Scholars Journal of Applied Medical Sciences (SJAMS)
Abbreviated Key Title: Sch. J. App. Med. Sci.
ISSN 2320-6691 (Online)
ISSN 2347-954X (Print)

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Respiratory Medicine

Role of ADA and Cytology in the Diagnosis of Exudative Pleural Effusion

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Original Research Article

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Article History
Received: 10.06.2018
Accepted: 27.06.2018
Published: 30.07.2018

DOI:
10.36347/sjams.2018.v06i07.001

Abstract: Exudative pleural effusions are a common diagnostic problem in clinical practice, as the list of causes is quite exhaustive, although sometimes they can be inferred from the clinical picture. Exudative effusions require to be separated into infectious causes, noninfectious causes and malignancy. The most common causes in most series are infections and malignancy. Tuberculosis was found to be the most common cause (74%) of pleural effusion in the region followed by malignancy (18%). Aims and objectives were Evaluate the role of ADA in diagnosis of exudative pleural effusion and Evaluate the role of cytology in diagnosis of exudative pleural effusion. This prospective study was carried out on 100 consecutive patients with exudative pleural effusions in the Department of Respiratory Medicine, Govt. Medical College and hospital Kota Over the period of October 2012 to September 2013. On the basis of history, clinical examination and various investigation with inclusion & exclusion criteria the study population finally divided into either tuberculous or non-tuberculous group. Statistical analysis was performed using a two way (time and group) analysis of variance followed by student't' test for parametric data. On comparison of the result of various tests and their combinations in our study, ADA emerged as a single best diagnostic test with sensitivity of 89.18%. The specificity can be increased from 84.62% with ADA alone to a good 96.15% by combining ADA and lymphocytes proportion of >50% in pleural fluid as a diagnostic criteria in tuberculous pleural effusions. However if ADA is between 40-63U/L it is highly suspicious of Tuberculous pleural effusion, and then the cytology report will aid in confirming the diagnosis. A lymphocyte exudates (50%) with high ADA value (40U/L) is highly suggestive of tuberculous pleural effusion.
Keywords: Exudative Plural Effusion, ADA, Cyotology.

INTRODUCTION

Exudative pleural effusions are a common diagnostic problem in clinical practice, as the list of causes is quite exhaustive [1], although sometimes they can be inferred from the clinical picture. Exudative effusions require to be separated into infectious causes, non-infectious causes and malignancy. The most common causes in most series are infections and malignancy.

The clinical, biochemical and cytological parameters of tubercular effusion are shared by malignancy, both being exudates and predominantly lymphocytic effusions. This can pose a significant diagnostic dilemma. Adenosine deaminase enzyme activity, gamma interferon, polymerase chain reaction, lysozyme measurement pleural fluid tuberculous protein antibodies and various tumour markers like CA15-3, squamous cell carcinoma antigen, etc have been used to differentiate TB from non TB [2].

Tuberculous pleural effusion also occurs in the absence of radiologically apparent tuberculosis. The pleural fluid mycobacterial cultures from the majority of patients with tuberculous pleural effusions are negative.

Differential diagnosis of exudative pleural effusions

A. Neoplastic diseases, B. Infectious disease ie - 1. Bacterial infections 2. Tuberculosis 3. Fungal infections 4. Parasitic infections5. Viral infections, C. Pulmonary embolization, D. Gastrointestinal disease, E. Collagen vascular diseases, G. Drug-induced pleural disease, H. Chylothorax, I. Hemothorax, J. Miscellaneous diseases and conditions.

DIAGNOSTIC APPROACH

When patient is found to have pleural effusion an effort should be made to determine the cause. The first step is to determine whether the effusion is transudate (or) exudate. The transudative and exudative pleural effusions are distinguished by measuring the LDH and protein levels in pleural fluid. Exudative pleural effusions meet at least one of the following criteria. Whereas transudative pleural effusions meet none;

- Pleural fluid protein/ serum protein > 0.5
- Pleural fluid LDH/serum LDH > 0.6
- Pleural fluid LDH more than two – thirds of normal upper limit for serum.

DIAGNOSTIC TESTS

If patients with exudative pleural effusion and normal levels of pleural fluid glucose and amylase, one must consider pleural fluid cytology examination. If this test is positive, the diagnosis of malignant pleural disease is established. If the pleural fluid cytological test is negative the differential cell count of pleural fluid should be examined. Malignant disease, pulmonary embolisation and tuberculosis are three most common causes of this picture. Lung scan should be obtained to rule out pulmonary embolisation because malignant disease and tuberculosis can be diagnosed by pleural biopsy; such patients should have at least two separate pleural biopsies with repeated pleural fluid cytological examinations if the lung scan is negative. Pleural biopsy specimens should be cultured for mycobacteria and patient should have a purified protein derivative (PPD) skin test.

Adenosine deaminase

Adenosine deaminase has two principle enzymes ADA-1 and ADA-2, which have different optimal pH, michaelis constant and relative substrate specificity patterns[3,4].

ADA-1 has roughly equal affinities for adenosine and 2 deoxyadenosine and is found in many tissues.

ADA-2 is the major component (73%) of the activity of total ADA in the serum of healthy persons. ADA-2 has much greater affinity for adenosine and is found only in macrophages and monocytes which release it when stimulated in the presence of the organisms.

Clinical application of serum ada in pleural effusion

ADA levels were found to be elevated in pleural fluid of patients with tuberculous pleural effusion way back in 1978 by Piras *et al*.

Review showed that levels of pleural fluid ADA were significantly higher than serum ADA levels in both tuberculosis and non tuberculoses pleural effusion suggesting a localised intrapleural production of ADA.

S.K.SHARNIA, V.SURESH, A. MOHAN *et al*. observed that CD4 + T cells are found to be present in the pleural fluid in greater proportion compared to blood in tuberculosis patients (with reverse being true for CD8 count) leading to a higher CD4 +/CD + 8 ratio in pleural fluid. These finding suggest that the antigen of M. Tuberculosis activate immune response leading to localised accumulation of T.helper cells. Which then augment the function of all forms of immune system against invading pathogen. However, there was no correlation between CD4 T lymphocyte proportions and the levels of ADA in pleural fluid of TB patients, contrary to what was observed by Baganta *et al*. This lack of correlation may be related to the fact that most of the ADA in pleural fluid may be derived from macrophages other than the T-Lymphocytes as the isozyme patterns of ADA in pleural fluid shows more of ADA2 isogyme which is not found in T-lymphocytes and is present in the macrophages[5].

Aims and objectives

- Evaluate the role of ADA in diagnosis of exudative pleural effusion.
- Evaluate the role of cytology in diagnosis of exudative pleural effusion.

MATERIALS AND METHODS

This prospective study was carried out on 100 consecutive patients with exudative pleural effusions in the Department of Respiratory Medicine, Govt. Medical College and hospital Kota. over the period of October 2012 to September 2013.

A. Inclusion criteria

1. Exudative effusion as per Light's criteria, 2. Age more than18 years, 3. Patients who have given informed consent, 4. Chest X-ray showing evidence of pleural effusion.

B. Exclusion criteria

1. Transudate effusion, 2. Patients who do not give their consent, 3. Patient who have undergone repeated pleurocentesis.

OBSERVATIONS

Table-1: Distribution of etiology of pleural effusion

S.N.	Parameters	No. Of Cases	%
1.	Tuberculosis	74	74
2.	Non Tuberculosis	26	26
	Malignancy	18	18
	Synpneumonic	6	6
	Others	2	2
	TOTAL	100	100

Tuberculosis was the most common cause of pleural effusions encountered (74%), followed by malignancy (18%), and followed by synpneumonic (6%). However malignant pleural effusion was the most common among non-tuberculous cases, as presented to our hospital.

Table-2: Appearance of pleural fluid colour in the study population

Colour of pleural fluid	TB		NTB	
	No.	%	No.	%
Straw	70	94.59%	2	7.69%
Hemorrhagic	4	5.4%	18	69.23%
Yellow and clear	-	-	4	15.38%
Yellow and turbid	-	-	2	7.69%
TOTAL	74	100%	26	100%

The appearance of pleural fluid in majority (94.59%) of tubercular pleural effusion was straw coloured. Among the non-tuberculous group majority were hemorrhagic (69.23%) whose etiology was later proved to be malignancy.

Table-3: Report of cytology of pleural fluid in study population

Mean Values	TB	NTB		
		Malignant	Synpneumonic	Others
Total WBC count (cells/mm^3)	1761.82	995.56	2366.67	200
Neutrophils (%)	24.50	24.67	77.17	13.50
Lymphocytes (%)	73.32	68.83	17.00	75.50
Mesothelial cells (%)	2.61	7.53	9.00	12.00
Malignant cells	-	+	-	-

(P value of lymphocyte %, TB v/s MALIGNANT P>0.05; TBv/sSYNPNEUMONIC P<0.001)

The mean total WBC count in the tubeculous group was 1761.82cells/mm^3 of which majority (73.32%) were lymphocytes Lymphocytes predominance was also seen in malignant pleural effusion.

All synpneumonic effusions showed neutrophil predominance (77.17%). 70 of 74 i.e. 94.59% of tuberculous cases and 14 of 18 i.e. 77.78% of malignant cases showed lymphocytic predominance(>50%) in pleural fluid cytology.

Atypical cells suggestive of malignant origin were found in 17 of 18(94.4%) cases of malignant pleural effusion.

Table-4: Occurrence of pleural fluid ADA level in study population

Study Group	Mean (U/L)± SD	Minimum	Maximum (U/L)
TB	76.29±30.91	13.00	157.00
NTB	26.77±19.09	5.4	62.40
M	23.95±14.71	7.20	62.40
SN	36.86±10.52	20.00	50.00
OTH.	9.15±5.30	5.40	12.90

P value of ADA
(P<0.001 (HS); p<0.01 when TB v/s M; TB v/s SN)

The pleural fluid ADA level was in the range of (13- 157 U/L) among the tuberculous cases with a mean value of 76.29±30.91 U/L.

The mean value of ADA among non-tuberculous cases was 26.77±19.09 U/L.

Table-5: Result of combination of ADA and cytology in diagnosis of tuberculous pleural effusion

	TB	NTB	TOTAL
POSITIVE	62	1	63
NEGETIVE	12	25	37
TOTAL	74	26	100

[Sensitivity - 83.78%, Specificity - 96.15%, Positive Predictive Value - 98.41%, Negative Predictive Value - 67.57%]

Table-6: Comparison of various tests and their combination in the diagnosis of tuberculous pleural effusion

TEST/CRITERIA	SENSITIVITY	SPECIFICITY	PPV	NPV
ADA>40 U/L	89.18%	84.62%	94.29%	73.33%
ADA> 63 U/L	67.57%	100%	100%	52%
ADA>40 U/L & >50% Lymphocytes in pleural fluid cytology	83.78%	96.15%	98.41%	67.57%

Pleural fluid ADA with sensitivity of (89.18%) and specificity of (84.62%) emerged as a single best diagnostic test for tuberculous pleural effusion with sensitivity of (89.18%) and specificity (84.62%) at 40 U/L cut-off level.

Combined use of pleural fluid ADA and lymphocytes predominance was found to increase the specificity to (96.15%).

DISCUSSION

The present study was a prospective study of 100 cases of exudative pleural effusion presenting to the department of Respiratory Medicine, Govt Medical College, kota between Oct. 2012 to Sept. 2013. The aim of study was a evaluate the Role of ADA and Cytology in the diagnosis of exudative pleural effusion.

On the basis of history, clinical examination and various investigation the study population finally divided into either tuberculous or non-tuberculous group. Of the 100 cases 74 were diagnosed as tuberculous and 26 non tuberculous etiology. In non-tuberculous group 18 are malignant effusions, 6 synpneumonic effusions and 2 are others group cases are included.

Tuberculosis was found to be the most common cause (74%) of pleural effusion in the region followed by malignancy (18%). Majority (86.47%) of tuberculous pleural effusion patients belonged to 20- 50 years of age group i.e. the most economically protective age group. Though (44.59) % of tuberculous pleural effusion cases occurred in the age group of 21-30 yrs. (47.29%) were above the age of 30 years with 3 patients (4.05%) more than 60 year of age. The mean age of tuberculous pleural effusion patients was found to be 37.40 years while the mean age of malignant pleural effusion cases was 59.78 years.

Berger *et al.*[6] also observed in their study of 49 patients of tubercular pleural effusion that 41% were Above 35 years with 7 patients (14.29%) above 70 years of age. S K Sharma *et al.* [7] 1997 in his study of 75 cases of pleural effusion found that the mean age of TPE patients was 33 years while that of malignant pleural effusion was 47 years.

In the present study the mean total WBC count in tubercular pleural effusion was1761.82 cells/mm^3 while it was 995.56 cells/mm^3 in malignant cases. Synpneumonic effusions had the highest of all cases, there mean WBC count being 2366.67 cells/mm^3.

Lymphocyte predominance defined as more than 50% of total WBC cells was found in majority of TPE and malignant pleural fluids. However it was not of much significance in differentiating TPE and malignant pleural effusion(94.59%) 70 of 74 TPE and (77.78%) 14 of 18 malignant effusion showed a lymphocytes predominance. Of the 74 cases of TPE, only 4 case had lymphocytes <50%. In these case ADA was >40 U/L. The means lymphocyte percentage was observed to be 73.32% among TPE cases and 68.83% in the malignant group. The difference in lymphocyte percentage between TPE and malignant effusions was not significant statistically (p>0.05). Hence it can be concluded in our study that neither lymphocytic predominance nor lymphocyte percentage is of any value in differential diagnosis of TPE from malignant effusions.

77.17% of synpneumonic cases showed neutrophil predominance in the pleural fluid. The mean lymphocyte percentage was only 17% which was found

to be statically significant when compared to TPE ($p<0.001$). Hence differential cytology is of help in differentiating synpneumonic cases from tubercolous cases.

Petterson *et al*. [8] in their study of 140 cases of pleural effusions of various etiology found a lymphocytic predominance in pleural fluid in 29 of 31 (93.5) cases of TPE and 18 of 24 (75%) of malignant cases. Ocana *et al*. [9] in their study which included 46 TPE and 46 malignant pleural effusions, they found that 86.5% of TPE and 74.3% of malignant cases had lymphocytic predominance of >68% of total WBC count.

In this present study, pleural fluid ADA among tuberculous cases ranged from 13-157U/L with a mean value of 76.29±30.91 U/L. The mean ADA among non-tuberculous cases was found to be 26.77±19.09 U/L. Though malignant cases showed lymphocyte predominance, the mean ADA was found to be 23.95±14.71U/L which is much lower than that of TPE. This gives us a simple test for differential diagnosis between TPE and malignant effusions. Synpneumonic cases mean ADA was found 36.86±10.52 U/L.

As the various studied in literature show a cut-off value of ADA for diagnosing TPE between33 to 50 U/L. The cut-off value for ADA can be set depending on whether we intend to use pleural fluid ADA as definite confirmatory test or an initial screening test. A screening test should have both acceptable sensitivity and specificity. We found both these parameters were fair enough at 40U/L cut off level. It is also level described in literatures at which it is suggestive of tubercular etiology. At this cut off value 66 cases of TPE gave a positive test leading to a sensitivity of 89.18% and specificity of 84.62%. PPV was good 94.29% and NPV was a fair 73.33%.

We obtained a100% specificity at a cut off value of 63U/L. At this value, the sensitivity decrease to a low 67.57% and NPV was 52% only. Thus 24 cases in tuberculous group were falsely classified as negative. We attained a PPV of 100% thus no non tuberculous cases were falsely categorized. This shows that at higher level of cutoff such as 63U/L as our study, pleural fluid ADA acts as a definite confirmatory test.

In setting like ours where tuberculosis still remains the commonest cause of pleural effusion, we need a test which is more sensitive rather than specific. As we know TPE, untreated though resolves spontaneously only to present as active TB elsewhere in future. Hence it is better to fix the cut off value at a lower range like 40 U/L.

To further increase the specificity of ADA, a combination of one or two other test is being evaluated by many studies. One such studied with much interest is the combined use of pleural fluid ADA and cytology i.e. lymphocytes percentage in particular.

In our study when we used a combination criteria of ADA>40U/L and a lymphocyte percentage >50% in the study population. Though 12 gave a false negative result. Only 1 patient among non-tuberculous group gave a false positive result. Thus decreasing the sensitivity to 83.78% but the specificity increased to 96.15%. PPV also increase to 98.41%.

Oliveria *et al*. [10] studied 276 patients of pleural effusion of which 54 were of tuberculous etiology. When they used a combined criteria of ADA >40U/L & >50% Lymphocytes, they observed a sensitivity of 90.7% and specifity of 97.7%.

Burgess *et al*. [11] studied 303 cases of pleural effusion of which 58% were tuberculous 19% malignant. At a cut off value of 50U/L for pleural fluid ADA, they found a sensitivity of 90% & specificity of 89%. 13 of 143 cases gave a false negative result. When they combined the criteria of lymphocyte to neutrophil ratio of >0.75 with ADA >50 U/L, sensitivity decreased to 88% but specificity increased to 95% from 89%. Hence they concluded that combined use of ADA and lymphocyte neutrophil ratio gave a more specific result in the diagnosis of TPE.

On comparison of the result of various tests and their combinations in our study, ADA emerged as a single best diagnostic test with sensitivity of 89.18%. The specificity can be increased from 84.62% with ADA alone to a good 96.15% by combining ADA and lymphocytes proportion of >50% in pleural fluid as a diagnostic criteria in tuberculous pleural effusions.

CONCLUSION

Finally, it can be concluded from the present study that pleural fluid ADA and cytology can be used in the differential diagnosis of pleural effusion as follows:

- In a clinically suspected cases of tuberculous pleural effusion if the pleural fluid ADA is >63U/L, it is essentially diagnostic of tuberculous pleural effusion.
- However if ADA is between 40-63U/L it is highly suspicious of Tuberculous pleural effusion, and then the cytology report will aid in confirming the diagnosis. A lymphocyte exudates(50%) with high ADA value (40U/L) is highly suggestive of tuberculous pleural effusion.

- A lymphocyte exudates (50%) with low ADA (<40U/L) is more in favour of non haematologicalmalignancies.
- A neutrophilic exudates with low ADA suggests synpneumonic effusion but a high ADA points towards para-infective empyema.

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