

Predictive models utilizing plural fluid adenosine deaminase (ADA) in optimizing the diagnosis of tuberculous plural effusion (TPE) in an area with a high incidence of tuberculosis and HIV.

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Abstract:

Background: Tuberculous Pleural Effusion (TPE) is the most common form of the extra pulmonary tuberculosis (EPTB) in India. The pleural fluid adenosine deaminase (ADA) is one of the best marker providing reliable basis for a treatment decision in TPE, due to its high sensitivity. The aim of this study was to evaluate the diagnostic usefulness of ADA along with other laboratory data using prediction models for TB decision in high prevalence of tuberculosis (TB) and HIV. **Methods:** 100 patients with suspected pleural effusion (41 TB and 59 non TB) were analyzed retrospectively. Two predictive models, one with pleural fluid ADA, lymphocyte % (model 1) and other with pleural fluid ADA, lymphocyte % and GeneXpert (model 2) were analyzed in 31 culture confirmed cases out of 41 suspected Tuberculous Pleural Effusion. The statistical modelling used analysis adjusted for the following covariates: gender, fever, chest pain, cough, sputum. The final regression tree was derived in. Model 1 selected two variables pleural fluid ADA (ADA >40 U/L) and lymphocytes (>50%) and Model 2 selected three variables, lymphocytes (>50%), pleural fluid ADA (ADA >40 U/L), Gene Xpert.

Both models were validated by combining with culture. **Results:** Model 1 correctly identified 23/31 TB effusions. Model 2 correctly identified 22/31 TB effusions. The sensitivity of models 1 and 2 was 96.9% and 99.9% respectively, specificity 36% and 100%. when combined with culture **Conclusions:** In areas with a high prevalence of tuberculosis and HIV although it is safe to predict TPE with lymphocyte % (>50%), pleural fluid ADA (ADA >40 U/L) and GeneXpert but culture as gold standard always needed for final decision.

Keywords – Adenosine deaminase, Predictive model, tuberculous plural effusion

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I. Introduction

Extrapulmonary TB (EPTB) is accounting for ~15% of cases, but this estimate increases to ~50% in high HIV prevalence settings [1]. Pleural TB, a common form of EPTB, remains a common problem for physicians practicing in high TB and HIV burden settings, particularly in resource limited setting. The diagnosis of pleural TB is challenging due to the paucibacillary nature of biological samples, and the need for diagnostic confirmation using invasive, expensive, and time consuming procedures such as blind pleural biopsy, imaging-guided-pleural biopsy, and medical or surgical thoracoscopy [2]. There are also limited data from high TB and HIV prevalence settings. In previous published data there is lack of comparative analysis with other commonly used biomarkers and correlation with bacterial load. Furthermore, there are also limited data from high TB and HIV prevalence settings.

A definite diagnosis of tuberculous pleural effusion (TPE) can be difficult to make because of the low sensitivity and/or specificity of noninvasive traditional diagnostic tools. In most series of patients with TPE, the results of pleural fluid staining for acid-fast bacilli are virtually always negative, and pleural fluid cultures are positive for mycobacteria in 25% of cases. On the other hand, a pleural biopsy specimen will demonstrate granulomatous pleuritic in 80% of patients with TPE, and when a culture of a biopsy specimen is combined with histologic examination, the diagnosis can be established in approximately 90% of cases.

Adeno-sine deaminase (ADA) catalyzes the conversion of adenosine to inosine, a stage of purine metabolism. Since 1978, when ADA activity was found to be high in tuberculous pleural exudates, ADA has

been used in the diagnosis of tuberculous pleural effusions [3–6]; overall, its sensitivity in this role has been 99% and its specificity 93%.

Usefulness of adenosine deaminase (ADA) estimation in pleural fluid has been shown as a reliable chemical bio-marker specially when there is suspicion of tuberculosis in endemic areas. Sometimes the increase is marked in early stages of the disease and in some other conditions with neutrophilic effusions like in Parapneumonic and empyema [7]. Researchers have established that ADA level rarely exceeds the cut-off set for tuberculous effusion in non-tuberculous lymphocytic effusions [8].

In practice, the ability to predict the presence or Absence of disease from test results is dependent on the prevalence of the disease in the population tested, as well as on the sensitivity and specificity of the test. Our aim of this work to assess whether utilizing in predictive models with ADA test which has high sensitivity and specificity will help to optimise Tubercular plural effusion in HIV and TB high prevalent population.

II. Materials and Methods

2.1 This retrospective study was approved by the Institutional Review Board. The study period was from November 2016 to November 2017.

2.2 We evaluated 100 consecutive cases of patients with suspected, symptomatic exudative lymphocytic pleural effusion according to Light's criteria.

2.3 After the routine procedure of informed consent, a detail history, a thorough physical examination and routine investigations were done in all the patients. The diagnostic criteria were adopted to label a case as tuberculous were as follows: 1) Presence of acid-fast bacilli. 2) Bacteriological confirmation of presence of Mycobacterium tuberculosis (direct smear or culture or histological finding).

2.4 At the time of the thoracentesis, pleural fluid was collected in a citrated tube for ADA quantification, in an ethylenediaminetetra-acetic acid tube for measurement of total and differential WBC counts, and in a plain tube for protein and lactate dehydrogenase (LDH) analysis. For ADA quantification, the pleural fluid aspirates were immediately centrifuged at 3,000 revolutions per minute for 20 min at 4°C. The supernatant was stored at 2–70°C until analysis.

2.5 Total ADA was measured by the colorimetric method of Giusti, [9] employing reagents optimized for ammonia measurement by Kaplan and the laboratory cutoff for tuberculous pleural effusion was 40 U/L identified from ROC (Receiver Operating Curve).

Patients were informed about study and written consent was taken before any investigation. All exudative pleural effusion cases were included and patients with transudative pleural effusion, malignant pleural effusion, empyema, hemothorax and patients on chemotherapy were excluded from this study.

2.6 Along with thorough clinical history and examination clinical record for symptoms and laboratory data was collected for following tests. Haematological examination record was used for Hemoglobin, Total Leucocyte count, Differential leucocyte count, Erythrocyte sediment rate. Radiological examination gave inputs of Chest X ray. Microbiological tests revealed results of ZN stain for AFB, Culture and GeneXpert analysis. Pleural fluid examination was essential for differential count and Lymphocyte/ Neutrophil ratio, LDH, Protein concentration which otherwise was routinely done. All biochemical measurements were performed on a clinical chemistry analyzer (ADVIA 2400, SIEMENS HEALTHCARE DIAGNOSTICS) using standard methodology.

2.7 We performed two regression tree models. The first included the ADA level in pleural fluid with >50% lymphocyte. (Model 1) while the second (Model 2) includes ADA with >50% lymphocyte and GeneXpert. The statistical modelling used analysis adjusted for the following covariates: gender, fever, chest pain, dyspnoea, cough, sputum, size and location of the effusion. (Figure1)

III. Results

In one year of the study period 150 cases of pleural effusion were studied out of 100 patients of suspected plural effusion were included in study by using simple random method. Out of 100 cases, 41 were confirmed cases (using any one criteria) of tubercular pleural effusion and rest 59 cases were non tubercular pleural effusion cases. (Figure1)

Among 100 suspected pleural effusion patients 52% were male and 48% females. Subjects were classified in age groups starting from 0-10 years to 50-60 and >60 and median age was 32. (Table 1) .

Using a cut-off point of the pleural fluid ADA (40 IU/L) with AUC of 96.7 (sensitivity 96.7%, specificity 84%, NPV 88%, PPV 95% and accuracy 91%) discrimination between tuberculous and other causes of pleural effusion occurred. Table 2 showed the Positivity rate by ADA 100% (41/41), by smear 46%(19/41), by culture 68% (28/41) and by GeneXpert 95% (39/41) in 41 cases of Tubercular pleural effusion in which a positive MTB (*Mycobacterium tuberculosis*) fluid culture and/or positive MTB biopsy culture and/or histology in keeping with MTB infection used as a reference to label as Tuberculous (TP).When we used the regression trees, the model that included the determination of ADA with >50% lymphocytes (model 1) only evaluated two variables: ADA (primary variable) and the culture.

The diagnostic yield of this model was (sensitivity 96.9%, specificity 34%). This regression tree is supported by the studies of Burgess and co-workers [10] and Diacon and colleagues, [11] as these authors used the same variables to establish the diagnosis of TPE in their series. (Table 3).

Model 2 of the regression trees used 3 variables ADA with >50% lymphocytes (ADAcut-off point 40%) as primary variable, GeneXpert and culture. With the primary variable, the model attempts to separate the effusions of infectious, non-tuberculous origin, which should have a low percentage of lymphocytes (only one false negative was observed with this variable). The diagnostic yield of this model was (sensitivity 100%, specificity 96%). (Table 3).

Figure 1: Regression trees for predicting tuberculous pleural effusions (TPE).

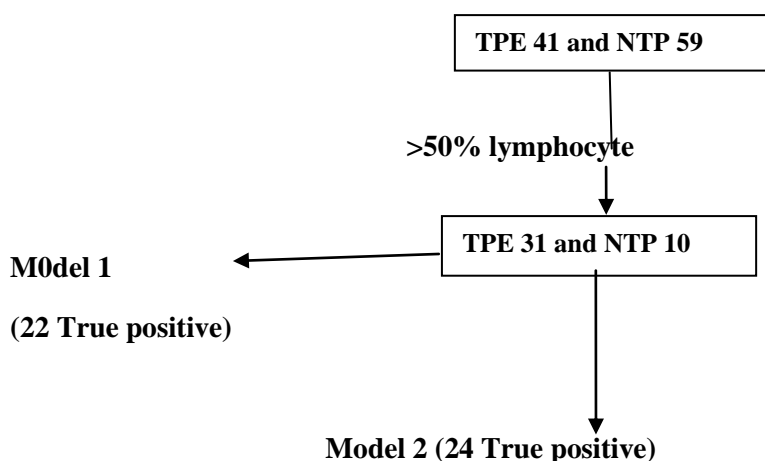


Table -1 Age and Sex distribution of patients of plural effusion

Age (Years)	Groups	Male %	Female%	Number of patients %
0-10		9	11	20
11-20		4	8	12
21-30		12	6	20
31-40		13	13	26
41-50		9	6	15
51-60		0	4	4
>60		3	0	3
Total		52	48	100

Table-2: Distribution of ADA, Smear, Culture and GeneXpert results among Tuberculous (TP) and nontuberculous (NTP) Group (n=100)

n=100	ADA >40 IU/L	ADA <40 IU/L	Smear positive	Smear negative	Culture Positive	Culture negative	GeneXpert Positive	GeneXpert negative
TPE (41)	41	00	19	21	28	10	39	02
NTP (59)	13	43	02	59	03	54	02	50

Table-3 Predictive analysis of Model 1 and Model 2

No.	Parameter	Model 1 ADA +>50% lymphocyte and culture	Model 2 ADA +>50% lymphocyte, GeneXpert and culture
1	Sensitivity% (95% CI)	96.9 %	95.6% ¹
2	Specificity% (95% CI)	34 %	100%
3	Positive Predictive Value	85.7%	100%
4	Negative Predictive Value	67%	50%
5	Accuracy	76 %	83 %

III. Discussion

ADA activity is higher in activated T lymphocytes as mitogenic and antigenic challenges. ADA activity depends on how rapidly the. Some authors believe that mycobacterial antigenic stimulation leads to the increased production of ADA, T lymphocytes proliferate which is essential to the accelerated T-cell blastogenesis and accumulation of CD4 subpopulation, commonly seen in tuberculous effusions.

Our study showed no correlation between the lymphocyte counts and ADA activity, but the predictive model utilizing ADA with more than 50% lymphocytic count, GeneXpert optimize the detection of Plural TB along with culture which is gold standard. ADA measurement was limited to lymphocytic pleural fluids which we thought to reduce false-positive diagnoses of TPE.

In areas with a high prevalence of tuberculosis, the proportion of false-positive results will be obviously less. However, a limitation of the test in this setting is that culture results are necessary to guide antituberculosis chemotherapy. Culture results are particularly necessary if drug-resistant tuberculosis is prevalent. Exudative lymphocytic pleural effusions are commonly encountered in clinical practice but often constitute difficult diagnostic problems. The diagnosis of tuberculous pleural effusion is important because tuberculosis is normally a treatable cause of exudative lymphocytic pleural effusion [10].

The primary difficulty in getting a diagnostic confirmation of tuberculous pleural effusion is the identification of mycobacteria in the pleural fluid.

Few authors had studied the diagnosis of TB pleural effusion using multivariate analysis and the results have some controversies. Carrion-Valero et al. [11] assessed the value of discriminate analysis as a method of optimizing the diagnosis of pleural TB but they did not included ADA in their model. A clinical score for differential diagnosis between TB and malignant pleural effusions, with retrospective data, was evaluated using multivariate analysis and the authors derived two models, with and without ADA [12]. Ghanei et al. [13] examined a statistical method by combining the diagnostic efficiency of ADA, pleural fluid protein, lactate dehydrogenase and cellular components to the diagnosis of pleural tuberculosis. Therefore, predictive models utilizing ADA in combination with other biomarkers seems to be a new and promising field of investigation.

IV. Conclusion

The predictive model utilizing ADA, in combination with other routine biomarkers like Xpert-MTB/RIF is particularly useful to clinicians as it prompts further work-up and tissue biopsy in patients who are unlikely to have TB, however further prospective testing is required.

Limitations of this work is the relatively small number of patients with pleural TB (usually quoted as part of a larger series of patients with EPTB), a paucity of biopsy-proven or culture positive samples as a gold standard. In conclusion, the predictive model utilizing ADA along with GeneXpert assay can optimize the diagnosis of Tubercular plural effusion but not alternative to biopsy and culture rather as a screening test followed by culture to guide further diagnostic procedures and management of an exudative pleural effusion.

ADA estimation being a simple, low cost, rapid and non-invasive test should become an integral part of the diagnostic work up of exudative pleural effusions in suspected cases of tuberculosis under national screening program.

References

- [1]. Light RW: Update on tuberculous pleural effusion. *Respirology* 2010, 15(3):451–458.
- [2]. Koegelenberg CF, Diacon AH: Pleural controversy: close needle pleural biopsy or thoracoscopy-which first? *Respirology* 2011, 16(5):738–746.
- [3]. Gecia-monco J C CNS Tuberculosis. *Neurologic clinics*. Marra C.M. ed.; 1999. 17(4):737-60
- [4]. Piras MA, Gakis C, Budroni M, Andreoni G. Adenosine deaminase activity in pleural effusions: an aid to differential diagnosis. *Br Med J* 1978; 2: 1751–1752
- [5]. Ocaña I, Martínez Vázquez JM, Segura RM, Fernández de Sevilla T, Capdevila JA. Adenosine deaminase in pleural fluids: test for diagnosis of tuberculous pleural effusion. *Chest* 1983; 84: 51–53
- [6]. Pettersson T, Ojala K, Weber TH. Adenosine deaminase in the diagnosis of pleural effusion. *Acta Med Scand* 1984; 215: 299–304
- [7]. Fontán J, Vereá H, Pérez J, Domínguez L, Martín MT, Montero MC. Diagnostic value of simultaneous determination of pleural adenosine deaminase and pleural lysozyme/serum lysozyme ratio in pleural effusions. *Chest* 1988; 93: 303–307
- [8]. Gakis C, Calia GM, Naitana AGV, Ortu AR, Contu A. Serum and pleural adenosine deaminase activity: correct interpretation of the findings. *Chest* 1991; 99: 1555–1556
- [9]. Burgess LJ, Maritz FJ, Le Roux I, Taljaard JJ. Combined use of pleural adenosine deaminase with lymphocyte/ neutrophil ratio. Increased specificity for the diagnosis of tuberculous pleuritis. *Chest* 1996; 109(2):414-419
- [10]. Material methods
- [11]. Giusti G. Adenosine deaminase. In: Bergmeyer HU, ed. *Methods of enzymate analysis*. New York, NY: Academic Press, 1974; 1092–1099
- [12]. M. Lamsal, N. Gautam, N. Bhatta, et al, Diagnostic utility of adenosine deaminase (ADA) activity in pleural fluid and serum of tuberculous and non-tuberculous respiratory disease patients, *South Asia J. Trop. Med. Public Health* 38 (2) (2007) 363–369
- [13]. Carrion-Valero F., Perpiña-Tordera M. Screening of tuberculous pleural effusion by discriminant analysis. *Int J Tuberc Lung Dis* 2001; 5(7):673-9.
- [14]. Porcel J.M., Vives M. Differentiating tuberculous from malignant pleural effusions: a scoring model. *Med Sci Monit* 2003; 9(5):CR227-32.
- [15]. Ghanei M., Aslani J., Bahrami H., Adhami H. Simple Method for Rapid Diagnosis of Tuberculosis Pleuritis: A Statistical Approach. *Asian Cardiovasc Thorac Ann* 2004; 12(1):23-9.

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