

ADENOSINE DEAMINASE ACTIVITY FOR THE DIAGNOSIS OF TUBERCULOSIS PLEURAL EFFUSIONS: A DIAGNOSTIC ACCURACY STUDY

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Abstract

Background: the diagnosis of tuberculosis pleural effusion continues to be a challenge in clinical practice in many parts of the world. In part due to the use of diagnostic tools with poor sensitivity and specificity. Biomarkers of pleural effusion due to inflammation caused by *Mycobacterium Tuberculosis* such as adenosine deaminase activity has the potential to optimize the diagnostic approach of tuberculosis pleural effusion. **Objective:** to describe the diagnostic accuracy of adenosine deaminase activity among

adult patients with pleural effusion. **Design:** a chart-review cross-sectional study. **Methods:** we retrospectively reviewed patient charts from the Internal Medicine Department Ambulatory Center in a Tertiary Care Hospital in Buenos Aires, Argentina from June-1993 to December-1995. We included 91 patients with pleural exudates in which adenosine deaminase activity measurement was performed. We evaluated the sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratios and accuracy of adenosine deaminase activity compared to the reference standard. **Results:** 34/91 pleural effusions were due to tuberculosis (39%). Mean \pm SD pleural effusion adenosine deaminase activity levels were higher in the tuberculosis group vs. Non tuberculosis group (99.2 ± 37.4 U/l vs. 51.6 ± 68.7 , $p < 0.0001$). The best cutoff value for pleural fluid adenosine deaminase activity using ROC curve results (AUC=0,86) was 50 u/l yielding sensitivity (97%), specificity (70%), positive predicted value 67%, negative predicted value (97%), positive likelihood ratio (3.3) and negative likelihood ratio (.04). **Conclusion:** given the successful use of the adenosine deaminase activity test in pleural fluid, makes this test a highly recommended option for the diagnosis approach in tuberculosis pleural effusion.

Keywords: Tuberculosis, pleural effusion, pleural fluid, adenosine deaminase activity sensitivity, specificity

Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) is the leading cause of death from infectious diseases worldwide, accounting for 3 million deaths per year (Sundre P, 1992). Argentina is a country with a medium incidence TB (Abbate EH, 2007), however mortality has remained constant with 1,050 deaths per year (INER Argentina, 2007). TB pleural effusion is an important extrapulmonary manifestation of the disease and in many parts of the world the most common cause following by lymph node involvement (Mlika-Cabanne N, 1995; Moudgil H, 1994).

The diagnosis of pleural TB is still challenging in clinical practice. Traditional diagnostic methods, based on high clinical suspicion, Chest XRay pattern (Siddiqi K, 2003) and a positive sputum smear, are very useful for the diagnosis of pulmonary TB; however, they have low yield in the case of pleural effusion due to TB. The diagnosis of TB pleural effusion is made by identifying the presence of tubercle bacilli in the sputum (acid-fast bacillus (AFB) smear), the pleural fluid, or the pleural biopsy specimen, or by demonstrating the existence of granulomas in the pleural biopsy specimen (Epstein DM, 1987). It increases the cost of patient care because it needs the presence of an adequate team and facilities for its performance, as well as a pathological anatomy laboratory and an experienced pathologist in this field

in order to interpret the findings (Diacon AH, 2003; Valdes L, 1993) and also all these tests are time consuming.

Recognition of the difficulty in diagnosing pleural TB led to a search for methods that would optimize the approach of pleural effusion in patients with a suspected TB infection. Of note among these procedures are those, such as Adenosine Deaminase Activity (ADA) in pleural fluid which identifies an inflammatory process triggered by MBT (Bañales JL, 1991; Ungerer JPJ, 1994; Seibert AF, 1991). The most recent study, a meta-analysis by Lian QL and co-workers, shows a very good accuracy in measuring ADA in pleural effusion to diagnose TB pleural effusion. Nevertheless the best cutoff they found was 40 U/L given a positive likelihood (LH) of 9, 03 and negative LH ratio of 0,01 which are important values to consider (Liang QL, 2008). However in our country the suggested cutoff value for ADA in pleural fluid is 60 U/L (Abbate E, 2009), which might be supported in the available literature (Riantawan P, 1999).

The present study was performed to add data in our country to find the best cutoff for ADA measurement in pleural fluid to diagnose A TB pleural effusion.

Materials and methods

Patients: We reviewed the records of patients admitted to the Internal Medicine Department in a Tertiary Care Hospital in Buenos Aires, Argentina, from June of 1993 to December of 1995, who had pleural effusion as the diagnosis made at the time of discharge. There were three internal medicine specialists, who reviewed the patient's charts. All of them who had exudates by Ligh criteria (Light RW, 1972) and modified Ligh criteria (Light RW, 1983) in pleural fluid samples taken at the time of admission and in which the ADA measurement were included. The patients included in this study in that period of time, were participating in a diagnosis protocol of pleural effusion at the hospital, where ADA and others markers were measured. These markers include glucose, LDH, cholesterol and proteins measured both in blood and pleural fluid. Cytology, pathology and cultures from pleural fluid were performed.

Gold Standard Criteria for Pleural Effusion Diagnosis: The diagnosis of a TB pleural effusion was made by the reference standard and was considered positive by the presence of one of the following criteria:

1. Smear positive for AFB and/or A positive culture for TB both in pleural fluid and/or histology from the pleura and/or an internal mammary chain lymph node.
2. Tuberculosis granuloma in both pleural and/or internal mammary lymph node, showing epithelioid macrophages and Langerhans giant cells along with lymphocytes, plasma cells, maybe a few

polymorphonuclear leucocytes, fibroblasts with collagen, and characteristic caseous necrosis in the center.

3. Smear positive for AFB or TB positive culture from both sputum and/or BAL.
4. Clinical response to treatment defined as diminution in the amount of pleural effusion without evidence of new active disease elsewhere, within two to four months from the beginning of the treatment and the patient becoming afebrile in the first few weeks.

ADA Measurements

ADA was measured the by spectrophotometry method based on the Guisti and Galanti method of enzymatic analysis (Giusti G and Galanti B, 1984; Giusti G, 1971). The sample was centrifuged; 25 μL of the pleural fluid supernatant was placed into a test tube together with 500 μL of an adenosine solution. The mixture was heated at 37°C for 60 minutes, and the reaction was then interrupted by the addition of a phenolnitroprusside solution and a hypochlorite solution. Then it was heated at 37°C for 30 minutes. The reading of the quantity of ammonia liberated by the ADA action was measured with the aid of a spectrophotometer at a wavelength of 620 nm and converted to U/L for statistics analysis.

Other causes of Pleural Effusion

Other causes of pleural effusion were classified as previously defined: cancer (Bielsa S, 2008; Diacon AH, 2003; Ferrer J, 2005), complicated para-pneumonic and empyema (Colice GL, 2000), pulmonary embolism (Romero-Candeira S, 2002) and uremic pleural effusions (Berger HW, 1975).

Statistical Analysis

We used a convenience sample for this study and did not perform a priori sample size calculation.

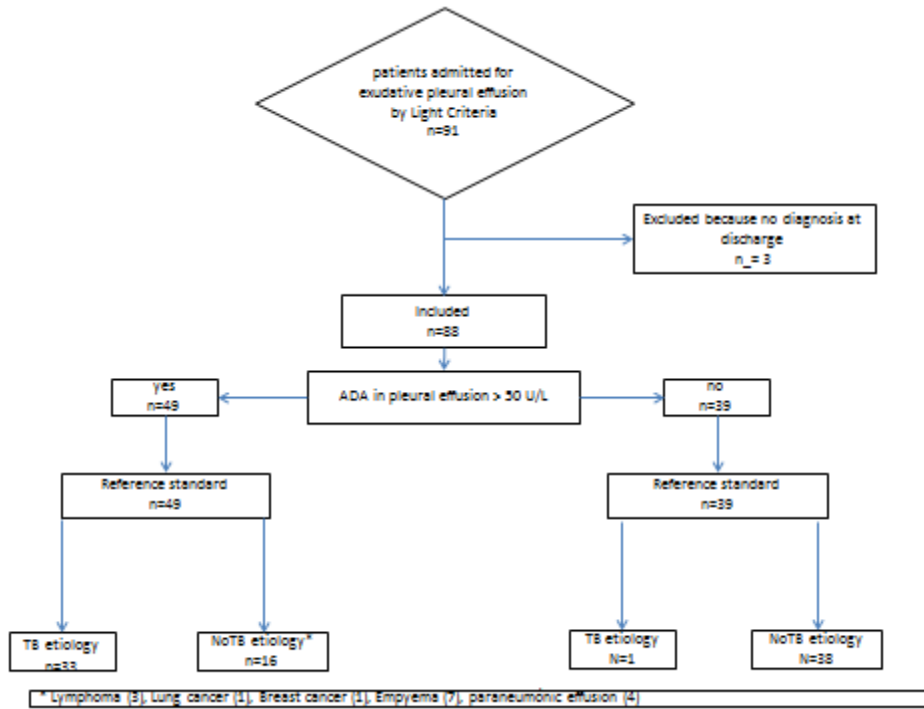
1. Primer analysis: a description of the population (age, sex, previous diagnosis) and
2. ADA levels across the reference groups. The results were expressed as mean \pm SD and compared using Student's T test.
3. ROC curves results (AUC)
4. The Scientific Geigy table determined the sensitivity, specificity, positive and negative predictive values, likelihood ratios

We used EPI INFO and IBM SPSS version 20 for all statistical analyses, The parameters were reported as % and 95% confidence interval; all P values were two sided and values of < 0.05 were considered statistically significant.

Results

Ninety-one patients with pleural effusions were included; see flow chart (Figure 1). Three of them were excluded because a diagnosis could not be done. Eighty eight patients were included.

Figure 1: Flow chart of the study



The etiologies into two groups were: those caused by TB and those caused by other etiology, named as Non TB. In the first group, we found 34 pleural effusions due to TB (39%) and in the former group 54 caused by Non TB etiologies (61%), cancer being the most common cause (28%) (Table 1).

Table 1: Pleural effusion characteristics, n=91

Groups	Etiology	N (%)	ADA (U/L)mean ±SD
TB (n=34)	Tuberculosis	34 (39)	99,3±37,4
Non-TB (n=57)	Cancer*	25 (28)	34,54 ±25,36
	Complicated Parapneumonic	16 (18)	40,70 ±26,46
	Emyema	10(11)	123 ±133,58
	Pulmonary embolism	2 (2)	18,25 ±10,25
	Uremic	1 (1)	23

*Lung (n=8); Breast (n=7); Lymphoma (n=5); Unknown origin (n=3); Pancreas (n=1) and Melanoma (n=1)

The pleural fluid ADA levels (Mean ± SD) in TB pleural effusion as 99.26 ± 37.44 U/L and in Non TB pleural effusion cases (Figure 2 and Table 2), it was 51.56 ± 68.67 (highly significant, $P < 0.0001$)

Figure 2: dispersion values of ADA levels in both groups

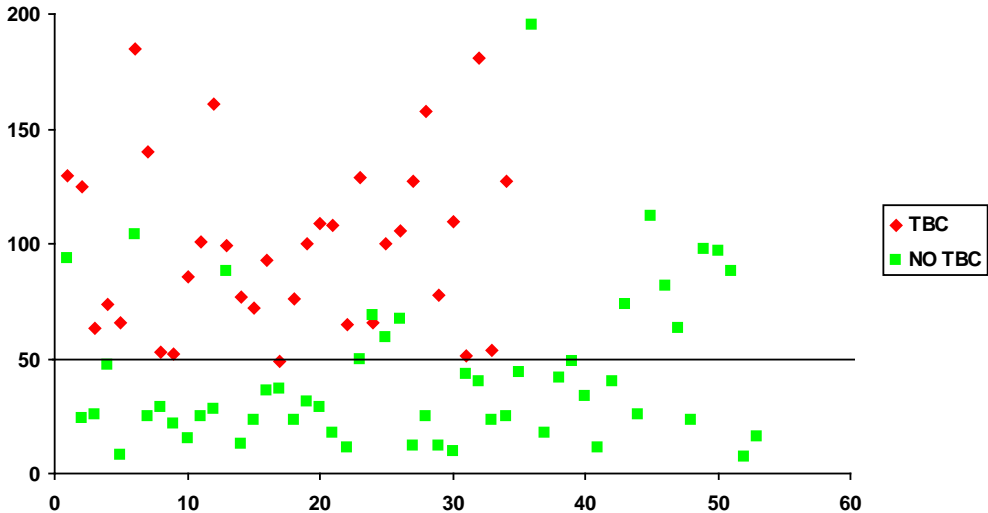
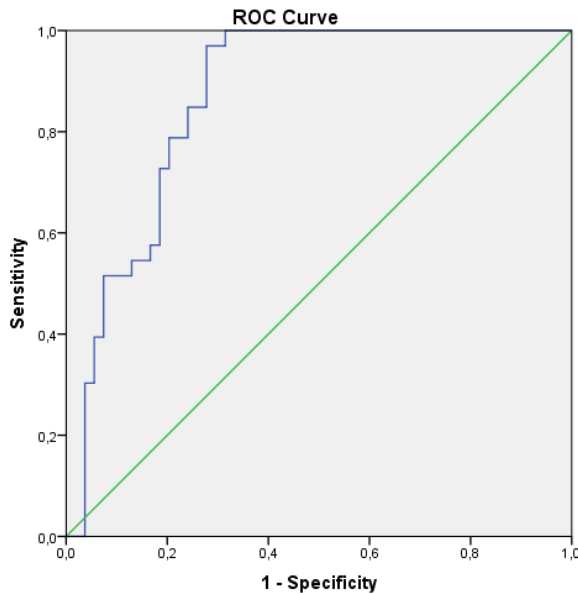


Table 2: ADA levels (Mean ± SD) in both groups

DATA	Non TB	TB
n	54.0	34.0
Media (U/L)	51.6	99.3
SD	68.7	37.4
Minim	7.0	49.0
Median	30.0	99.0
Maxim	478.0	185.0
$P < 0.0001.$		

The best cut off value of pleural fluid ADA level confirming on ROC’s curve was 50 U/L (Figure 3).

Figure 3: ROC curve for ADA values



Taken into account the cutoff value of 50 U/L the false positive etiologies, which means non TB etiologies, were: cancer, empyema (mostly Pneumococcus etiology) and parapneumonic effusions; being the most common empyema (Table 3)

Table 3: False positive etiologies

Etiology	ADA U/l
Lymphoma	94
Lung cancer	104
Paraneumónico	88
Empyema (due to actinomyces)	478
Parapneumonic effusions	50
Lymphoma	69
Breast cancer	59
Parapneumonic effusions	67
Empyema (due to pneumococcus)	195
Empyema (due to pneumococcus)	74
Empyema (due to pneumococcus)	112
Empyema (due to pneumococcus)	82
Lymphoma	63
Parapneumonic effusions	98
Empyema	97
Empyema	88

Table 4 shows the diagnostic accuracy: sensitivity, specificity, positive predicted value (PPV), negative predicted value (NPV), positive Likelihood ratio (PLR) and negative Likelihood ratio (NLR) for an ADA´s cut off of 50 U/L in pleural fluid, being sensitivity, specificity, PPV, NPV, PLR and NLR of 0,97, 0,70, 0, 67, 0,97, 3,28 and 0,04 respectively.

Table 4: ADA diagnostic accuracy

Parameter	%	(95% CI)
Sensitivity	97.1	(91.4-100)
Specificity	70.4	(58.2-82.5)
PPV	67.4	(54.2-80.5)
NPV	97.4	(92.5-100)
Likelihood ratio (+)	3.3	(2.7-3.9)
Likelihood ratio (-)	0.04	(.01-0.30)
Accuracy (AUC)	0,86	(0,79-0,94)
Prevalence	39	

Discussion

Despite the efforts to control tuberculosis and reduce its spread, the lack of accurate laboratory diagnosis hinders this effort. The diagnosis of tuberculosis is still the detection of the appropriate organism and/or a positive culture of *M tuberculosis* (Kim TC, 1984), which is not only time consuming, but also has a low rate of finding the bacillus, depending on the sample evaluated. The problem is more difficult in the diagnosis of extra pulmonary tuberculosis such as a TB pleural effusion. For the etiological confirmation of TB pleural disease, cytology and culture, Mantoux test, pleural biopsy (Donohoe RF, 1958) and histopathology evaluation are still the most common used tools. In order to add evidence in the usefulness of adenosine deaminase activity in pleural fluid (Piras MA, 1978) for the diagnosis of tuberculosis pleural effusion, we carried out this retrospective study in which the values of pleural fluid ADA level in tuberculosis were significantly elevated when compared with other etiologies. In our study, the cut-off for ADA's activity was established on 50 U/L, because it has not only a high sensitivity and specificity, but also a high NPV. The NPV is low as well, giving the idea that those patients with ADA level below 50 U/L unlikely have TB as offending agent. The prevalence of pleural TB in our work was of 39 % being the most prevalent infection in the population we evaluated. Our results in respect to the diagnostic value of the dosage of ADA in the pleural fluid could be applied in a population with a different prevalence if we consider the statistical data not dependent of the dosage of ADA. The negative LH ratio was 0.04, which would discard TB in the majority of the pleural effusions with ADA values of 50 u/l.-

In respect of the false positive we found, highlight purulent pleural effusions (45.75 %), paraneumonic pleural effusions (25 %) and lymphomas (18.75%), which coincides with literature (Valdes L, 1993; Bañales JL, 1991).

Regarding the diagnostic methods used, we had the major yield with the pleural biopsy in patients with TB (16 positive biopsies 25= 64%) while the culture of the pleural fluid was positive only in 2% of the cases.

We highlight the high yield of cytology in the pleural fluid to diagnose cancer, it was positive in 90 % of the cases (18 of the 20 patients)

The patients considered as tuberculosis individuals based on the response to empiric treatment had a clinical high pre test in all the cases and 5 of the 9 patients had as well pathological findings compatible with TB such as high lymphocytic count, few mesothelial cells in the fluid and a chronic infiltrate in the pleural biopsy.

There are other publications where the cut off is below this value (40 U/L) and nearly the same sensitivity and NPV (Bañales JL, 1991; Ungerer JPI, 1994; Chander A, 2012; Kaisemann MC, 2004; Ocaña I, 1983). In Argentina, the determination of the ADA levels are not performed in the majority of public hospitals and the cut off recommendation is 60 U/L given a sensitivity and specificity of 0, 95 and 0, 96 respectively; nevertheless there are no studies in our country to support this value. The recommendation in our country is to perform the test in selected cases¹⁴. The results of this study indicate that in a population with relatively high prevalence of tuberculosis and those patients with high risk of TB pleurisy, the analysis of ADA levels in pleural fluid constitutes a useful marker for the diagnosis of TB, which, in addition, can be made quickly and at a low cost (Agrawal S, 2012; Maldhure BR, 1994). We defined high risk patients as the ones having TB or those with an epidemiology and a clinical course compatible with TB and also with a large amount of lymphocytes and a few mesothelial cells in pleural fluid and/or chronic indeterminate infiltrate in pleural histology. We treat them as TB serositis, until proven otherwise.

Given the knowledge regarding the diagnosis of a TB pleural effusion and the publications showing the successful use of the ADA test in pleural fluid instead of others methods (Moon JW, 2005; Liu KT, 2007; Morisson P, 2008), its simplicity, low cost and quickly available results, makes this test a very good option in patients like the above mentioned. We encourage physicians and other health care professional, to use this method in our country and take an ADA cut-off value of 50 U/L based in this study or the best current evidence of 40 U/L.

One limitation of our study is that all the cases in the TB group were not confirmed by the gold standard method (histopathological evaluation of pleural biopsy) used to confirm TB pleurisy. This could have introduced some bias in the selection of this group of patients.

Conclusion

In our population and our laboratories the cut off was significant for an ADA value of 50 u/l.

The most important finding in our paper is the highly negative predictive value (97.94 %), which lets us discard with high certainty the TB etiology in pleural effusions with ADA values lower than 50 u/l.

The sensitivity we found of 97.06% allows us to start empiric treatment while we wait for the definite diagnosis with a low possibility of making a mistake. These finding in the epidemiologic context and a compatible clinical presentation, are to be considered as a possible TB infection in patients with a pleural effusion with ADA values higher than 50 U/L. This is sustained by the fact that the false positive results that were found correspond to other pathologies easily diagnosed by other methods. The importance of this is based on the fact that there are multiple studies that shows the elevated probability to develop active pulmonary TB in patients with these characteristic and who did not receive treatment (Siebert AF, 1991).

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