

## Diagnostic accuracy of different cut-off values of adenosine deaminase levels in tuberculous pleural effusion

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### Abstract

**Objective:** To assess the diagnostic accuracy of different cut-off values of pleural fluid adenosine deaminase levels as a diagnostic method for tuberculous pleural effusion.

**Method:** The prospective study was conducted from 2014 to 2016 at the Aga Khan University Hospital, Karachi, and comprised pleural fluid samples of adult patients with and without tuberculosis which were tested for adenosine deaminase levels, and divided into tuberculosis group A and non-tuberculosis group B. Sensitivity, specificity, negative predictive value and positive predictive value were calculated using different cut-offs. Data was analysed using IBM SPSS (Statistical Package for Social Sciences) version 21.0 (IBM Corp., Armonk, NY).

**Results:** Of 155 patients, 46(29.7%) had tuberculosis; 30(65.2%) males and 16(34.8%) females. Those who did not have tuberculosis were 109(70.3%); 69(63.3%) males and 40(36.7%) females. The adenosine deaminase levels were elevated in group A compared to group B ( $p < 0.001$ ). The cut-off of 30U/L showed the highest sensitivity (71.7%) and negative predictive value (87.4%), and a specificity of 82.6%. The cut-off of 50U/L showed the highest specificity (89.9%) with sensitivity 52.2%, and the cut-off of 40U/L showed the highest positive predictive value of 68.9% with sensitivity 67.4% and specificity 87.2%.

**Conclusion:** Pleural fluid adenosine deaminase testing for diagnosing tuberculosis pleuritis revealed highest sensitivity and moderate specificity for cut-off value of 30U/L.

**Keywords:** Tuberculosis, Pleural effusion, Adenosine deaminase, Diagnosis.

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### Introduction

Tuberculosis (TB) is one of the leading infectious causes of morbidity and mortality worldwide. In 2018 there were 10 million new cases and 1.45 million deaths caused by TB globally.<sup>1</sup> In Pakistan, the estimated incidence reported was 562,000 (265/100,000) and 44,000 patients died in 2018.<sup>1</sup> Pulmonary TB is the most common form of TB, but other parts of the body can also be affected by Mycobacterium tuberculosis (MTB) (extra-pulmonary TB).<sup>2</sup> One of the more common manifestations of extra-pulmonary TB (EPTB) is pleural TB which accounts for pleural effusion in up to 25% TB patients.<sup>3</sup> A delayed type hypersensitivity reaction in response to rupture of sub-pleural focus of MTB infection that increases vascular permeability has been noted to be the cause of TB effusion.<sup>4</sup>

Patients with pleural TB mostly present with fever, cough and pleuritic chest pain.<sup>5</sup> Pleural TB is usually diagnosed by pleural fluid microscopy or culture of acid-fast bacilli (AFB) or by pleural biopsy. Caseating granulomas are the

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characteristic finding on histopathology.<sup>6,7</sup> However, these methods are not only invasive, but also time-consuming.<sup>8</sup> It takes around 4-6 weeks for culture results to be reported which has an impact on immediate clinical decisions to be taken, differentials to be ruled out, and ultimately delay in timely treatment.<sup>8</sup> Moreover, specimens from pleural space have a low bacillary burden and decreases the sensitivity of culture results.<sup>8</sup>

Due to the lack of sensitivity of diagnostic assays and relative difficulty in pleural tissue sampling compared to thoracentesis, biomarkers are being increasingly looked upon in pleural effusions of patients with TB.<sup>8,9</sup> Among many biomarkers identified, adenosine deaminase (ADA), an enzyme that converts (deoxy)adenosine to (deoxy)inosine, was found to be the most relevant marker to be used as a diagnostic tool.<sup>10</sup> ADA has two isoenzymes; ADA-1, which is present in almost all cells, and ADA-2, which is mainly expressed in monocytes and macrophages and is released following intracellular infection.<sup>11</sup> Activity of both isoenzymes is increased in pleural TB, but predominantly in ADA-2.<sup>11</sup> ADA helps monocytes to mature into macrophages and is also known to be responsible for proliferation and differentiation of lymphoid cells, especially T cells, and is shown to contribute in purine metabolism.<sup>12</sup> Hence, ADA is an

essential component of cellular immunity.<sup>12</sup> Previous studies have shown that activity of ADA increases in pleural effusions of patients with TB because of mycobacterial stimulation of T cells, therefore, ADA has been proposed to be a potential marker for the detection of TB in body fluids, such as pleural, pericardial and peritoneal fluid.<sup>13</sup>

Multiple studies have favoured the utilisation of ADA measurement as a quick, simple and low-cost diagnostic tool for pleural TB with high sensitivity and specificity.<sup>11</sup> Meta-analyses from different countries have revealed high mean sensitivity (ranging 90-94%) and specificity values (88-92%).<sup>10,14-16</sup> Diagnostic ADA levels have also been correlated with age, with 72U/L for those aged 55 years or below, and 26U/L for those >55 years.<sup>17</sup>

The current study was planned to assess the sensitivity and specificity of different cut-off values of pleural fluid ADA levels for the diagnosis of TB effusion in suspected patients in a high-burden country.

## Patients and Methods

The prospective study was conducted from 2014 to 2016 at the Aga Khan University Hospital, Karachi after receiving approval from the institutional ethics review committee. The sample size was calculated using PASS (Power Analysis and Sample Size Software) version 13.0 (NCSS, LLC, Kaysville, Utah, USA). It was determined with 80% power using a two-sided binomial test comparing sensitivities of 0.50 and 0.75 under the null and alternate hypotheses, respectively. The sample size was also calculated with 100% power using a two-sided binomial test comparing specificities of 0.50 and 0.85 under the null and alternate hypotheses, respectively. The target significance level was 0.05 for both tests. The actual significance level achieved by the sensitivity test and specificity test was 0.026 and 0.035, respectively. The prevalence of tuberculous pleural effusion was 29.7%. For sample size calculation, sensitivity and specificity of 0.75 and 0.85 respectively, were assumed to match the lowest values observed in previously available literature from this region in order to obtain the highest sample size.<sup>14,18</sup> Non-probability sampling technique was used. Adult patients with and without TB were included, while patients with transudate pleural effusion were excluded.

After taking written informed consent from the subjects, diagnosis of TB effusion was made on the basis of criteria mentioned in literature.<sup>19</sup> Pleural fluid AFB smear or culture positive, and/or caseating granuloma on pleural biopsy. Alternatively, clinical features indicative of TB (fever and/or cough >3 weeks, night sweats, weight-loss), signs of pleural effusion on chest examination, and chest X-ray showing pleural effusion; exudative lymphocytic pleural effusion on pleural fluid analysis as per Light's

criteria;<sup>20</sup> and treatment response assessment which was done at 2 months and 6 months of anti-TB therapy in patients with clinically diagnosed TB effusion. Criteria for successful treatment was resolution of symptoms and resolution of effusion on chest X-ray.

Pleural fluid samples of the subjects were tested for adenosine deaminase levels tested for ADA levels. Results of ADA were blinded to avoid bias in the determination of patients' clinical diagnoses. The subjects were subsequently divided into TB group A and non-TB group B. Demographic, clinical and microbiological data was recorded.

For specimen processing, an aliquot of pleural fluid from each sample for ADA activity was centrifuged at 2,000 RPM for 10 minutes. Supernatant was separated and stored at -80°C until further processing.<sup>21</sup>

ADA levels were determined using Adenosine Deaminase Assay Kit (Diazyme Laboratories, San Diego, CA, USA) as per the manufacturer's instructions in pleural fluid samples stored at -80°C. The assay used was based on Berthelot reaction, as described by Giusti and Galanti.<sup>22</sup> The cut-off defined by the manufacturer for a positive result was 30U/L where one unit of ADA corresponded to the amount of enzymatic activity required to release 1µmol of ammonia per minute from adenosine under defined conditions.<sup>21</sup>

Data was analysed using IBM SPSS (Statistical Package for Social Sciences) version 21.0 (IBM Corp., Armonk, NY) and Graphpad PRISM. Data was presented as mean values with standard deviation (SD). Student's t test and Mann-Whitney U test were used to compare the groups. The optimal cut-off value for ADA was taken as 30U/L. Predictive value analyses were performed using MedCalc 20.110 (MedCalc Software, Ostend, Belgium).  $P < 0.05$  was considered statistically significant.

## Results

Of 155 patients, 46(29.7%) had TB; 30(65.2%) males and 16(34.8%) females. Those who did not have TB were 109(70.3%); 69(63.3%) males and 40(36.7%) females. Within group A, 20(43.5%) cases had TB diagnosis microbiologically confirmed, and 26(56.5%) were clinically diagnosed.

Besides, a decreased trend of total leucocyte count was observed in group A patients compared to group B; protein levels were significantly raised in group A compared to group B ( $p < 0.001$ ); glucose levels were lower in group A ( $p > 0.05$ ), and there was increased lactate dehydrogenase (LDH) activity in group A compared to group B (Table-1).

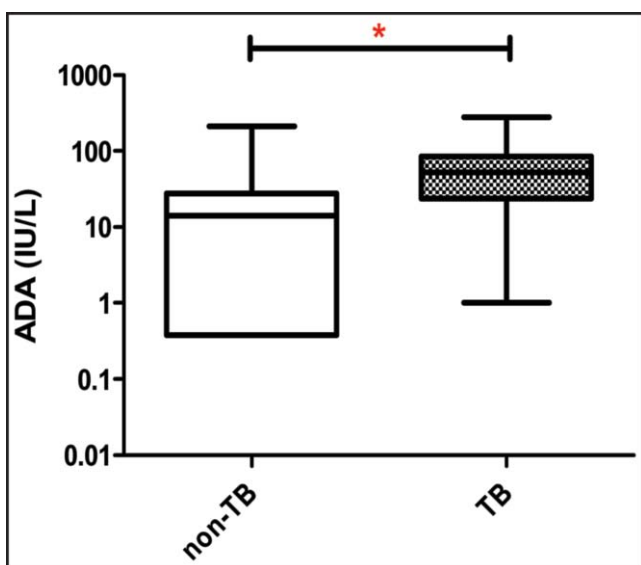
ADA levels were elevated in group A  $72.17 \pm 68.13$  compared to group B  $23.21 \pm 36.54$  ( $p < 0.001$ ) (Figure).

**Table-1:** Characteristics of study population.

Parameters	Non-TB (n=109)	TB (n=46)	P value
Age (Years) (mean±SD)	51.4±21.9	46.6±19.6	0.207
Gender	0.821		
Male (n=99)	69 (63.3%)	30 (65.2%)	
Female (n=56)	40 (36.7%)	16 (34.8%)	
Pleural fluid protein concentration (g/dl)	4353.8±1024.0	5030.2±881.7	<0.001
Pleural fluid LDH (IU/L)	901.2±1459.0	4226.7±12202.8	0.142
Total leucocyte count (cells/mm3)	11.7±11.7	8.2±4.6	0.076
Pleural fluid glucose concentration (g/dl)	107.9±62.4	86.8±48.3	0.057

TB: Tuberculosis, M:F: Male:female, LDH: Lactate dehydrogenase test. SD: Standard deviation. Statistical analysis was done using t test. P values <0.05 were considered statistically significant.

The cut-off of 30U/L showed the highest sensitivity (71.7%) and negative predictive value (NPV) (87.4%), and a specificity of 82.6%. The cut-off of 50U/L showed the highest specificity (89.9%) with sensitivity 52.2%, and the cut-off of 40U/L showed the highest positive predictive value (PPV) of 68.9% with sensitivity 67.4% and specificity



**Figure:** Adenosine deaminase (ADA) activity in tuberculous (TB) and non-TB pleural effusions; Box and whiskers plots indicate data between 10th and 90th percentiles with median values of each group represented by a horizontal line. \* denotes statistically significant differences (p<0.05) between the two groups.

**Table-2:** Diagnostic accuracy of various cut-off values of adenosine deaminase (ADA) in diagnosing pleural tuberculosis (TB).

Statistic	Cutoff 30 U/L		Cutoff 35 U/L		Cutoff 40 U/L		Cutoff 45 U/L		Cutoff 50 U/L	
	Value	95% CI	value	95% CI	value	95% CI	Value	95% CI	value	95% CI
Sensitivity	71.7%	56.5% to 84.0%	67.4%	52.0% to 80.5%	67.4%	52.0% to 80.5%	52.2%	37.0% to 67.1%	52.2%	37.0% to 67.1%
Specificity	82.6 %	74.1% to 89.2%	83.5%	75.2% to 89.9%	87.2%	79.4% to 92.8%	87.2%	79.4% to 92.8%	89.9%	82.7% to 94.9%
Positive Predictive Value	63.5%	52.6% to 73.1%	63.3%	51.9% to 73.3%	68.9%	56.6% to 79.0%	63.2%	49.4% to 75.0%	68.6%	53.9% to 80.3%
Negative Predictive Value	87.4 %	81.3% to 91.7%	85.9%	79.9% to 90.3%	86.4%	80.6% to 90.6%	81.2%	76.0% to 85.5%	81.7%	76.6% to 85.8%
Accuracy	79.4%	72.1% to 85.4%	78.7%	71.4% to 84.9%	81.3%	74.3% to 87.1%	76.8%	69.3% to 83.2%	78.7%	71.4% to 84.9%
Youden Index	0.524	-	0.509	-	0.492	-	0.403	-	0.421	-

CI: Confidence interval.

87.2% (Table-2).

## Discussion

Among other findings, the current study favoured a cut-off value of 30U/L in terms of diagnostic accuracy of pleural fluid ADA levels as a diagnostic method for TB pleural effusion. A broad range of cut-off values have been described in literature, but the most commonly used threshold has been between 30U/L and 60 U/L.<sup>9,15</sup> The cut-off in the current study was lower because the population was highly predictive of TB. A meta-analysis of studies conducted in Spain revealed a high sensitivity (93%) and specificity (92%) with no significant differences detected for different cut-off values ranging from 23-35U/L to 43-45U/L.<sup>10</sup> A meta-analysis of Brazilian studies on the subject stated a mean sensitivity value of 91.8% and a mean specificity value of 88.4% with ADA cut-off values ranging from 30U/L to 60U/L (mean: 40.7 U/L).<sup>16</sup> Another meta-analysis of Indian studies gave a pooled estimate of 94% and 89% for sensitivity and specificity, respectively.<sup>14</sup> Suggesting that ADA thresholds of 38-42U/L were more useful for excluding disease, and thresholds >42U/L were more useful as a confirmatory marker, the study was unable to comment on the diagnostic performance of high ADA levels.<sup>14</sup> Similar estimates were seen in other meta-analyses of global data.<sup>15,23</sup> To further emphasise on South Asian population, a recent study in India showed sensitivity and specificity of 93.8% and 93.2% respectively when ADA levels were measured in patients suspected of TB pleuritic, and the cut-off was set at 40U/L.<sup>24</sup> A study in Nepal showed sensitivity and specificity of 76.1% and 100% respectively using ADA as a diagnostic test in pleural fluid specimens of patients with TB compared with study subjects with other respiratory diseases when a cut-off of 45U/L was used.<sup>18</sup>

The variation in the results of the current study compared to other studies could be due to different methods used or different cut-offs set for analysis of results by different groups. The characteristics of the population studied also has an impact on the results obtained.

The diagnostic accuracy of ADA can be enhanced by

using different ADA isoenzymes. ADA-1 is increased in bacterial empyema and ADA-2 is more increased in TB effusions. Utilisation of ADA-2 can increase the specificity of ADA for TB diagnosis.<sup>11</sup>

The current study has its limitations as it was conducted in a single tertiary care hospital in Karachi. Since it is a private hospital, the patient population belongs to upper social classes and the complexity of the cases might not be the same. Hence, the findings might not be truly representative of the entire population. Additionally, the study had a relatively small sample. Large-scale, multi-centre studies are needed to evaluate the more sensitive and specific ADA cut-off level for diagnosing TB pleuritis.

## Conclusion

Pleural fluid ADA testing for diagnosing TB pleuritis revealed highest sensitivity and moderate specificity for cut-off value of 30U/L. Considering that different ADA isozymes are elevated in different pathologies, with ADA-2 predominantly increased in TB effusions, the diagnostic accuracy of pleural fluid ADA testing in patients with TB pleuritis can be potentially increased by using ADA-2 values instead.

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