

RESEARCH COMMUNICATION

Predictive Role of Adenosine Deaminase for Differential Diagnosis of Tuberculosis and Malignant Pleural Effusion in Turkey

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Abstract

Tuberculous pleural effusion (TPE) is a common problem for differential diagnosis from malignant effusion (MPE) in epidemic areas of tuberculosis (TB). Prediction based on adenosine deaminase (ADA) is dependent on age as well as the tuberculosis incidence. The aim of the study was to evaluate cutoff values for ADA with sensitivity and specificity results for the differential diagnosis of MPE and TPE in a population with intermediate incidence of TB. We retrospectively analysed 196 patients with a definitive diagnosis of TPE (n=114) and MPE (n= 82). The optimal cutoff value of ADA was determined using the receiver operating characteristic (ROC) curve. There was a statistically significant difference according to the levels of pleural fluid ADA between TPE and MPE groups ($p<0.0001$). The cutoff value for diagnosing TPE was $>55\text{U/L}$, with a sensitivity = 86.8%, specificity = 86.6%, positive predictive value (PPV) = 90%, negative predictive value (NPV) = 82.6% and accuracy = 82.6%. We then combined $\text{ADA}>55\text{U/L}$ and $\text{age}<50$ and were able to discriminate the TPE group with increased specificity (95.7 %) and PPV (98.8%) results. The model could correctly classify 21 MPE out of 23 and 82 TPE out of 94 patients. A pleural fluid ADA value $<31\text{U/L}$ suggests that TPE is highly unlikely with a sensitivity = 43.9 %, specificity = 100%, PPV = 100%, NPV = 71.3% and accuracy = 76.6%. It can be concluded that ADA is a very useful parameter for the differential diagnosis of TPE and MPE, specifically in youngsters with a higher incidence of tuberculosis.

Keywords: Tuberculosis - pleural effusion - adenosine deaminase - malignant effusion

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Introduction

TPE is the most common form of the extra pulmonary tuberculosis in our country. Its definitive diagnosis is established by determining of the tuberculosis in the phlegm, pleural fluid or pleural tissue. Acid fast bacilli can be determined by less than 25% with direct examination of the pleural fluid. In TPE, granulomatous pleuritis is determined in 80% of the cases with the pleural biopsy, while the histological examination combined with culture of the biopsy material increases the rate of diagnosis to 90% (Light, 1995). The diagnosis cannot be established in 10-20% of the patients with these methods even in the best conditions. Therefore, many studies have been conducted to demonstrate the role of pleural fluid levels of ADA in the differential diagnosis of pleuritis in recent decades. High sensitivity and specificity values reported (Ocaña I et al., 1983; Valdés L et al., 1993; Krenke and Korczyński, 2010). These values may vary according to the incidence of the tuberculosis and proportional frequency of the diseases included in the differential diagnosis of the community in which the measurements were done. The level of ADA is

used more commonly in the countries with a moderate to high incidence of tuberculosis in the differential diagnosis of TPE. Because it is a less invasive and more inexpensive method that can be accessed more quickly and accurately specifically in young patients with a high prevalence of TPE. Hence, ADA values needs to be detected in a series of the data collected from populations which is intermediate/high prevalent for tuberculosis.

In this study, we aimed to determine the optimum levels of ADA in the differential diagnosis of TPE and MPE those are the two most common causes of lymphocytic pleural fluid.

Materials and Methods

Total 196 patients with pleuritis, including 114 patients with TPE and 82 patients with MPE diagnosed between January 2006 and December 2009 in our clinic were retrospectively studied. The pleural fluid was exudative with predominantly mononuclear cells (lymphocyte count $> 50\%$) in all patients.

The conditions considered for TPE diagnosis were:

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1. Determination of the necrotizing granulomatous inflammation in the pleural sampling carried out with closed biopsy or Video Assisted Thoracoscopic Surgery; 2. Reproduction of *Mycobacterium tuberculosis* in the pleural fluid; 3. While there was not any other reason to explain the pleural fluid, Ziehl-Neelsen stains or Lowenstein culture of pleural fluid/tissue, sputum, or needle aspiration were positive together with clinical and radiologic appearance suggesting TB.

For the diagnosis of MPE, malignancy in cytology of the pleural fluid and/or on histology of the pleural tissue was considered. ADA activity in the pleural fluid was studied with De Giusti method and the results were recorded as IU/L in all the patients (Giusti, 1974).

The patients who had not met the above mentioned diagnostic criteria for TPE and MPE and also those had diagnosed with the heart failure, kidney failure and liver cirrhosis or those with nephrotic syndrome and transudate were not included to the study. All subjects included in this study signed written informed consent and was conducted according to Good Clinical Practice.

Statistics

The evaluations were carried out using SPSS 10.0 software (SPSS, Inc. Chicago, IL, USA). In the analysis of the categorical variables, Chi-square test was used. Non-parametric Mann-Whitney U test was used for the variables without normal distribution, and t-test was used for normal distributed variables in comparisons between the groups. An optimum cutoff value was established by using the receiver operating characteristic (ROC) curve methodology. The results were expressed as the mean ± standard deviation. The values for P < 0.05 were considered as statistically significant.

Results

A total of 196 patients (144 male and 51 female; mean (SD) age 45 ± 20 years) were included to the study. TPE group consisted of 87 male and 27 female; the mean (SD) age was 35 ± 18 years. MPE group consisted of 58 men and 24 women; the mean (SD) age was 59 ± 12 years. Distribution of the etiological causes in the MPE patients are given in Table 1. Diagnostic methods used in the TPE diagnosis were given in Table 2. Mean age of the MPE group was significantly higher than the TPE group (p<0.0001). There was no statistically significant difference between the two groups in the gender distribution (p>0.05). There was negative correlation between age and ADA levels in effusion (Pearson's r=-0.478, p<0.0001).

Effusion was located in 57 patients (50 %) on the right, 52 patients (46%) on the left side in TPE group. Remaining 4 percent of the patients have bilateral effusion. Most common site (61 %) for pleural effusion was right in MPE group. Whereas 34 percent of the patients have left sided and 5.3 percent of the patients have bilateral effusion. No difference was detected between study groups in terms of the localization of the pleural effusion (p> 0.05).

No significant difference according to the amount of the effusion between the two group (p>0.05), except the

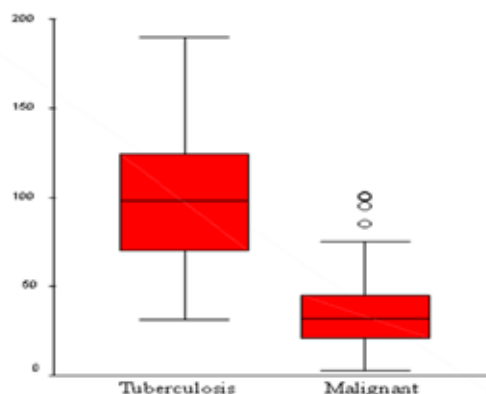


Figure 1. Comparison of the Fluid Level of ADA in the Tuberculosis and Malignant Cases

Table 1. Diagnostic Distribution of the Malignant Pleural Effusions

Diagnosis	N
Lung cancer	55
Lymphoma	6
Mesothelioma	13
Others	8
Total	82

Table 2. Diagnostic Methods Used for Tuberculous Pleurisy

Diagnostic methods		n
Positive Lowenstein Culture	Pleural fluid	2
	Sputum	2
	Transthoracic needle aspiration	1
Histopathological (granulomatous pleuritis)	Conventional pleural biopsy	107
	Pleural biopsy by VATS	3
	Mediastinal lymph Node Sampling	1

Table 3. Mean and Minimal-maximal (min-max) ADA Levels of the Pleural Fluid

Group	ADA level (mean ± SD) (IU/L)	ADA min-max range (IU/L)
TPE	102 ± 40.37	31-190
MPE	36.81±21.74	3-101

Table 4. Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of the Tuberculous Pleurisy according to the ADA Level of the Pleural Fluid

Cutoff levels of ADA (U/L)	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
55	86.8	86.6	90	82.6	86.7
31	43.9	100	100	71.3	76.6
>55 + (age<50)	87.2	95.7	98.8	64.7	88.9

higher ratio of massive effusion was detected in MPE group (31% versus 15.9%) (p=0.014). Pleural fluid LDH/ blood LDH ratio, lactate dehydrogenase, protein and albumin levels in effusion was higher in MPE group than TPE group. (p=0.001)

The mean level (SD) of ADA was 102 ± 40.4 U/L in TPE and 36.8 ± 21.7 U/L in MPE. ADA level of the pleural fluid was significantly higher in TPE (p<0.001) (Figure 1). Mean levels of ADA with minimum and maximum values were given in Table 3. Considering all patients, a significant negative correlation was defined between the fluid's ADA activity and age (p<0.0001; r=-0.478). When the groups were analyzed separately (TPE and

MPE), there was not any correlation between the fluid's ADA levels and age ($p > 0.05$). The cutoff value of ADA for diagnosing TPE was > 55 U/L, with a sensitivity = 86.8%, specificity = 86.6%, positive predictive value (PPV) = 90%, negative predictive value (NPV) = 82.6% and accuracy = 82.6%. The lower cutoff value of ADA to exclude TPE was < 31 U/L, with a sensitivity = 43.9%, specificity = 100%, PPV = 100%, NPV = 71.3% and accuracy = 76.6%.

In patients under 50 years old, a cutoff value of ADA (> 55 U/L) for diagnosing TPE, with a sensitivity = 87.2%, specificity = 95.7%, PPV = 98.8%, NPV = 64.7% and accuracy = 88.9%. When the level of ADA and age were considered together, diagnostic value of the test was remarkably increased. Sensitivity, specificity, PPV, NPV and accuracy for the critical ADA cutoff levels are given in Table 4.

Discussion

The prevalence of tuberculosis estimated as 45 at 100.000 in Turkey according to World Health Organization (WHO) report (WHO, 2005). It is indicated that tuberculosis is moderately common in our country, and recently it follows a stable course. TPE is the most common extrapulmonary form of the tuberculosis in our country. Although it is usually seen in the young ages, yet it may be seen also in the advanced ages and its differential diagnosis with MPE might be a problem (Zarić et al., 2008)

Histopathological and microbiological analysis of the pleural fluid or tissue may seem as the most ideal method, but definitive diagnosis cannot be reached in approximately 20% of the patients (Zarić et al., 2008) (Maskell and Butland, 2003). Hence many markers that may be helpful in the differential diagnosis were studied in the pleural fluid. Two of these, ADA and interferon gamma are the most widely used and currently the most accepted tests (Krenke and Korczyński, 2010). Especially ADA has been more commonly preferred for the diagnostic algorithms in the countries with a moderate to the high incidence of tuberculosis because it is a more inexpensive method that can be accessed more quickly (Porcel et al., 2008; Sales et al., 2009; Valdés et al., 2010).

ADA is an enzyme catalyzing the conversion of the adenosine and deoxyadenosine to the inosine and deoxyinosine in the purine degradation pathway. Its quantity increases in the immature and non-differentiated T-lymphocytes following mitogenic and antigenic stimulation (Light, 2001). While the increase of ADA activity in the MPE has been associated with the predominance of CD8, prominent rise observed in TPE has been tried to be explained with the presence of gradually increasing CD4 blastogenesis after the mycobacterial antigenic stimulus (Baganha et al., 1990). There are numerous studies in the literature indicating the increase of pleural fluid levels of ADA in the TPE. High values of the sensitivity and specificity have been reported in the countries with the high prevalence of TPE, particularly in the young patients (Ocaña I et al., 1983; Valdés L et al., 1993; Valdés et al., 1996). However, false positive results

increased and the diagnostic value decreased with the decrease of the prevalence of disease. Therefore, recently studies are conducted on which levels of ADA can be used to rule out the tuberculosis (Jiménez Castro et al., 2003; Chen et al., 2004; Zarić et al., 2008). In a study conducted by Castro et al. evaluating consecutive 410 nontuberculous lymphocytic pleural fluid samples, were identified the level of ADA above 40 U/L only in seven cases (1.71%). They can accurately use ADA levels < 40 U/L to rule out the tuberculosis (Jiménez Castro et al., 2003). In our study, we found the lower cutoff value of ADA as 31 U/L with 100% specificity and negative predictive value to rule out the tuberculosis. Whereas we identified 5 cases (4%) have pleural ADA level below 40 U/L, so level was suggested previous studies is not really useful to rule out tuberculosis in our patient group.

In another study was conducted in Japan, reported that the ADA level < 50 U/L was used to rule out the tuberculosis, but they performed the thorascopic pleural biopsy on 50 patients with ADA level under 50 U/L and defined tuberculosis in 6 (12%) of these patients (Sakuraba et al., 2009). This high rate of the false negative results may be due to extremely higher cutoff value they used in this study. As a similar results with this study, when we use the cutoff as an 55 U/L, we missed 15 (10%) of the TPE patients. Thus, the cutoff value that we found in our study to rule out tuberculosis has attracted attention with being lower than the values reported in the countries with the tuberculosis is seen less.

In a study conducted in Serbia in which the tuberculosis is moderately common similar to our country, 54 TPE and 67 MPE cases were prospectively evaluated. On the TPE diagnosis, sensitivity was found as 89.2%, specificity 70.4%, PPV 84.4% and NPV 78.4% for the cutoff value of ADA 49 U/L. Furthermore, they reported the ADA level of 16 U/L that was even lower than in our study (31 U/L) could be used to rule out the tuberculosis. All patients underwent pleuroscopy-guided pleural biopsy to confirm the diagnosis in the same study. Sensitivity of this process was calculated as 66.7%, specificity 100%, PPV 100% and NPV as 78.8% (Zarić et al., 2008). In another retrospective study, Chen et al., (2004) evaluated 63 TPE and 147 nontuberculous pleurisy cases and reported higher diagnostic values like the sensitivity of 87.3%, specificity 91.8%, PPV 82.1% and NPV 94.4% for the cutoff value similar to ours (55.8 U/L) in the TPE diagnosis. They concluded total ADA value of the pleural fluid is an appropriate and fast diagnostic tool for the diagnosis of tuberculosis. In the present study, we retrospectively evaluated 114 TPE and 82 MPE cases who had a definitive diagnosis. We found slightly higher diagnostic values with sensitivity of 86.8%, specificity 86.6%, PPV 90% and NPV 82.6% for the cutoff value of ADA 55 U/L. Our cutoff value obtained from ROC analysis was similar with previous studies (Chen et al., 2004; Zarić et al., 2008). But, interestingly lower cutoff was detected in our study to exclude all of the tuberculosis cases.

Age is used most commonly along the ADA in the diagnostic algorithms. Diagnostic value of the pleural fluid levels of the ADA increases when it was used together with age. Since the mean age in the MPE group of our

study was high, higher diagnostic values were obtained for the same cutoff value (55 U/L) in the TPE diagnosis of the patients under 50 years old. For these patients, the sensitivity was 87.2%, specificity 95.7%, PPD 98.8%, NPV 64.7% and accuracy 88.9%. When we analyze MPE patients if age < 50, > 55 U/L cutoff value can discriminate tumor cases successfully 21 patients out of 23. Only 2 nonhodgkin lenfoma patient in the MPE group out of 23 were missed when we use combined age < 50 and ADA > 55 U/L. When we use same algorithm for all patients, 2 false positive (9%) and 12 false negative (13%) result was detected. This results considering that combination of age and ADA increased specificity and PPD values of test and it is very useful for the regions in the presence of increased risk of TPE.

Out of the tuberculosis, high levels of ADA in the lymphocytic pleural effusion have been also reported in the fungal infections such as coccidioidomycosis and histoplasmosis. However, our country is not among the regions that have endemic mycosis and this is seen very rarely and usually in the immunosuppressive patients. Among the noninfectious cases, high levels of the ADA are seen in the malignancies and collagen vascular diseases (eg. rheumatoid arthritis and systemic lupus erythematosus (Laniado-Laborín, 2005). The conditions other than malignancy are more easily differentiated than TPE with clinic, laboratory and fluid profile. However, differentiation of the MPE and TPE is more difficult, since they present similar clinical features and pleural fluid profiles. In this study, the level of ADA in 11 patients (13%) with MPE was above the cutoff value of 55 U/L. Diagnoses of these patients were adeno carcinoma (n=4), mesothelioma (n=3), metastasis (n=2) and lymphoma (n=2). No specific type of tumor was detected suffered from false negative results for MPE diagnosis.

This study has some limitations. First, we included only the patients with TPE and MPE in the differential diagnosis. However, as mentioned above, most problems occur in the differential diagnosis of these two diseases in the exudative lymphocytic effusions. Second, since our study was retrospective, ADA was not studied again in the tuberculous effusions with low levels of ADA. Querol et al. (Querol et al., 1990) found the level of ADA below the threshold value (43 U/L) in 9 patients with TPE. They reported that the ADA level measured in 5 of these patients after a few days, raised above the threshold value (Querol et al., 1990). Also ADA isoenzymes in the MPE group with a high level of ADA could not be studied. While some studies reported this contributes to the differential diagnosis (Gorguner et al., 2000), the others stated isoenzymes did not provide an important contribution to the total level of ADA, and that was more expensive and not readily available (Laniado-Laborín, 2005). On the other hand, the advantage of our study being retrospective is the both groups to consist of the sufficient number of the patients with a definitive diagnosis.

In conclusion, ADA level of the pleural fluid is a non invasive, inexpensive and repeatable test that provides the results quickly. This study indicates that the ADA levels of the pleural fluid can be used with high diagnostic rates in the diagnosis and exclusion of the tuberculosis in the

patients whom the pleural tissue could not be obtained with various causes in the differential diagnosis of TPE and MPE and those with waiting for the laboratory outcomes of the pleural tissue.

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