Original article

Indian Journal of Pathology: Research and Practice

Role of Pleural Fluid ADA & the Combination Tests in Tubercular Pleural Effusion

Akshata J.S.*

Manjunath K.N.**

*M.D, Asst. Prof. Dept. of Pulmonary Medicine, SDSTBRC & RGICD, Bengaluru, Karnataka, INDIA

**M.S., D.N.B., M.ch, Asst. Prof. Dept. of Plastic & Reconstructive Surgery, MSRMC, Bengaluru, Karnataka, INDIA

Accepted on 11.07.2012

Abstract

Tuberculosis is an oldest and common infectious disease. Detected as far back as 10,000 BC it still remains a major public health problem world wide The use of biological markers in the diagnosis of tuberculous pleural effusion (TPE) is a breakthrough Adenosine deaminase(ADA) has been proposed to be a useful surrogate marker for TB in pleural, peritoneal and pericardial fluids. Although sensitive it is not a specific diagnostic tool, but combined with other tests the sensitivity can be increased. Materials&methods; 100 patients of suspected TPE were studied .Sensitivity and specificity of pleural fluid ADA alone and in combination with Mantoux test, pleural fluid cytology studied. Conclusion; pleural fluid ADA (>63U/L) alone was essentially diagnostic of TPE, between 40-63U/L it is highly suspicious of TPE along with >50% lymphocytes in pleural fluid confirms the diagnosis.

Keywords: Tubercular pleural effusion; ADA; Mantoux test; Pleural fluid cytology.

Introduction

Tuberculosis is an ancient disease which continues to haunt us even into the new millennium. It is one of the oldest and commonest infectious diseases. Detected as far back as 10,000 BC it still remains a major public health problem world wide.

Tuberculosis commonly affects the lung but extrapulmonary tuberculosis is not uncommon. Tuberculous pleuritis is one of the two most common extrapulmonary manifestations of TB, the other being lymphatic involvement. Tuberculosis remains the most common cause of pleural effusion in countries where it is highly endemic and with the increase of HIV infection, the incidence of pleural effusion in tuberculosis patients is on rise. Tuberculous pleural effusion (TPE) traditionally affected adolescents and young adults between 28-40 yrs.

Diagnosis of pulmonary TB is confirmed mainly by sputum examination for AFB. However, the diagnosis of tuberculous pleural effusion requires special investigations like pleural fluid biochemistry and cytology,as Pleural fluid staining for AFB is usually negative. The use of biological markers in the diagnosis of tuberculous pleural effusion is a breakthrough Adenosine deaminase (ADA) has been proposed to be a useful surrogate marker for TB in pleural, peritoneal and pericardial fluids. Many studies in different

Corresponding Author:

Dr Manujunath K.N., M.S., D.N.B., M.ch, Asst. Prof. Dept. of Plastic & Reconstructive Surgery, MSRMC, Bengaluru, Karnataka, INDIA. Mob: 09535971056

E-mail : drknmanjunath@gmail.com

parts of the world have confirmed high sensitivity (77-100%) and specificity (86%) at a predetermined cut off value for early diagnosis of TPE. Although sensitive it is not a specific diagnostic tool, but combined with other tests the sensitivity can be increased.

Hence this study is being undertaken to evaluate the role of combined use of pleural fluid ADA, cytology and Mantoux test (MT) in diagnosis of TPE.

Materials & Methods

100 patients both out patients and inpatients suspected to be a tuberculosis were studied. A detailed history was taken and a thorough physical examination was done. Following investigations was performed on all:

1. Chest radiograph, PA view (lateral or decubitus)- site noted and size of pleural effusion was graded (Minimal-fluid<1/3 rd of hemithorax, Moderate-1/3 rd - 2/3rd of hemithorax, Massive- >2/3 rd of the hemithorax)
2. Where ever indicated three samples sputum smear for acid fast bacilli by Ziehl-Neelson staining.
3. Mantoux test using 5TU (intermediate strength)
4. Pleuralfluid

ADA level estimated by spectrophotometer (Galanti and Guisti)

- Cytology: totalcount, differential count, any atypical cells.
- Pleura fluid for AFB culture using Lowenstein Jensen medium whenever feasible.

All the cases were finally grouped as tuberculous or non tuberculous.

Tuberculous

Absolute criteria:

1. Pleural fluid/sputum smear revealed Mycobacterium tuberculosis on Ziehl-Neelsen staining.
2. Pleural fluid/pleural biopsy specimen/ sputum grew Mycobacterium tuberculosis on Lowenstein-Jensen culture.
3. Histopathological evidence suggestive of tuberculosis in pleural biopsy specimen/ palpable lymph nodes or other tissue sites.

Suggestive criteria

1. History of fever, pleuritic chest pain, malaise, anorexia, weight loss and features of toxemia consistent with the clinical presentation of TPE.
2. Good response to antituberculosis treatment.

TPE was diagnosed if any one of the absolute criteria or both of the suggestive criteria were present.

Non tuberculous

1. Malignant pleural effusion- Cytological or histopathologically proven cases.
2. Parapneumonic-with suggestive clinical symptoms and good response to antibiotic course.
3. Transudates due to congestive cardiac failure and relieved with diuretics.

Patients under tuberculous group were put on anti tubercular drugs either DOTS CAT I or III accordingly. Follow-up was done at 15 days, 1, 2 months and clinical and radiological improvement noted. Those with suspected parapneumonic were put on antibiotics.

Results

In our study, Tuberculosis was the most common cause of pleural effusions encountered (74%), followed by malignancy (18%). However, malignant effusion was the most common among non tuberculous cases. (table 1)

74.32% of the tuberculous pleural effusion cases were observed below the age of 50 years.

Table 1: Distribution of Etiology of Pleural Effusion in the Study Population

Parameters	No. of Cases	%
Tuberculous (TB)	74	74
Non Tuberculous (NTB)	26	26
Malignancy (M)	18	18
Synpneumonic (SP)	6	6
Transudate (T)	2	2

Table 2: Clinical Symptoms in Study Population

Symptoms	TB		NTB	
	No.	%	No.	%
Cough Dry	37	50.00%	5	19.23%
Productive	35	47.30%	6	23.08%
Chest pain	47	63.51%	15	57.69%
Dyspnea	30	40.54%	20	76.92%
Fever	64	86.49%	14	53.85%
Anorexia	58	78.38%	18	69.23%
Weight loss	17	22.97%	13	50.00%
Hemoptysis	9	12.16%	4	15.38%

Table 3: Result of Pleural Fluid ADA at 63 U/L Cutoff Values

	TB	NTB	Total
Positive	50	0	50
Negative	24	26	50
Total	74	26	100

Sensitivity	67.57%
Specificity	100%
Positive Predictive Value	100%
Negative Predictive Value -	52%
False Negatives	0%
False Positives	32.43%

Table 4: Result of Combination of ADA and Cytology in Diagnosis of Tuberculous Pleural Effusion (ADA > 40U/L and Cytology >50% Lymphocytes)

	TB	NTB	Total
Positive	62	1	63
Negative	12	25	37
Total	74	26	100

Sensitivity	83.78%
Specificity	96.15%
Positive Predictive Value -	98.41%
Negative Predictive Value -	67.57%

Table 5: Result of combining cytology and Mantoux test in Diagnosing TPE (Lymphocytes >50% and Mantoux test positive)

	TB	NTB	Total
Positive	35	3	38
Negative	39	23	62
Total	74	26	100

Sensitivity	47.30%
Specificity-	88.46%
Positive Predictive Value -	92.11%
Negative Predictive Value -	37.10%

Table 6: Result of Combining ADA and Mantoux test in Diagnosing TPE (ADA >40 U/L and Mantoux test positive)

	TB	NTB	Total
Positive	35	2	37
Negative	39	24	63
Total	74	26	100

Sensitivity	47.30%
Specificity	92.31%
Positive Predictive Value -	94.59%
Negative Predictive Value -	38.10%

Table 7: Combined Role of ADA, Cytology and Mantoux test in the diagnosis of TPE (ADA > 40 U/L + Cytology >50% lymphocytes + Mantoux test – positive)

	TB	NTB	Total
Positive	34	1	35
Negative	40	25	65
Total	74	26	100

Sensitivity	45.95%
Specificity	96.15%
Positive Predictive Value	97.14%
Negative Predictive Value	38.46%

Table 8: Comparison of Various Tests and Their Combinations in the Diagnosis of TPE

Test/Criteria	Sensitivity	Specificity	PPV*	NPV**
ADA >40 U/L	89.18%	84.61%	94.29%	73.33%
ADA >63 U/L	67.57%	100%	100%	52%
Mantoux test	51.43%	76.92%	85.71%	37.03%
ADA >40 U/L+>50% lymphocytes	83.78%	96.15%	98.41%	67.57%
ADA >40 U/L+ positive Mantoux test	47.30%	92.31%	94.59%	38.10%
MT positive + >50% lymphocytes	47.30%	88.46%	92.11%	37.10%
ADA >40 U/L, positive Mantoux test & >50% lymphocytes	45.95%	96.15%	97.14%	38.46%

*PPV- positive predictive value; **NPV- negative predictive value

Majority (44.59%) were found in the age group of 21-30 yrs. Mean age was 39.45yrs (Range 15-75).Majority of cases among malignant etiology (66.67%) were found above the age of 50 years with a mean age of 58 years. Male preponderance was observed in the study both among tuberculous and non tuberculous group. In both the groups' majority of patients hailed from a rural locality. In the study population, 65% were either smokers or ex-smokers. 24% were either alcoholic or ex-alcoholics. 27% of them were indulges in both smoking and alcoholism. Among the tuberculous group cough was the commonest symptom (97.3%) followed by fever and anorexia (86.49%), chest pain (63.51%).In malignant cases, dyspnea was the most common (76.92%) followed by chest pain (57.69%) and weight loss (50%).Hemoptysis was little more common in malignant cases (15.38%) than the Tuberculosis group (12.16%). (table 2)

18 patients (24.32%) of tuberculous pleural effusion presented with associated parenchymal lesions in the chest x-ray.Majority (20.27%) showed minimal infiltration shadows. Consolidation was the major associated finding (34.62%) in the non-tuberculous group. Majority (58.11%) of the tuberculous pleural effusion were of moderate size occupying 1/3rd to 2/3rd of the hemi thorax. All the 7 (26.92%) massive pleural effusion among non-tuberculous group were proved to be of malignant etiology. Among the tuberculous pleural effusion cases 51.42% gave positive result while only 23.07% were Mantoux test positive among the non tuberculous group. The mean total WBC count in the tuberculous group was 1761.82cells/ mm^{3}of which majority (73.32%) were lymphocytes. Lymphocyte predominance was also seen in malignant pleural effusion. All synpneumonic effusions showed neutrophil predominance (77.17%). 70 of 74 i.e. 94.59% of tuberculous cases and 14 of 18 i.e. 77.78% of malignant cases showed lymphocyte predominance (>50%) in pleural fluid cytology. A typical cells suggestive of malignant origin were found in 17 of 18 (94.4%) cases of malignant pleural effusion.

The pleural fluid ADA level was in the range of (13-157 U/L) among the tuberculous cases with a mean value of 76.29±30.91 U/L.The mean value of ADA among non tuberculous cases was 26.77±19.09 U/L. A definitive diagnosis of tuberculous pleural effusion can be made at ADA level above 63U/L, as Specificity of 100% was obtained at a cut off value of 63U/L, but the sensitivity of the test dropped to a low 67.57%. (table 3)

Results of various tests, and the individual sensitivity and specificity are given below. The combination of tests also compared with each other, after all tests Pleural fluid ADA with sensitivity of 89.18% and specificity of 84.61% emerged as a single best diagnostic test for tuberculous pleural effusion with sensitivity of 89.18% and specificity 84.61% at 40 U/L cut-off level.(table.4,5,6,7,).

Combined use of pleural fluid ADA and lymphocyte predominance was found to increase the specificity to 96.14% with 98.41% positive predictive value.(table.8)

Discussion

Definitive diagnosis of TPE depends on the demonstration of tubercle bacilli in the sputum, pleural fluid or pleural biopsy specimen or the demonstration of granulomas in the pleural biopsy.Smears of pleural fluid for mycobacteria are usually negative and pleural fluid cultures are positive for Mycobacteria in fewer than 40%[1,2]. BACTEC culture provides higher yield and faster results (18 days; 3-40 days) when compared to conventional cultures (33.5days; 21-48 days) [3]. But, All these above tests are either time consuming or invasive. Hence was the need for a simple and sensitive test for the diagnosis of TPE.

Mantoux test

Ocana et al[4] of Spain in their study of 221 cases of pleural and peritoneal fluids of which 46 were TPE noted that 33(68.7%) were tuberculin positive.Epstein et al[5], found that of 23 TPE cases 11 of the 13 patients (73%) for whom MT result was avaible were positive. Seibert et al[6] from Alabama studied 70 patients of TPE and found that 93% were MT positive. The positivity of MT was 95% in patients associated with parenchymal lesions also and 91% in patients with isolated pleural effusion. Pedro et al[2] of Spain conducted a study on 254 patients of TPE and found that tuberculin skin test was positive in only 109(66.5%) of the cases. Maria Virjinia et al[7] in their study of 140 cases of pleural effusion noted that MT was positive in 50% confirmed and 70.6% probable TPE cases. It was also found that 35% of the patients with effusion due to non tuberculous etiology also gave a positive response to MT giving it a sensitivity of 50% and a specificity of 64.4% in the diagnosis of TPE.

All these studies used only MT as diagnostic tool and found that it had low sensitivity and specificity

Pleural fluid cytology

Pleural fluid analysis is useful in the diagnosis of tuberculous pleuritis. Grossly it's a clear, straw colored odourless non viscid fluid commonly and haemorrhagic at times. Invariably an exudate with protien level >5g/dl frequently with raised LDH and decreased glucose level [8]. If eosinophils are found in pleural fluid in significant numbers (>10%) one can virtually exclude the diagnosis of TPE, unless patient has a pneumothorax or has had a previous aspiration [8]. Studies have confirmed that patients with TPE rarely contain >5% mesothelial cells in their pleural fluid [910]. Unfortunately, the absence of mesothelial cells is not diagnostic of TB. Any condition in which the pleural surfaces are extensively involved by an inflammatory process, mesothelial cells are not found in the pleural fluid.

Pettersson et al[11] studied the diagnostic value of total and differential WBC counts in 140 pleural effusion patients. Total counts were higher in exudates. A lymphocyte predominance (>80% of all leukocytes) was seen in 29 of the 31 (93.5%) of TPE cases. However 18 of 24 (75%) fluids of malignant origin also showed lymphocyte predominance. None of the TPE cases had >10% eosinophils. Hence they concluded that lymphocyte predominance was not characteristic particularly of TPE but also in those of malignant etiology. So it is not disease specific. Epstein et al[5] in their study found that of 23 TPE cases only 54% had lymphocyte predominence which was much less as compared to previous studies.

Pedra et al[2] studied 254 cases of TPE and found that about 93% had >50% lymphocytes in their differential counts with mean value of 77%.In a much recent study at Peshawar, Anwar et al[12] performed pleural biopsy using Abraham's needle to establish the etiology of 74 patients with lymphocyte predominant exudative pleural effusion. In 71.1% of cases in which a definite histopathological diagnosis was established. 52.7% had tuberculous and 18.9% had a malignant etiology to their pleural effusions. They concluded that tuberculosis followed by malignancy were the most common causes of lymphocytic exudative pleural effusion.

Pleural fuild ADA

ADA level is ten times higher in lymphocytes than in erythrocytes and particularly in T-lymphocytes with variations according to cellular differentiation. ADA plays a part in the differentiation of lymphoid cells and the maturation of monocytes to macrophages [13]. For this reason ADA has been looked on as a marker of cell mediated immunity which encompasses the delayed hypersensitivity reaction.

Ocana et al[2,4] measured ADA in 221 patients of pleural and peritoneal effusions. All patients with a pleural fluid ADA level of >70 U/L had tuberculous etiology and no patient with ADA <40 U/L had TPE. In D.K. Gupta et al[14] study of 53 cases of pleural effusion found that the mean ADA level in those of tuberculous origin was 77.7 U/L in contrast to 14.5 U/L in cases of malignant effusion.Rajendra Prasad et al[15] studied 47 cases of pleural effusion and found that at 30 U/L cut off value the sensitivity and specificity of ADA for diagnosing TPE was 100%.L Valdes et al[40] in their study of 405 patients of pleural effusion of various etiology found the mean value of ADA in cases of TPE 107.5 U/L. Also a sensitivity of 100% and specificity of 95% at 47 U/L cut off value. In a recent study, Gaga et al[16] of Athens found that with calorimetric determination of ADA by Guisti, its mean value was noted as 94 U/L in tuberculous and 28 U/L in non tuberculous cases. The sensitivity and specificity for TPE was calculated to be 97% both.

Likewise several studies have been conducted by many since 1983. It has been suggested that an elevated pleural fluid ADA level predicts TPE with a sensitivity of 90-100% and a specificity of 89-100% when the Giusti method. The reported cut off value varies between 33-50 U/L[20]. In regions with a high prevalence of TB and in patient groups with a low risk of other causes of pleurisy the positive predictive value of this marker is increased. In lower incidence of TB the positive predictive value decreases and so there is higher likelihood that a test would give a false positive test.

The majority of ADA in TPE is ADA2 whereas ADA1 is found to be the predominent form in parapnuemonic and empyema cases[17]. Thus this separation of ADA activity into ADA1 and ADA2 will alleviate a major limitation in distinguishing TPE from parapnuemonic empyemas. The ADA1 to ADA ratio will slightly increase the sensitivity and specificity of ADA in diagnosing TPE but is found not to be cost effective in vast majority of cases. Nevertheless an ADA1 to total ADA ratio in fluid > 0.42 is a good indicator of TB [17, 18]. This test is still not widely available in the market. Studies have reported that pleural fluid ADA level didn't vary with the HIV status of the patient which is another plus point for countries gripped by the HIV and AIDS pandemic [19].

As noted though ADA has both good sensitivity and specificity, the specificity needs to be further increased. Hence use of another test in combination is being evaluated since a few years. The combination tests need to be simple, easily available and cost effective. As an answer to the search, many workers have been evaluating the use of cytology and cell counts in pleural fluid. It has been noted that in addition to ADA of >40 U/L if the diagnostic criteria for TPE also includes a pleural fluid lymphocyte to neurophil ratio >0.75, the specificity is increased [8].

Burgess L.J. et al[20] studied 303 cases of pleural effusion of which 143(58%) were TPE. The ADA in TPE at cut off value of 50 U/L showed a sensitivity of 91% and specificity of 81%. 6 non tuberculous cases were misclassified with ADA >50 U/L criteria alone. So when lymphocyte neutrophil ratio of > or equal to 0.75 was also considered, 17 patients of TPE were omitted. But 13 of these were also misclassified when only an ADA criterion was used. Though the sensitivity decreased from 91% to 88%, the specificity of the tests increased from 81% to a gross of 95%. Maria et al[7] of Columbia in their study of 140 pleural effusion cases, 42 were of confirmed tuberculous origin and 19 were probably TPE. The mean ADA value was higher in TPE with a sensitivity and specificity of 88% and 86% respectively at a cut off value of 47 U/L. They also noted that MT performed in the cases gave a sensitivity of 50% and specificity of 64.4%. When both

criteria of ADA >47 U/L and a positive response to MT was considered together, the sensitivity reduced to 46.9% but the specificity increased to a high 94.9%. These studies show that ADA with a high sensitivity and specificity is a good screening as well as a confirmative test in TPE. Its specificity can be further increased by combining pleural fluid cytology and/or MT thus decreasing the few false positive results.

In our present study of 100 cases of pleural effusions carried out in during 1 year period, the role of combined use of pleural fluid ADA, cytology and Mantoux test in the diagnosis of tuberculous pleural effusion was evaluated.

Mean ADA of pleural fluid was 76.29±30.91 U/L in TPE and 26.77±19.09 U/L in non-tuberculous cases. The difference in ADA values were statistically significant between TPE v/s Malignancy ($p<0.001$) and TPE v/s Synpneumonic ($p<0.01$).

In TPE, pleural fluid ADA at a cut off level of 40U/L showed a sensitivity, specificity, positive predictive value and negative predictive value of 89.18%, 84.61%, 94.29% and 73.33% respectively, specificity of 100% was obtained at a higher cut off value of 63U/L but the sensitivity decreased to 67.57%. On combining the criteria of ADA > 40U/L and lymphocyte predominance (>50%) in TPE cases, though the sensitivity decreased to 83.78%, the specificity increased to 96.15%. PPV was a high 98.41% and NPV was 67.57%. When the criteria of ADA > 40U/L and positive Mantoux test were used in combination in TPE, the sensitivity was a low 47.30% and specificity was found to be 92.31%. PPV and NPV were 94.59% and 38.10% respectively.

All the three criteria – ADA > 40U/L, lymphocyte predominance (>50%) and positive Mantoux test when used in combination resulted in sensitivity 45.95% and specificity of 96.15%. It shows that sensitivity further decreased without any increase in specificity. Thus combination of Mantoux test with pleural fluid ADA and cytology had no added value.

At the end of this study, pleural fluid ADA emerged as a single best sensitive test with fair specificity as well. It being a simple and less time consuming test is an added advantage. Tuberculosis still remains the leading cause of pleural effusion and ADA with good positive predictive value is a valuable time sparing diagnostic tool. But since it can still give false positive results caution is advised. The use of combination of pleural fluid ADA and differential cell counts will help in further increasing the specificity.

Conclusion

Finally, it can be concluded from the present study that pleural fluid ADA, cytology and Mantoux test combination can be used in the differential diagnosis of pleural effusion as follows:

- In a clinically suspected case of tuberculous pleural effusion if the pleural fluid ADA is >63U/L, it is essentially diagnostic of TPE.
- However if ADA is between 40-63U/L it is highly suspicious of TPE, and then the cytology report will aid in confirming the diagnosis. A lymphocytic exudate (>50%) with high ADA value (>40U/L) is highly suggestive of tuberculous pleural effusion.
- Pleural fluid ADA >40U/L and a positive Mantoux test also increases the specificity of diagnosis.
- A lymphocytic exudate (>50%) with low ADA (<40U/L) is more in favour of non haematologic malignancies.

References

1. Berger HW, Mejia E. Tuberculous pleurisy. *Chest.* 1973; 63: 88-92.
2. Pedro P, Valdes L, Alvarez D, San Jose E et al. Tuberculous pleurisy: a study of 254 patients. *Arch Intern Med.* 1998; 158: 2017-21.
3. Maartens G, Bateman ED. Tuberculous pleural effusions: increased culture yield with bedside

inoculation of pleural fluid and poor diagnostic value of ADA. *Thorax.* 1991; 46: 96-9.

4. Ocana JM, Martinz-Vazquez, Segura RM et al. Adenosine deaminase in pleural fluids, a test for diagnosis of TPE. *Chest.* 1983; 84: 51-53.
5. Epstein DM, LR Kline, Albelda SM et al. Tuberculous pleural effusion. *Chest.* 1987; 91: 106-109.
6. Seibert AF, Haynes J, Middleton R et al. Tuberculous pleural effusion-a twenty year experience. *Chest.* 1991; 99; 883-886.
7. Maria Virginia Villegan, Luz Angela Labrada, Nancy Saravia et al. Evaluation of polymerase chain reaction, adenosine deaminase and interferon gamma in pleural fluid for differential diagnosis of pleural tuberculosis. *Chest.* 2000; 118: 1355-1364.
8. Richard W Light. Pleural Diseases 4th edition, Tuberculous pleural effusion. 182-92.
9. Light RW, Erozan YS, Ball WC. Cells in pleural fluid:their value in differential diagnosis. *Arh Intern Medicine.* 1973; 132: 854-860.
10. Hurwitz S, Leiman G, Shapiro C. Mesothelial cells in pleural fluid:TB or not Tb? *S Afr Med J.* 1980; 57: 937-939.
11. Petterson T, Riska H. Diagnostic value of total and differential leukocyte counts in pleural effusions. *Acta Med Scand.* 1981; 210: 129-35.
12. Anwar R, Farooqi JI. Causes of lymphocytic exudative pleural effusion as revealed by percutaneous pleural biopsy: experience from Peshwar. *Pak J Med Sci.* 2003.
13. Jacobus P et al. Significance of ADA activity and its isoenzymes in tuberculous pleural effusion. *Chest.* 1994; 106.
14. Gupta DK, Suri JC, Goel A. Efficacy of Adenosine deaminase in the diagnosis of pleural effusions. *Ind J Chest Dis And All Sci.* 1990; 32: 205-208.
15. Rajendra Prasad, Tripathi RP, Mukerji PK et al. Adenosin deaminase activity in pleural fluid-a diagnostic test of tuberculous pleural effusion. *Ind J Chest And All Sci.* 1992; 34: 123-126.
16. Gaga M, Papamichalis G et al. Tuberculous Effusion: ADA activity correlates with CD4 cell numbers in the fluid Respiration 2005; 72: 160-162.
17. Perez-Rodriguez E, Castro DJ. The use of ADA and ADA isoenzymes in the diagnosis of tuberculous pleuritis. *Curr Opin.* 2000; 6: 259-266.
18. Valdes L, San Jose E, Alvarez D et al. ADA isoenzyme analysis in pleural effusions: Diagnostic role and relevance to the origin of increased ADA in tuberculous pleurisy. *Eur Respir J.* 1996; 9: 747-751.
19. Riantawan P, Chaowalit P et al. Diagnostic value of pleural fluid ADA in tuberculous pleuritis with reference to HIV coinfection and a Bayesian analysis. *Chest.* 1999; 116: 97-103.
20. Burgess LJ, Maritz FJ, Le Roux I et al. Combined use of pleural adenosine deaminase with lymphocyte / neutrophil ratio; increased specificity for the diagnosis of tuberculous pleuritis. *Chest.* 1996; 109: 414-419.