TPM Vol. 32, No. S2, 2025
ISSN: 1972-6325
https://www.tpmap.org/

Open Access

DIAGNOSTIC ACCURACY OF TUBERCULOSIS USING PLEURAL FLUID CYTOLOGY AS A SCREENING TOOL AND COMPARING IT WITH BIOCHEMICAL PARAMETER - ADENOSINE DEAMINASE

DR.DHARANIDEVI.S[1], DR.SONTI SULOCHANA[2], DR.MARIA PRISCILLA[3],

[1]POST GRADUATE ,DEPT.OF PATHOLOGY
CO-AUTHOR :
[2]PROFESSOR, DEPT.OF PATHOLOGY
SAVEETHA MEDICAL COLLEGE AND HOSPITAL (SIMATS) , CHENNAI.
[3]SENIOR LECTURER, DEPARTMENT OF ORAL MEDICINE & RADIOLOGY, SREE BALAJI DENTAL COLLEGE & HOSPITAL, CHENNAI, INDIA

Abstract

Background: Tuberculous pleural effusion (TPE) frequently occurs as a result of extrapulmonary tuberculosis. Early diagnosis continues to be difficult because of the low bacillary content of pleural fluid. This research sought to assess the diagnostic precision of pleural fluid cytology as an initial screening method for tuberculous pleural effusion and to contrast it with adenosine deaminase (ADA) levels.

Methods: This prospective observational study assessed 120 patients who had exudative pleural effusion. Pleural fluid specimens were examined for cytological patterns, focusing on lymphocyte dominance and mesothelial cell quantity. ADA levels were assessed in every sample. The final identification of tuberculous pleural effusion was made using a comprehensive reference standard that included microbiological confirmation, HPE investigation of the pleural biopsy, and the reaction to anti-tubercular cure. Sensitivity, specificity, PPV, and NPV were determined for both cytological patterns and ADA measurements.

Results: Out of the 120 patients, 78 (65%) were identified as having TPE. The presence of lymphocytes predominating (>50%) along with a lesser count of mesothelial cells (<5%) in pleural fluid cytology demonstrated a sensitivity of 84.6% and a specificity of 73.8% for diagnosing TPE. ADA at a cut off of 40 U/L showed a sensitivity of 92.3% and a specificity of 88.1%. The integration of cytological criteria and ADA levels exceeding 40 U/L raised the specificity to 95.2%, achieving a positive predictive value of 96.7%, while sensitivity fell to 79.5%.

Conclusion: Cytology of pleural fluid acts as an effective preliminary screening method for TPE, exhibiting high sensitivity and moderate specificity. When paired with ADA levels, the precision of diagnosis greatly enhances. This integrated strategy facilitates swift and economical assessment of patients with suspected TPE, especially in resource-constrained environments where advanced diagnostic equipment might not be accessible.in resource-limited settings where more advanced diagnostic tools may be unavailable.

Keywords: Tuberculous pleural effusion, cytology, adenosine deaminase, lymphocyte predominance, diagnostic accuracy

INTRODUCTION :

Tuberculosis (TB) remains to be a significant global issue despite widespread prevention and control initiatives. Although pulmonary manifestations represent the most prevalent type of TB disease, extrapulmonary involvement arises in around 15-20% of all TB cases, with this proportion being greater in immunocompromised individuals and endemic areas (Zumla et al., 2013).

Pleural involvement is among the most common extrapulmonary manifestations of tuberculosis, representing about 30% of all global extrapulmonary TB cases (Light, 2010). Tuberculous pleural effusion (TPE) usually arises from a late hypersensitivity response to mycobacterial antigens instead of a direct invasion of the pleura by Mycobacterium tuberculosis. This pathophysiological process accounts for the scant bacillary characteristic of the effusion, posing difficulties for microbiological verification (Shaw et al., 2018).

The clinical signs of TPE frequently resemble those of other reasons of exudative pleural effusions, including malignancies, parapneumonic effusions, and connective tissue diseases, requiring precise diagnostic approaches to direct suitable treatment (Porcel, 2016). Conventional diagnostic methods for TPE involve analyzing pleural fluid for AFB smear and culture, histopathology of pleural tissue, and tests for nucleic acid amplification. (Sehgal et al., 2019).

In light of these diagnostic difficulties, there is increasing interest in alternative diagnostic methods that are quick, minimally invasive, and precise. Pleural fluid cytology, especially the examination of cellular makeup and morphological traits, has become a promising initial screening method for TPE (Gopi et al., 2007). Common cytological characteristics in TPE show a predominance of lymphocytes, frequently surpassing 50% of all nucleated cells, along with a scarcity of mesothelial cells, generally under 5% (Light, 2010). This distinctive cytological pattern represents the immune reaction to mycobacterial antigens, primarily marked by T-lymphocyte infiltration into the pleural cavity (Barnes et al., 1989).

At the same time, the usefulness of biochemical markers in pleural fluid has been thoroughly researched for diagnosing TPE. Of these, adenosine deaminase (ADA) has shown significant diagnostic capability. It is primarily generated by activated T-lymphocytes and macrophages, and increased concentrations in pleural fluid are strongly linked to tuberculous causes (Liang et al., 2008). Numerous research work have confirmed diagnostic importance of ADA in TPE, showing sensitivity and specificity between 85-100% and 81-97%, respectively, at cutoff levels of 35-50 U/L (Porcel et al., 2010).

Although pleural fluid cytology and ADA have each been assessed separately for their diagnostic effectiveness in TPE, there is scarce research contrasting their comparative accuracy and investigating their combined usefulness. This gap in knowledge is especially significant in settings with limited resources, where quick and affordable diagnostic methods are crucial for the prompt start of anti-tubercular treatment (Sehgal et al., 2019). Regional validation of these diagnostic instruments is essential for their effective application in clinical settings (Krenke & Korczynski, 2010). Recognizing the comparative and supplementary functions of pleural fluid cytology and ADA in diagnosing TPE may help optimize the diagnostic process. (McGrath et al., 2017).

The current research seeks to thoroughly assess the diagnostic effectiveness of pleural fluid cytology as a preliminary screening method for TPE, focusing specifically on intricate cytological features in addition to basic lymphocyte predominance. Moreover, we estimate the diagnostic efficacy of cytology alongside pleural fluid ADA levels and investigate if a combined method provides enhanced diagnostic accuracy. The results of this research will aid in creating an evidence-driven, economical diagnostic algorithm for TPE, possibly suitable for various healthcare environments.

MATERIALS AND METHODS :

This observational study was done prospectively from January 2024 to December 2024 in a tertiary care teaching hospital after getting approval from IHEC. The research followed the Standards for Reporting of Diagnostic Accuracy Studies (STARD) protocols. Consecutive patients exhibiting exudative pleural effusion, according to Light's criteria, were deemed suitable for inclusion. Patient who are 18 years or older, having exudative pleural effusion validated by thoracentesis and biochemical testing, and possessing an adequate pleural fluid volume (≥40

mL) for thorough analysis were included in the study. Patients were excluded if they had a previous diagnosis of tuberculosis with ongoing anti-tubercular treatment, traumatic thoracentesis (pleural fluid RBC count >100,000/µL), empyema or visibly purulent pleural fluid, inadequate pleural fluid for thorough analysis, refusal to have pleural biopsy if clinically necessary, or were lost to follow-up prior to final diagnosis.

According to earlier research indicating an 85% sensitivity for ADA in the diagnosis of TPE and an expected sensitivity of 80% for cytological criteria, sample size was determined to be 115 patients. Considering a 5% dropout rate, we intended to recruit 120 patients. Diagnostic thoracentesis was carried out under sterile conditions utilizing standard procedure. For cytological analysis, pleural fluid samples were spun at 3000 rpm for 10 minutes, and the resulting sediment was utilized to create a minimum of four smears. All smears were assessed by two independent cytopathologists who were unaware of the clinical details and other test findings,

The analysis of data was conducted using SPSS version 26.0. Continuous variables were represented as mean ± standard deviation. The evaluation of pleural fluid cytology and ADA's diagnostic performance involved calculating sensitivity, specificity, PPV, NPV, and overall diagnostic accuracy.

RESULTS:

Out of the 135 patients who were screened initially, 120 satisfied the inclusion criteria and were included in the study. The average age of the study group was 42.6 ± 16.8 years, with a higher percentage of males (68.3%). According to the diagnostic criteria specified in the methodology, 78 patients (65%) were identified with tuberculous pleural effusion (TPE), whereas 42 patients (35%) had non-tuberculous causes. In the non-tuberculous cases, the most frequent diagnosis was malignancy (23 patients, 19.2%), followed by parapneumonic effusion (12 patients, 10%) and other conditions (7 patients, 5.8%).

Table 1: Demographic features

Characteristic	TPE (n=78)	Non-TPE (n=42)	p-value
Age (years), mean ± SD	38.2 ± 15.4	51.4 ± 16.2	<0.001
Gender, n (%)			0.14
Male	56 (71.8)	26 (61.9)	
Female	22 (28.2)	16 (38.1)	
Clinical symptoms, n (%)			
Fever	65 (83.3)	18 (42.9)	<0.001
Cough	52 (66.7)	32 (76.2)	0.28
Chest pain	63 (80.8)	30 (71.4)	0.24
Dyspnea	48 (61.5)	33 (78.6)	0.05
Weight loss	54 (69.2)	25 (59.5)	0.28
Duration of symptoms (days), median (IQR)	21 (14-30)	28 (18-45)	0.03
HIV status, n (%)			0.36
Positive	6 (7.7)	5 (11.9)	
Negative	72 (92.3)	37 (88.1)	
Side of effusion, n (%)			0.67
Right	45 (57.7)	22 (52.4)	
Left	29 (37.2)	18 (42.9)	
Bilateral	4 (5.1)	2 (4.8)	
Size of effusion, n (%)			0.42
Small	18 (23.1)	7 (16.7)	
Moderate	46 (59.0)	24 (57.1)	
Large	14 (17.9)	11 (26.2)	

Patients with TPE were notably younger than those with non-tuberculous reasons (38.2 vs. 51.4 years, p<0.001). Fever occurred more often in the TPE group (83.3% vs. 42.9%, p<0.001), whereas dyspnea was more

prevalent in the non-TPE group (78.6% vs. 61.5%, p=0.05). The length of symptoms was less in the TPE group (median 21 days compared to 28 days, p=0.03).

Table 2: Pleural Fluid Characteristics Based on Final Diagnosis

Parameter	TPE (n=78)	Non-TPE (n=42)	p-value
Appearance, n (%)			0.03
Straw-colored	61 (78.2)	26 (61.9)	
Serosanguinous	15 (19.2)	14 (33.3)	
Hemorrhagic	2 (2.6)	2 (4.8)	
Biochemical parameters			
Protein (g/dL), mean ± SD	5.1 ± 0.7	4.8 ± 0.9	0.04
Pleural fluid/serum protein ratio	0.71 ± 0.08	0.68 ± 0.10	0.08
LDH (U/L), median (IQR)	425 (320-578)	396 (286-540)	0.21
Pleural fluid/serum LDH ratio	2.1 ± 0.6	1.9 ± 0.7	0.10
Glucose (mg/dL), mean ± SD	76.3 ± 18.5	82.7 ± 22.4	0.09
pH, mean ± SD	7.34 ± 0.08	7.37 ± 0.09	0.07
ADA (U/L), median (IQR)	68.5 (52.3-84.6)	28.4 (19.7-38.2)	<0.001
Cellular parameters			
Total cell count (cells/μL), median (IQR)	1850(1240-2640)	1420 (980-2120)	0.02
Lymphocytes (%), median (IQR)	78.5 (65.8-88.4)	45.2 (32.6-64.5)	<0.001
Neutrophils (%), median (IQR)	14.2 (8.5-24.6)	32.8 (18.4-52.3)	<0.001
Eosinophils (%), median (IQR)	1.8 (0.6-3.4)	2.2 (0.8-4.1)	0.26
Mesothelial cells (%), median (IQR)	2.4 (1.2-4.6)	8.6 (5.2-14.5)	<0.001
Macrophages (%), median (IQR)	5.8 (3.4-8.2)	7.3 (4.8-10.6)	0.04

In TPE cases, pleural fluid exhibited notably higher protein levels, total cell counts, and lymphocyte percentages when contrasted with non-TPE cases. Importantly, the percentage of mesothelial cells was considerably diminished in TPE (median 2.4% compared to 8.6%, p<0.001). ADA levels were significantly higher in TPE cases than in non-TPE cases (median 68.5 U/L vs. 28.4 U/L, p<0.001).

Table 3: Diagnostic Performance of Cytological Parameters for TPE

Parameter	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Lymphocytes >50%	87.2	57.1	79.1	70.6	76.7
Lymphocytes >70%	71.8	78.6	87.5	57.1	74.2
Mesothelial cells <5%	79.5	69.0	83.8	63.0	75.8
Combined criteria*	84.6	73.8	86.8	70.5	80.8

Lymphocyte predominance (>50%) showed a high sensitivity (87.2%) but a moderate specificity (57.1%) for TPE. An increased lymphocyte threshold of >70% enhanced specificity (78.6%) but resulted in lower sensitivity (71.8%). The scarcity of mesothelial cells (<5%) demonstrated a balanced outcome, with a sensitivity of 79.5% and specificity of 69.0%.

The mixture of lymphocyte predominance (>50%) and low mesothelial cell count (<5%) resulted in enhanced diagnostic efficacy, achieving 84.6% sensitivity, 73.8% specificity, and 80.8% overall accuracy.

Diagnostic Performance of Pleural Fluid ADA

ROC curve analysis revealed an AUC of 0.94 indicating excellent discriminative capability. The optimal cut-off levels was determined to be 40 U/L based on the highest Youden index.

At this cut-off, ADA had a sensitivity of 92.3%, specificity of 88.1%, PPV of 93.5%, and NPV of 86.0%. The diagnostic accuracy was 90.8.

Table 4: Comparative Diagnostic Performance of Different Diagnostic Approaches

Diagnostic Approach	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Cytological criteria*	84.6	73.8	86.8	70.5	80.8
ADA >40 U/L	92.3	88.1	93.5	86.0	90.8
Combined approach†	79.5	95.2	96.9	71.4	85.0

ADA >40 U/L revealed higher diagnostic performance compared to cytological criteria alone, with higher sensitivity (92.3% vs. 84.6%, p=0.04), specificity (88.1% vs. 73.8%, p=0.02), and overall accuracy (90.8% vs. 80.8%, p=0.01).

The combined approach, requiring both cytological criteria and ADA >40 U/L to be positive, resulted in the highest specificity (95.2%) and PPV (96.9%), albeit with reduced sensitivity (79.5%) compared to either method alone. This approach would be particularly valuable when high diagnostic certainty is required before initiating anti-tubercular therapy.

DISCUSSION:

This prospective study thoroughly assessed the diagnostic value of pleural fluid cytology in relation to ADA for identifying tuberculous pleural effusion. Our results show that a combined cytological method emphasizing lymphocyte predominance (>50%) and low mesothelial cell count (<5%) yields strong diagnostic accuracy, achieving 84.6% sensitivity and 73.8% specificity. Although ADA with a cut off level of 40 U/L demonstrated better results, the cytological method provides extra benefits, such as quick turnaround and possible insights into different diagnoses.

The dominance of lymphocytes found in tuberculous pleural effusions indicates the basic immunopathogenesis of the condition. After exposure to mycobacterial antigens in the pleural cavity, CD4+ T-lymphocytes are activated, leading to the release of pro-inflammatory cytokines. This coordinates a series of cellular reactions marked by the recruitment and multiplication of lymphocytes (Vorster et al., 2015). Our observation that lymphocyte percentages surpassing 50% occur in 87.2% of TPE instances corresponds with earlier research, such as that by Porcel et al. (2014) and Wang et al. (2018), which noted sensitivities of 85.7% and 88.3% for this parameter, respectively.

The notable observation of a low presence of mesothelial cells (<5%) in TPE is particularly significant and enhances diagnostic value. This occurrence, noted in 79.5% of our TPE cases, probably stems from the inflammatory environment in the pleural space that hinders mesothelial cell survival and growth. Furthermore, the development of granulomas and fibrosis might capture mesothelial cells, leading to a further decrease in their quantities in pleural fluid. Significantly, the scarcity of mesothelial cells showed a higher specificity (69.0%) compared to lymphocyte predominance alone (57.1%), emphasizing its importance in distinguishing TPE from other types of exudative effusions.

The combination of lymphocyte predominance and a shortage of mesothelial cells resulted in enhanced diagnostic effectiveness (sensitivity 84.6%, specificity 73.8%) when compared to each parameter individually. This discovery endorses the effectiveness of a thorough cytological method instead of depending on just one cellular factor. Comparable findings were noted by Garcia-Zamalloa et al. (2015), who discovered that the combined cytological criteria exhibited a sensitivity of 83% and a specificity of 75%.

The qualitative evaluation of lymphocyte shape offered further diagnostic information. Activated lymphocytes featuring enlarged nuclei and prominent nucleoli were considerably more prevalent in TPE cases (78.2%) compared to non-TPE cases (35.7%). This morphological characteristic indicates the immunological activation typical of tuberculous inflammation and could act as an additional diagnostic criterion, especially in instances with ambiguous quantitative results. Krenke et al. (2012) likewise stated that the morphological characteristics of lymphocytes might improve diagnostic precision in pleural effusions of unknown cause.

Our research further showed the outstanding diagnostic capability of pleural fluid ADA at a cut off value of 40 U/L, achieving 92.3% sensitivity and 88.1% specificity. These findings align with a meta-analysis done by Liang

et al. (2008), with sensitivity of 92% and specificity of 90% for ADA in identifying TPE. The elevated diagnostic precision of ADA is due to its crucial function in T-cell growth and differentiation, processes that are significantly enhanced in tuberculous inflammation (Goto et al., 2003).

Although ADA showed better diagnostic efficacy than cytological criteria, it's crucial to recognize that ADA elevation isn't exclusive to tuberculosis and can also be seen in other conditions like empyema, lymphoma, and rheumatoid effusions (Porcel, 2016). In our research, increased ADA levels (>40 U/L) were noted in 5 non-TPE cases (11.9%), which comprised 2 lymphoma cases, 2 parapneumonic effusions, and 1 rheumatoid effusion.

The integrated method, which requires positive cytological indicators along with increased ADA, achieved the greatest specificity (95.2%) and positive predictive value (96.9%) in our research. This method would be especially beneficial in situations where the implications of incorrect positive diagnoses are substantial, like in areas with low TB rates or when there are worries about possible negative impacts of anti-tubercular treatment. Nonetheless, the compromise is lower sensitivity (79.5%), which could postpone diagnosis in certain actual TPE instances.

Notably, in our cohort, the diagnostic effectiveness of pleural fluid cytology (sensitivity 84.6%) significantly surpassed that of traditional microbiological techniques (sensitivity 41%). This difference emphasizes the restrictions of microbiological methods in pleural TB and stresses the importance of cytological testing as a primary screening tool. The paucibacillary characteristics of tuberculous pleural effusions, along with the compartmentalized immune response, frequently lead to a low mycobacterial load in pleural fluid, diminishing the effectiveness of direct microbiological techniques (Shaw et al., 2018).

It is important to mention that pleural fluid cytology offered diagnostic insights beyond TPE. In the non-TPE group, cytological analysis detected malignant cells in 78.3% of cancer cases and specific findings in 75% of parapneumonic effusions. This dual diagnostic function—for both confirming TPE diagnosis and recognizing other causes—improves the significance of cytology in the diagnostic process for pleural effusions.

Multivariate analysis revealed multiple independent predictors of TPE, with ADA >40 U/L being the most significant (adjusted OR 7.94), accompanied by lymphocyte predominance (adjusted OR 4.06), fever (adjusted OR 3.82), and a low count of mesothelial cells (adjusted OR 3.28). This discovery indicates that an inclusive method that integrates clinical characteristics, cytological factors, and biochemical indicators offers the best diagnostic precision.

CONCLUSION:

In summary, cytology of pleural fluid, especially when assessing lymphocyte dominance and a lack of mesothelial cells, acts as an effective preliminary screening method for tuberculous pleural effusion. Although ADA shows better diagnostic effectiveness as an individual test, the integration of cytological criteria with ADA yields the greatest diagnostic confidence. This integrated method enables swift, economical assessment of patients with suspected TPE, possibly lessening the requirement for more invasive interventions in certain instances. Considering the worldwide impact of tuberculosis and the difficulties in identifying pleural involvement, it's essential to enhance the utilization of accessible diagnostic methods like cytology and ADA to ensure prompt commencement of suitable treatment and better patient results.

REFERENCES:

1. Barnes PF, Mistry SD, Cooper CL, Pirmez C, Rea TH, Modlin RL. (1989). Compartmentalization of a CD4+ T lymphocyte subpopulation in tuberculous pleuritis. Journal of Immunology, 142(4), 1114-1119.
2. Garcia-Zamalloa A, Ruiz-Irastorza G, Aguayo FJ, Gurrutxaga N. (2015). Diagnostic accuracy of adenosine deaminase and lymphocyte proportion in pleural fluid for tuberculous pleurisy in different prevalence scenarios. PLoS One, 10(3), e0121669.
3. Gopi A, Madhavan SM, Sharma SK, Sahn SA. (2007). Diagnosis and treatment of tuberculous pleural effusion in 2006. Chest, 131(3), 880-889.

4. Goto M, Noguchi Y, Koyama H, Hira K, Shimbo T, Fukui T. (2003). Diagnostic value of adenosine deaminase in tuberculous pleural effusion: a meta-analysis. Annals of Clinical Biochemistry, 40(4), 374-381.
5. Krenke R, Korczynski P. (2010). Use of pleural fluid levels of adenosine deaminase and interferon gamma in the diagnosis of tuberculous pleuritis. Current Opinion in Pulmonary Medicine, 16(4), 367-375.
6. Krenke R, Safianowska A, Paplinska M, Nasilowski J, Dmowska-Sobstyl B, Bogacka-Zatorska E, et al. (2012). Pleural fluid cytological and biochemical parameters in patients with pleural effusions of different etiologies. Pneumonologia i Alergologia Polska, 80(3), 199-206.
7. Liang QL, Shi HZ, Wang K, Qin SM, Qin XJ. (2008). Diagnostic accuracy of adenosine deaminase in tuberculous pleurisy: a meta-analysis. Respiratory Medicine, 102(5), 744-754.
8. Light RW. (2010). Update on tuberculous pleural effusion. Respirology, 15(3), 451-458.
9. McGrath EE, Blades Z, Anderson PB. (2010). Chylothorax: aetiology, diagnosis and therapeutic options. Respiratory Medicine, 104(1), 1-8.
10. Porcel JM. (2016). Advances in the diagnosis of tuberculous pleuritis. Annals of Translational Medicine, 4(15), 282.
11. Porcel JM, Esquerda A, Bielsa S. (2010). Diagnostic performance of adenosine deaminase activity in pleural fluid: a single-center experience with over 2100 consecutive patients. European Journal of Internal Medicine, 21(5), 419-423.
12. Porcel JM, Vives M, Esquerda A, Ruiz A. (2014). Biomarkers of infection for the differential diagnosis of pleural effusions. European Respiratory Journal, 44(5), 1383-1389.
13. Sehgal IS, Dhooria S, Aggarwal AN, Behera D, Agarwal R. (2019). Diagnostic Performance of Xpert MTB/RIF in Tuberculous Pleural Effusion: Systematic Review and Meta-analysis. Journal of Clinical Microbiology, 57(1), e00025-18.
14. Shaw JA, Diacon AH, Koegelenberg CFN. (2018). Tuberculous pleural effusion. Respirology, 23(3), 241-250.
15. Vorster MJ, Allwood BW, Diacon AH, Koegelenberg CF. (2015). Tuberculous pleural effusions: advances and controversies. Journal of Thoracic Disease, 7(6), 981-991.
16. Wang W, Zhou Q, Zhai K, Wang Y, Liu JY, Wang XJ, et al. (2018). Diagnostic accuracy of interleukin 27 for tuberculous pleural effusion: two prospective studies and one meta-analysis. Thorax, 73(3), 240-247.
17. World Health Organization. (2023). Global tuberculosis report 2023. Geneva: World Health Organization.
18. Zumla A, Raviglione M, Hafner R, von Reyn CF. (2013). Tuberculosis. New England Journal of Medicine, 368(8), 745-755.
19. Klimiuk J, Krenke R, Safianowska A, Korczynski P, Chazan R. (2015). Diagnostic performance of different pleural fluid biomarkers in tuberculous pleurisy. Advances in Experimental Medicine and Biology, 852, 21-30.