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REVIEW ARTICLE

Diagnostic aspects of pleural fluid analysis

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ABSTRACT

A sample of pleural fluid is obtained by the procedure of thoracentesis. Fifty to 100 ml of fluid are usually removed and sent for analysis. Specimen is examined for chemical content. Presence of microorganisms. Gram stain and culture to identify bacterial infections in Microbiology examined. Countries such India with high prevelance of tuberculous effusion have a high degree of specificity and sensitivity for ADA test which makes it an integral part of a diagnostic workup of lymphocyte rich exudative body fluids. In a scenario where the diagnosis of tuberculous pleural effusion is difficult because of the low sensitivity of various diagnostic tools, the reliability of the early diagnosis of pleural TB has been greatly improved by the use of biochemical markers such as ADA which has a high degree of specificity and sensitivity ,which makes it an integral part of a diagnostic workup of lymphocyte rich exudative body fluids.

KEY WORDS: Pleural effusion, Microorganisms, Mycobacterium tuberculosis, AdenosineDeaminase.

Introduction

Pleural effusion is diagnosed by chest x-ray. Chest films acquired in the lateral decubitus position (with the patient lying on her side) are more sensitive, and can pick up as little as 50 ml of fluid. Once accumulated fluid is more than 500 ml, there are usually detectable clinical signs in the patient, such as decreased movement of the chest on the affected side, dullness to percussion over the fluid, diminished breath sounds on the affected side, decreased vocal fremitus and resonance, pleural friction rub, and egophony. Once a pleural effusion is diagnosed, the cause must be determined. Pleural fluid is drawn out of the pleural space in a process called thoracentesis. A needle is inserted through the back of the chest wall into the pleural space. The fluid may then be evaluated for the following [1]:

- Chemical composition including protein, lactate dehydrogenase(LDH), albumin, amylase, pH and glucose
- Gram stain and culture to identify bacterial infections
- Cell count and differential
- Cytology to identify cancer cells, but may also identify some infective organisms
- Other tests as suggested by the clinical situation – lipids, fungal culture, viral culture, specific immunoglobulins

Analysis of pleural fluid

Normal pleural fluid has the following characteristics: clear ultrafiltrate of plasma, pH 7.60-7.64, protein content less than 2% (1-2g/dL), fewer than 1000 WBCs per cubic millimeter, glucose content similar to that of plasma, lactate dehydrogenase level less than

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50% of plasma and sodium, and potassium and calcium concentration similar to that of the interstial fluid [2]

Biochemistry

A sample of pleural fluid is obtained by the procedure ofthoracentesis. Fifty to 100 ml of fluid are usually removed and sent for analysis. Specimen is examined for chemical content (for example, protein, glucose, certain enzymes)

Glucose: A low pleural fluid glucose level is one less than 60 mg/dL. The differential diagnosis includes TB, malignancy, rheumatoid pleurisy, complicated parapneumonic effusion, empyema, hemothorax, paragonimiasis, Churg-Strauss syndrome, and occasionally, lupus pleuritis [2]. A very low serum glucose concentration (<30 mg/dL) indicates rheumatoid pleurisy or empyema, and a low serum glucose concentration (30-60 mg/dL) suggests malignant effusion, tuberculouspleuritis, or lupus pleuritis.

In addition to these tests, pleural fluid pH and glucose should be measured during the initial thoracentesis in most situations. Pleural fluid pH is highly correlated with pleural fluid glucose levels, and, for parapneumonic effusions, pleural fluid pH is more predictive of complicated effusions than is pleural fluid glucose. In parapneumonic effusions, pleural fluid pH less than 7.1-7.2 indicates the need for urgent drainage of the effusion, and pleural fluid pH more than 7.3 suggests that the effusion may be managed with systemic antibiotics alone [3]. In malignant effusions, pleural fluid pH less than 7.3 is associated with more extensive pleural involvement, higher yield on cytology, decreased success of pleurodesis, and shorter survival times [3].Examine the serum-to–pleural fluid albumin gradient (serum level minus pleural fluid level). A gradient of less than 1.2 g/dL indicates exudative effusion, one greater than 1.2 g/dL, transudative effusion [4].

Amylase: An elevated pleural fluid amylase level is one greater than the upper limit for serum levels or one that results in a pleural fluid–to–serum amylase ratio of more than one. Pleural fluid amylase levels can be elevated in acute pancreatitis, pancreatic pseudocyst, esophageal rupture, malignancy, and ruptured ectopic pregnancy. Among these, pancreatic pseudocyst as the highest amylase levels (frequently > 100000 IU/L) Determination of the amylase isoenzyme level is useful in distinguishing effusions caused by pancreatic disease (pancreatic isoamylase) from effusion caused by esophageal rupture or nonpancreaticcarcinoma (salivary isoenzyme) [2].

LDH: The LDH level is an indicator of the degree of pleural inflammation. The higher the value, the more inflamed the pleural surface. High concentrations (>1000 IU/L) occur with complicated parapneumonic effusions and paragonimiasis. Rheumatoid pleuritis is associated with moderately high (>700 IU/L) LDH levels [2].

Microbiology

Presence of microorganisms. Gram stain and culture to identify bacterial infections are studied here. In the appropriate clinical setting the following may be helpful: Gram staining, acid-fast bacilli staining, fungal (KOH) staining, culturing and sensitivity testing for aerobic and anaerobic organisms and fungi. Immunologic studies of the pleural fluid, such as evaluation of the rheumatoid factor titer and antinuclear antibody level, are the most useful tests for suspected rheumatoid and lupus pleuritis, respectively. Additional tests of the pleural fluid, such as amylase isoenzyme determination or immunohistochemistry, can be performed. Adenosine deaminase (ADA) is a cytosolic enzyme of the purine salvage pathway and catalyzes the hydrolytic and irreversible deamination of 2 - deoxyadenosine

in to 2 deoxyinosine and of adenosine into inosine a critical step in the production of essential metabolites for the synthesis of nucleic acid [5]. This enzyme is widely distributed in human tissues. Further metabolisation of these deaminated nucleosides leads to hypoxanthine, which can be either transformed into uric acid by xanthine oxidase salvaged into mononucletides by the action of hypoxanthine- guanine phosphoribosyl – transeferense

Sources, Secretion, Isoenzymes, and Origin

In humans the highest ADA activity is found in thymus and other lymphoid tissues (~800 IU/mg), the lowest in erythrocytes (~1 IU/mg) [6]. Among nonlymphoid tissues in humans, relatively high levels of ADA are found in the villi of epithelial cells lining the duodenum; levels are lower in other portions of the gastrointestinal tract. The tissues with most consistent high activity are duodenum and spleen. This enzyme is widely distributed in human tissues. The activity of ADA is ten times greater in lymphocytes than in erythrocytes and in relation to lymphocytes, it is greater in T-lymphocytes than B-lymphocytes and varies during T-cell differentiation. ADA is significantly increased in immature (or) un differentiated states [7].Therefore ADA is considered a marker of cell mediated immunity [7]. Two ADA isoenzymes are known: ADA-1 and ADA-2. Human tissue extracts contained ADA-1 predominantly and ADA-2 was the main component of serum ADA. Therefore, ADA activity measured in serum reflects ADA 2 activity. It was found that the ADA2: ADA ratio decreased in acute hepatitis, but increased in chronic active hepatitis and liver cirrhosis [8]. This enzyme may have its origin in lymphocytes.

Tuberculosis

The cause of tuberculosis, Mycobacterium tuberculosis (MTB), is a slow-growing aerobic bacterium that divides every 16 to 20 hours. This is extremely slow compared to other bacteria, which tend to have division times measured in minutes (among the fastest growing bacteria is a strain of E. coli that can divide roughly every 20 minutes). It is not classified as either Gram-positive or Gram-negative because it does not have the chemical characteristics of either, although it contains peptidoglycan in the cell wall. It is a small rod-like bacillus which can withstand weak disinfectants and can survive in a dry state for weeks but, spontaneously, can only grow within a host organism [9]. There are three major types of tubercle bacilli that affect humans. The human type (Mycobacterium tuberculosis), first identified in 1882 by Robert Koch, is spread by people themselves. It is the most common one. The bovine type (M. bovis) is spread by infected cattle but is no longer a threat in areas where pasteurization of milk and the health of cattle are strictly supervised. The avian type (M. avis) is carried by infected birds but can occur in humans. The tubercle bacillus can live for a considerable period of time in air or dust. The most common means of acquiring the disease is by inhalation of respiratory droplets [10]. Nontuberculous mycobacteria (NTM) are other mycobacteria (besides M. leprae which causes leprosy) which may cause pulmonary disease resembling TB, lymphadenitis, skin disease, or disseminated disease. These include Mycobacterium avium, M.kansasii, and others[9].

ADA is an enzyme found in most cells [11], which has two types of isoenzymes [12] ADA1 and ADA2. ADA is involved in proliferation and differentiation of lymphocytes, especially T-lymphocytes. They release ADA when stimulated in the presence of live intracellular microorganisms for this reason, ADA has been

looked on as a marker of the activation of T-lyphocytes[11]. ADA1 probably originates from lymphocytes and neutrophils [13].

Diagnosing tuberculous pleural effusion (pTB) is often difficult because the culturing of tubercle bacilli results in a negative test in the majority of cases[14].Adenosine deaminase activity is considered in many clinics to be a valuable biochemical test of this pathology. Determination of ADA and its isoenzymes can help to differentiate the causes of pleural effusion. The adenosine deaminase level in tuberculous pleural effusions was higher than in non-tuberculous pleural effusions [14].The elevation of ADA activity in tuberculous pleural effusions does not always reflect the activation of cell-mediated immunity [15]. Increased ADA2 activity is a striking marker of tuberculous effusionsthan total ADA activity, which has similar sensitivity and a little better specificity compared with pleural ADA, which is originated from the only known sources of monocytes and macrophages.Among effusions with high total ADA the 2'-deoxyadenosine deaminase/ADA activity ratio differentiates tuberculous effusions from empyemas and parapneumonic effusions [16] , but fails to discriminate well between tuberculous and neoplastic effusions[11]. ADA1 (both ADA1m and ADA1c isoenzymes) was significantly elevated in parapneumonic effusions [17-18]. Determinations of the activity level of the ADA1 and ADA2 isoenzymes provide no diagnostic advantage over total ADA activity [16]. Tuberculosis effusion had a much higher ADA activity than cancerous effusion, and high ADA activity mainly originated from the increase in ADA2 activity. Further, total ADA activity in tuberculous pleural decreased after antituberculosis treatment, because of the decreased in ADA2 activity.

Discussion and Conclusion

Countries such India with high prevelance of tuberculous effusion have a high degree of specificity and sensitivity for ADA test which makes it an integral part of a diagnostic workup of lymphocyte rich exudative body fluids [19]. The effusion is usually a result of delayed hyper sensitivity to protein of the mycobacteria and the actual bacterial load in the pleural space is often low as a result pleural biopsy and pleural fluid culture findings are often negative [20]. ADA has gained increasing popularity as a diagnostic test for tuberculouspleurities since 1978, especially in countries where the prevalence of TB is high. ADA is an enzyme involved in purine catabolism and is found in many cells but particularly in lymphocytes and is related to lymphocytic differentiation and proliferation and activity increases during antigenic response of lymphocytes [21].

Pleural effusion ADA has been shown to be a useful biochemical marker of TB effusion and provides reliable basis for treatment decision particularly in areas where the disease is prevalent such as in India [22]. However the elevation may be limited in early stages of disease and it has been shown recently that ADA levels in non tuberculosis lymphocytic pleural effusion seldom exceed the cutoff set for TB effusion. Countries such as India with a high prevalence of TB pleural effusion have a high degree of specificity and sensitivity for the ADA test, which makes it an integral part of diagnostic workup of lymphocyte rich exudative body fluids [20]. With this we can safely say that ADA activity detection in pleural fluid alone is very useful, cost effective and reliable test to detect or to rule out tuberculosis etiology of pleural effusions in low income countries with high prevalence of TB. When a clinician uses a laboratory test to help establish a diagnosis, knowing the test sensitivity and specificity can assist with proper interpretation. The sensitivity of an assay is the fraction of those subjects with a specific disease that the assay correctly predicts. The specificity is the fraction of those individuals

without the disease that the assay correctly predicts.

Identifying tuberculouspleuritis is a common clinical problem with multiple pitfalls. One third of patients with this condition can have a negative tuberculi skin test. Pleural fluid culture results can be positive in < 25 % of cases.ADA analysis in pleural fluid is a relatively simple and inexpensive colorimetric test, with several studies reporting that an elevated pleural fluid ADA level predicts tuberculouspleuritis with a sensitivity of 90-100% and specificity of 89-100% when the Giusti method is use [23-27]. In a scenario where the diagnosis of tuberculous pleural effusion is difficult because of the low sensitivity of various diagnostic tools, the reliability of the early diagnosis of pleural TB has been greatly improved by the use of biochemical markers such as ADA which has a high degree of specificity and sensitivity ,which makes it an integral part of a diagnostic workup of lymphocyte rich exudative body fluids.

References

1. Light RW. 2001. Pleural Diseases, 4thed, New York: Lippincott Williams & Wilkins. ISBN 0-7817-2777-4.
2. Fredrick M Abrahamian, Michael S Beeson, MD, MBA,Francisco Talavera, PharmD, PhD, Paul Blackburn, DO, John Halamka, MD, andRobert E O'Connor, MD, MPH. 2005.Pleural Effusion; Editor(s).; 6: 27.
3. Jeffrey Rubins, MD, Stephen P Peters MD, PhD,Francisco Talavera, PharmD, PhD, Harold L Manning, MD, Timothy D Rice, MD, ZabMosenifar, MD.2005. Pleural Effusion.4: 21.
4. Tarn AC and Lapworth R. 2001. Biochemical analysis of pleural fluid: What should we measure? Annals of Clinical Biochemistry. 38:311-322.
5. Carrenra J, Porras JA, Vidal F, Pinto B, Richart C.1995. Evaluation of Serem adenosine deaminase of a prognostic marker in the treatment of human immunodeficiency virus infection with Zidovudine. Rev. Clin. Esp.2:195(2).
6. Harshfield M.S.: Mitchell B.S. 1995. Immunodeficiency disease caused by adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency. In: Scriver, C.R.; Beaudet, A.L.: Sly, W.S.; Valle, D. (eds.).The Metabolic and Molecular Basses of Inherited Disease. 7:1725-1768.
7. Pras M.A, Gakis C, Brundoni M, Andreoni G.1978. Adenosine deaminase activity in pleural effusions : an aid to differential diagnosis. Br Med J.2:1751-1752
8. Kobayashi F, Ikeda T, Marumo F, Sato C. 1993. Adenosine deaminaseisoenzymes in liver disease. Am J Gastroenterol. 88:266-271.
9. Core Curricuclum on Tuberculosis: What the Clinician Should Know, 4th edition (2000). Division of Tuberculosis Elimination,updated Aug 2003.
10. R. Dubos, The White Plague.1955. and S. A. Waksman.1964. The Conquest of Tuberculosis.
11. Valdes L, San Jose E, Alvarez D, Valle JM, and Inase N, Tominaga S, Yasui M, Tsukada Y, Oukouchi M, Miura H.2001. Adenosine deaminase 2 in the diagnosis of tuberculouspleuritis. Chest.120: 356-361.
12. Gorguner M, Cerci M, Gorguner I.2005. Determination of adenosine deaminase activity and its isoenzymes for diagnosis of pleural effusions. JAMA .8:293(22):2776-84.
13. Ungerer JP, Oosthuizen HM, Retief JH, Bissbort SH. 2005.Significance of adenosine deaminase activity and its isoenzymes in tuberculous effusions. 15; 171(6):554-62.
14. Morimoto T, Takanashi S, Hasegawa Y, Fujimoto K, Okudera K, Hayashi A, Taima K, Okumura K. 2005.Level of antibodies against mycobacterial glycolipid in the effusion for diagnosis of tuberculous pleural effusion. Gene Ther. 2:03
15. Shibagaki T, Hasegawa Y, Saito H, YamoriS,Shimokata K.2000. Adenosine deaminaseisozymes in tuberculous pleural effusion. . Thorax. 55:887-901
16. Andreasyan NA, Hairapetian HL, Sargisova YG,Mardanyan SS, Badalyan LT, KhanoyanAS. Clinical Biochemistry. 2001. Activity of adenosine deaminase and its isoforms in pleural fluid in tuberculouspleuritis. Clinical Biochemistry. 38:311-322
17. Gorguner M, Cerci M, Gorguner I.2005. Determination of adenosine deaminase activity and its isoenzymes for diagnosis of pleural effusions. JAMA .8:293(22):2776-84.

18. MountainsBeyondMountains: The Quest of Dr.Paul Farmer. 2000. A Man Who Would Cure the World. Tracy Kidder, Random House. ISBN 0-8129-7301-1.A nonfiction account of treation TB in Haiti, Peru and elsewhere.

19. Yash P. Kataria MD, FCCP, ImtiazKhurshid, MBBS, Greenville, NC. 2000.CHEST 8:120.

20. Y. C. Gray Lee, Jeffrey T. Rogers, R.Michael Rodriguez, Kent D. Miller MD and Richard W. Light.2001. Adenosine Deaminase levels in Nontuberculous Lymphocytic Pleuarl Effusions. Chest.120: 356-361.

21. David Jiménez, MD; GemaDíaz, MD and Esteban Pérez-Rodríguez, MD.2002. Diagnosis of Pleural Tuberculosis;Chest.121:1005.

22. Anete Trajman. 2006. Diagnosing Pleural Tuberculosis; www.chestjornal.org by on August 14.

23. Valdes, L, Alvarez, D, San Jose, E.1998. Tuberculouspleuritis; a study of 254 patients. Arch intern Med. 158:2017-2021 .

24. Villena, V, Navarro-Gonzalvez, JA, Garcia – Benayas, C.1996. Rapid automated determination of adenosine deaminase and lysozyme for differentiating tuberculous and non-tuberculous pleural effusions clinchem. 42:218-221 [abstract]

25. Burgess, LJ, Marti Z, FJ, LeRoux, I. 1995.Use of adenosine deaminase as a diagnostic tools pleurisy EurRespir J.9, 747, Thorax 50: 672 – 674 [abstract]

26. Banales, JL, Pineda, PR, Fitzgerald, JM. 1991. Adenosine deaminase in the diagnosis of tuberculous pleural effusions; a report of 218 patients and review of the literature. Chest. 99:355-357 [abstract]

27. Valdes, L, Alvarez, D, San Jose, E.1995. Value of adenosine deaminase in the diagnosis of tuberculous pleural effusion in young patients in a region of high prevalence of tuberculousis. thorax 50: 600 – 603. [abstract]