variable)
Less common causes (10% overall) Volume overload/hypervolemia Hypoalbuminemia Nephrotic syndrome Atelectasis Peritoneal dialysis Pulmonary arterial hypertension Trapped lung ^a Constrictive pericarditis Cerebrospinal fluid leak Extravascular migration of CVC Urinothorax ^a	Less common causes (25% overall) Post-surgery (cardiothoracic, abdominal) Pericardial diseases Trauma (hemothorax) Viral pleuritis Pulmonary embolism Drugs Abdominal diseases (e.g., pancreatitis) Autoimmune rheumatic diseases Benign asbestos pleural effusion Chylothorax ^b Uremic pleural effusion Gynecologic (OHS ^c , -endometriosis)

^aThey may also be exudates.^bThey may also be transudates (e.g., cirrhosis).^cA complication of ovulation induction with human chorionic gonadotropin (hCG) in the setting of *in vitro* fertilization.

CVC: central venous catheter; OHS: ovarian hyperstimulation syndrome.

Table 3. Light criteria for the differentiation of pleural exudates and transudates

A pleural effusion is classified as exudative if it meets one or more of the following conditions, while a transudate meets none

- A pleural fluid/serum protein ratio > 0.5
- A pleural fluid/serum LDH ratio > 0.6
- A pleural fluid LDH concentration > two-thirds (67%) of the normal upper limit for serum LDH

LDH: lactate dehydrogenase.

identification of exudates (94% sensitivity and 88% specificity)²¹⁻²³.

A transudate with a pleural protein concentration < 1 g/dL suggests one of the following diagnoses²⁴: (1) extravascular migration of a central venous catheter connected to a saline or glucose infusion system, (2) diaphragmatic fistula, (3) peritoneal dialysis, (4) urinothorax (i.e., urine in the pleural space, usually due to obstructive uropathy), (5) ventriculopleural shunt, or (6) migration of a ventriculoperitoneal shunt to the pleura. At the opposite extreme, 70% of patients with tuberculous PEs exhibit PF protein concentrations > 5 g/dL²⁵, while 40% of those with multiple myeloma have levels > 7 g/dL²⁶.

The LDH concentration in PF is not only useful for differentiating exudates from transudates but also for

consistently reflecting the degree of pleural inflammation, which is of particular interest in managing pleural infections. In the context of parapneumonic effusions, the finding of a pleural LDH concentration > 2000 U/L (approximately 4 times the upper limit of serum LDH levels) is an indicator of poor clinical outcome that merits consideration for the need of a chest drainage tube (likelihood ratio [LR] positive 3.4)²⁷. Moreover, in patients with lymphocytic exudates of unclear etiology, observing a decrease of LDH concentrations in subsequent thoracenteses reduces the probability of a malignant cause²⁸.

Leukocyte cell count

While the number of PF leukocytes is of limited value in the differential diagnosis of PE, parapneumonics encompasses more than 80% of counts higher than 10,000/ μ L²⁹. However, only one-third of parapneumonic PEs reach such high counts and, paradoxically, empyemas might exhibit scarce leukocyte cellularity due to neutrophil autolysis. On the other hand, the differential leukocyte count is of interest in all pleural exudates because the predominance of a specific cell type, either neutrophils or lymphocytes, can help to narrow down the differential diagnosis.

Neutrophilic PE

The delay in performing a thoracentesis from the onset of the pleural lesion determines the predominant type of leukocyte present in the PF. In acute inflammatory processes affecting the pleura, neutrophils predominate (> 50% of the total leukocytes). Although pneumonia is the most frequent cause of neutrophilic PEs, they can also be associated with abdominal diseases (e.g., pancreatitis or subphrenic abscess), pulmonary embolism, acute-phase viral and tuberculous infections, neoplasms, or asbestos exposure (benign asbestos PE). In particular, 10% of tuberculous PEs can be neutrophilic predominant²⁵, a fact which is associated with a higher yield of mycobacterial cultures of sputum (50% vs. 25%) and PF (50% vs. 10%) as compared to patients whose PF is predominantly lymphocytic³⁰.

Lymphocytic PE

In pleural transudates, lymphocyte counts greater than 50% (a finding that occurs in approximately 90% of the cases)³¹ have no diagnostic significance. However, pleural lymphocytosis is of great importance in the differential diagnosis of exudates, given that this finding usually indicates a chronic disease. The two main diagnoses to consider for exudates of lymphocytic predominance are cancer and tuberculosis, which together represent more than two-thirds of all cases. Thus, about 90% of tuberculous PEs and 80% of malignant PEs are lymphocytic³¹. Less frequent causes of lymphocyte-predominant exudates are post-cardiac surgery in the late phase, pulmonary embolism, chylothorax, rheumatoid pleuritis (chronic), yellow nail syndrome, sarcoidosis, and acute rejection of a lung transplant, among others. Notably, around 17% of parapneumonic PFs have a predominance of lymphocytes, which is probably influenced by the use of antibiotics before thoracentesis rather than the elapsed time between the start of symptoms and the pleural tap³².

Eosinophilic PE

PF eosinophilia is defined as having greater than 10% of eosinophils in the total PF cell count. In a recent systematic review that included 687 cases of eosinophilic PEs, the most frequent causes were neoplasms (26%), idiopathic (25%), parapneumonic (13%), blood or air in the pleural cavity (13%), tuberculosis (7%), and transudates (7%), among a variety of additional

causes³³. As the percentage of eosinophils in the PF increases (e.g., > 30%), the probability of a neoplasm decreases while the probability of idiopathic causes increases^{33,34}. For instance, neoplasms only explained 7% of the PEs with eosinophil counts > 32% in the aforementioned systematic review³³.

Conventionally, eosinophilic PEs are thought to be the result of air or blood in the pleural cavity (e.g., hemothorax, pneumothorax, or repeated thoracentesis). Yet, in some reports only 5% of PEs became eosinophilic after a second thoracentesis was performed³⁵.

Erythrocyte count

A concentration of 5000-10,000 erythrocytes/ μ L is required for PF to have a reddish appearance. Only 1 mL of blood in a moderate-sized PE is needed to produce a blood-tinged PF. The diagnostic value of reddish PEs is limited, as this appearance is present in about 15% of transudates and one-third of exudates³¹. A PF erythrocyte concentration greater than 10,000/ μ L is observed in > 75% of post-traumatic PEs, 56% of PEs secondary to pulmonary embolism, 40% of malignant PEs, and more than one-third of parapneumonic PEs³¹.

Of greater interest is the finding of a bloody PE, which corresponds to PF erythrocyte counts greater than 100,000/ μ L. A PE with these characteristics is suggestive of three possible diagnoses: cancer, trauma, or pulmonary embolism. These high red blood cell counts characterize, however, only around 10% of PEs secondary to cancer or pulmonary embolism^{16,31}. It has been traditionally thought that PF hematocrit should be determined for all purely bloody PEs. If it exceeds 50% of that in the peripheral blood, a hemothorax is present, the majority of which are caused by trauma. Nevertheless, a confident approximation of the PF hematocrit level can be obtained by dividing the erythrocyte count in the PE by 100,000.

pH

Under physiological conditions, PF pH is alkaline (7.60-7.66) due to the accumulation of bicarbonate in the pleural cavity. Pleural transudates have a pH that tends to vary between 7.40 and 7.55, while it ranges between 7.30 and 7.45 in the majority of exudates³⁶. However, a group of exudative PEs does exhibit pH values < 7.30, which represent a substantial accumulation of hydrogen ions in the pleural space. The etiology of these PEs, which tend to be associated with a low PF glucose content (< 60 mg/dL), includes²⁴: (1)

complicated parapneumonic PEs and empyemas, which are the most frequent cause of pleural acidosis, (2) malignant PEs, (3) tuberculous PEs, (4) chronic rheumatoid PEs, and (5) esophageal rupture (anaerobic empyema). Marked pleural acidosis ($\text{pH} < 7.20$) has been described in around 60%, 6%, 9%, 70%, and 100% of these etiologies, respectively^{25,27,31}. The only transudative PE (although this condition may also meet exudative criteria) presenting with a $\text{pH} < 7.30$ is urinothorax, which results from the migration of acidic urine from the capsule of an obstructed kidney to the pleural space through ipsilateral diaphragmatic defects³⁷.

PF pH is valuable for the management of patients with parapneumonic PEs. By definition, an uncomplicated parapneumonic PE resolves with antibiotic treatment alone. In contrast, a complicated parapneumonic PE requires a tube thoracostomy for resolution. Empyema, characterized by the presence of pus in the pleural cavity, is a type of complicated parapneumonic PE. The challenge for the clinician is to identify those parapneumonic PEs with a non-purulent appearance that requires a drainage tube. The pleural acidosis that can develop in this type of PEs results from an increase in metabolic activity in the pleural space (neutrophilic phagocytosis and bacterial metabolism) and the accumulation of products derived from glucose, CO_2 , and lactic acid. A parapneumonic non-purulent PE with a $\text{pH} < 7.15$ is unlikely to resolve without the insertion of a chest tube ($\text{LR} = 6.2$)²⁷. However, pH alone is not sensitive enough (66%) to identify complicated parapneumonic PEs²⁷, and about 10% of parapneumonic PEs with marked PF acidosis may even resolve with antibiotics alone^{27,38}. In a retrospective study of 641 parapneumonic PEs, findings increasing the probability of chest tube usage the most were²⁷: PEs occupying half or more of the hemithorax on a chest radiograph ($\text{LR} = 13.5$), PF $\text{pH} \leq 7.15$ ($\text{LR} = 6.2$), PF glucose ≤ 40 mg/dL ($\text{LR} = 5.6$), pus ($\text{LR} = 4.8$), positive PF cultures ($\text{LR} = 3.6$), and PF LDH > 2000 U/L ($\text{LR} = 3.4$). It should be noted that in a multiloculated parapneumonic PE, different locules of fluid can have different pH measurements³⁹. The measurement of pH or any assay, other than a Gram stain and culture, for a purulent PE is of no value and should not be conducted as empyemas virtually always require a tube thoracostomy or, if small, maximal aspiration when technically feasible.

PF acidosis in malignant PEs results from an extensive tumor infiltration of the pleura that inhibits the flow of glucose products from the pleural space. Thus, a low pH is typically found in advanced malignant PEs and

is associated with poorer response to pleurodesis⁴⁰ and survival⁴¹, and increased probability of requiring a definitive therapy for the pleural space (i.e., pleurodesis or indwelling pleural catheters)⁴².

Glucose

The significance of low PF glucose is similar to that of low pH and, at a cutoff point of < 40 – 60 mg/dL, it is a good substitute for pH to predict the need for pleural drainage in parapneumonic PEs ($\text{LR} = 5.6$)^{27,43}. Extremely low glucose levels are characteristic of complicated parapneumonic PEs, empyemas, paragonimiasis, and rheumatoid PEs (80% of rheumatoid PEs have this characteristic)⁴⁴. In pleural infections, there is an increased utilization of glucose by neutrophils and bacteria of PF, whereas in rheumatoid pleurisy the underlying mechanism is a decreased diffusion of glucose from blood to PF as a result of thickened pleural membranes. Approximately, 9% of malignant PEs³¹ and 25% of tuberculous pleuritis²⁵ also exhibit PF glucose levels lower than 60 mg/dL. On the other hand, when glucose values in PF exceed those in serum, two potential diagnoses should be considered²⁴: (1) extravascular migration of a central venous catheter through which a glucose solution is being instilled, and (2) migration of peritoneal dialysate fluid from the peritoneal space to the pleural cavity.

ADA

In geographical areas where tuberculosis has a moderate or high incidence, the determination of ADA levels in PF is routine for diagnosing a tuberculous PE and has replaced pleural needle biopsy⁴⁵. In areas with low disease burden, ADA is still of value in that a low PF level almost entirely rules out tuberculosis. According to recently published meta-analyses, pleural ADA values greater than 35–40 U/L have an approximate sensitivity of 92%, a specificity of 90%, a positive LR of 9, and a negative LR of 0.09 for establishing the tuberculous origin of a PE^{46,47}. Perhaps lower thresholds should be considered in older patients, since ADA concentrations decrease with age⁴⁸. The majority of false-positive ADA results have been attributed to parapneumonic PEs and empyemas that, in contrast to the majority of tuberculous PEs, predominantly contain neutrophils. ADA activity still remains elevated in tuberculous PEs with neutrophilic predominance³⁰. Approximately 16% of non-complicated parapneumonic PEs, 44% of complicated non-purulent parapneumonic PEs, 70% of

empyemas, and 10% of malignant PEs (a percentage that increases to 35% in diffuse large B-cell lymphomas) exhibit high pleural ADA values^{49,50}. When ADA concentrations are extremely elevated (> 250 U/L), an empyema (easily diagnosed due to the purulent appearance of the PF) or lymphoma, rather than tuberculosis, should be a strong consideration⁴⁹. Measurement of the isoenzyme ADA2 is not standardized and adds little to total ADA measurement in the majority of cases³⁰.

Optional analysis of PF

Natriuretic peptides

Natriuretic peptides, in particular brain natriuretic peptide (BNP) and N-terminal pro-BNP (NT-proBNP), are hormones synthesized by cardiomyocytes in response to parietal stress secondary to volume or pressure overload. In a meta-analysis of 12 studies that included 599 PEs due to HF and 1055 non-cardiac PEs, NT-proBNP levels in PF had a sensitivity of 94%, a specificity of 91%, a positive LR of 10.9, and a negative LR of 0.07 in identifying PEs with a cardiac origin⁵¹. Respective figures for blood NT-proBNP extracted from four studies were 92%, 88%, 7.8, and 0.10⁵¹. The most widely used cutoff point is 1500 pg/mL^{52,53}, though with the advent of more sensitive new generation tests this threshold needs to be reevaluated. NT-proBNP is a more useful biomarker of HF than BNP when measured in PF⁵³. In addition, NT-proBNP concentrations allow clinicians to correctly identify $> 80\%$ of those cardiac PEs misclassified as exudates by Light's criteria⁵³. Furthermore, natriuretic peptides are optimal for differentiating between cardiac and hepatic (cirrhosis) transudates, as the pathophysiological mechanisms underlying PF formation differ in the two processes. Detection of elevated PF levels of NT-proBNP in patients with established non-cardiac causes of PE (e.g., pneumonia, cancer, and pericardial disease) is currently a frequent situation which may reflect some degree of underlying decompensated cardiac disease contributing partially to PF development.

Tumor markers

A number of soluble-protein biomarkers have been studied in PF for the diagnosis of malignancy. For tumor markers to be diagnostically useful, they must be 100% specific (i.e., threshold levels should not be exceeded by any benign PEs) which inevitably results in low

diagnostic sensitivity. For example, one study evaluated the diagnostic accuracy of PF carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3) in 1575 patients with non-purulent exudates⁵⁴. Using cutoff values with 100% specificity, it was found that 41%, 40%, and 60% of malignant PEs had PF levels of CEA > 45 ng/mL, CA 15-3 > 77 IU/L, or either, respectively. More importantly, more than one-third of cytology-negative malignant PEs could be identified by at least one marker⁵⁴. However, the use of tumor markers does not eliminate the need for obtaining a definitive cytohistological diagnosis.

There is great interest in the discovery of biomarkers for malignant mesothelioma, a tumor which is difficult to diagnose. Soluble mesothelin is the most widely accepted biomarker. A meta-analysis on the diagnostic value of PF soluble mesothelin in 3359 patients (including 759 mesothelioma cases) estimated sensitivity, specificity, LR positive and LR negative at 68%, 91%, 7.8, and 0.35, respectively⁵⁵. The threshold value commonly employed is 20 nmoL/L. Although measuring PF mesothelin levels doubles the yield of conventional cytology to diagnose mesothelioma, the histological confirmation of this neoplasm is mandatory.

C-reactive protein (CRP)

The presence of a PF with a predominance of polymorphonuclear leukocytes and a CRP concentration > 45 mg/L strongly suggests that the cause of the PE is a bacterial infection⁵⁶. In a recent meta-analysis of 18 publications, the diagnostic performance of PF CRP for the identification of parapneumonic PEs was as follows: sensitivity 80%, specificity 82%, LR positive 4.51, and LR negative 0.25⁵⁷. Moreover, the diagnostic indexes of PF CRP in differentiating complicated from uncomplicated parapneumonic PEs, using a cutoff of around 100 mg/dL, were: sensitivity 65%, specificity 85%, LR positive 4.26, and LR negative 0.41⁵⁷.

Lipids

The PF analysis of a chylothorax shows an exudate in 85% of the cases, mostly by the protein but not the LDH criterion (referred to as protein-discordant exudate). A transudative chylothorax (15%) should lead to a suspicion of cirrhosis⁷. Lymphocytes are predominant in 80% of the cases, and PF triglyceride concentrations are greater than 110 mg/dL in 85%⁵⁸. A PF triglyceride concentration that is lower than 50 mg/dL strongly argues against the diagnosis of chylothorax ($< 3\%$ of cases). If pleural

Figure 1. A proposed algorithm for diagnosing pleural effusions based on pleural fluid analyses. Modified from Porcel 2010⁶⁸, with permission. ADA: adenosine deaminase; CRP: C-reactive protein; PF: pleural fluid; PF/S: pleural fluid to serum ratio; S-PF: gradient (difference) between the serum and pleural fluid; TB: tuberculosis.

triglyceride values are intermediate (50-110 mg/dL) and there are doubts regarding the existence of a chylothorax, the finding of chylomicrons in PF is definitive. However, if a lipoprotein analysis is not available and there are still concerns about the diagnosis, the administration of a high-fat meal will result in a dramatic change in the appearance and triglyceride content of the PF.

In contrast, cholesterol PEs are exudates, often lymphocyte-predominant (60%), with a PF cholesterol/triglyceride ratio > 1 (97%), cholesterol crystals visible under a polarized light microscope (90%), PF cholesterol concentrations > 200 mg/dL (75%), and the absence of chylomicrons⁵⁹.

Antinuclear antibodies (ANAs)

PEs can present clinically in one-third of patients with systemic lupus erythematosus (SLE). However,

PEs in this population may be caused not only by lupus pleuritis, but also by infections, pulmonary embolism, nephrotic syndrome, HF, or neoplasms. All patients with lupus pleuritis have positive serum ANAs, which raises questions about the need to measure their levels in PF for diagnostic purposes. However, the presence or absence of ANAs in PF may be of interest in the diagnosis of individuals with known SLE and a PE of uncertain etiology. Specifically, the absence of PF ANAs is a strong argument against lupus pleuritis and should motivate the search for alternative diagnoses⁶⁰. Conversely, the presence of ANAs (titers $\geq 1/160$), anti-dsDNA, or anti-ENA antibodies in PF strongly supports the diagnosis of lupus pleuritis (LR = 17)⁶⁰. Approximately 6% of SLE-unrelated PEs, primarily malignant PEs, show significant ANA titers in PF^{60,61}.

Amylase

The most frequent causes of increased PF amylase levels (values greater than the upper limits of normal for serum amylase) are malignancy (55%) and tuberculosis (13%)^{62,63}. This enzyme is elevated in approximately 15% and 10% of patients with malignant (more commonly in lung cancer) and tuberculous PEs, respectively. However, only when an acute or chronic (pancreatic pseudocyst) pancreatic disease or esophageal rupture is suspected is it justified to request a PF amylase measurement. PF amylase is of the salivary type in neoplasms and esophageal rupture, while the pancreatic isoenzyme predominates in pancreatic diseases.

Unstimulated interferon- γ and interleukin-27 (IL-27)

PF unstimulated interferon- γ and IL-27, both measured by enzyme-linked immunosorbent assays, have an accuracy similar to ADA for identifying tuberculous PEs. However, the latter is simpler, cheaper, and less time-consuming.

A meta-analysis of 67 publications, comprising 2657 tuberculous and 4496 non-tuberculous PEs, yielded summary estimates of sensitivity (93%), specificity (96%), LR positive (22.8), and LR negative (0.07) for PF interferon- γ in diagnosing tuberculosis⁶⁴. Discriminative threshold values have ranged from < 2 IU/mL to > 5 IU/mL. As is the case for ADA, empyemas and lymphomas can cause “false elevations” in the level of this cytokine.

In contrast to unstimulated IFN- γ , interferon- γ release assays (IGRA), which quantify IFN- γ released by T lymphocytes in response to stimulation by specific mycobacterial antigens, are generally thought to be of little value for diagnostic purposes⁶⁵. It has been suggested, however, that IGRA (e.g., T-SPOT) might be diagnostically helpful in patients with tuberculous PE whose ADA is lower than 40 U/L⁶⁶.

In a meta-analysis of 11 studies with 502 tuberculous and 952 non-tuberculous PEs, the pooled sensitivity, specificity, positive LR, and negative LR of PF IL-27 assays for tuberculosis were 95%, 91%, 13.9, and 0.07, respectively⁶⁷. Optimal cutoff values, however, need to be determined.

Conclusions

The routine biochemical analysis of PF obtained by thoracentesis should include total and differential cell

counts, protein, LDH, glucose, pH, and ADA. The differentiation of transudates and exudates using Light's criteria is the first diagnostic step for all PEs. If the PE is a transudate, HF is usually the cause and diuretics should be instituted. Not infrequently, however, diuretics remove more water than protein and LDH from the pleural space, resulting in a misclassification of the PE as an exudate. In these circumstances, a serum-effusion albumin (or protein) gradient should be obtained as a simple strategy to reveal the true transudative nature of the PE.

If the PE is an exudate, different analytical tests can narrow down the differential diagnosis. Specifically, an ADA concentration > 35 U/L generally indicates tuberculosis in PFs with a lymphocytic predominance, while a pH < 7.15 or a glucose concentration < 40 mg/dL allows for the identification of complicated parapneumonic PEs. Other PF assays are optional and facilitate the diagnosis of PEs which have uncertain etiologies. For example, natriuretic peptides (NT-proBNP) are good biomarkers of HF, triglycerides, and cholesterol facilitate the identification of lipid-rich PEs, elevated PF concentrations of CEA and/or CA 15-3 strongly suggest a diagnosis of malignancy, and a PF CRP concentration > 100 mg/L indicates a parapneumonic PE that will most likely require drainage. Figure 1 provides an algorithm for diagnosing PEs based on PF analysis⁶⁸.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

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