E-ISSN: 2706-9575
P-ISSN: 2706-9567
IJARM 2019; 1(2): 168-172
Received: 10-10-2019
Accepted: 12-11-2019

Dr. Baburao Eruku
Assistant Professor, Department of Respiratory Medicine, Mamata Medical College, Khammam, Telangana, India

Ch. Praveen Kumar
Assistant Professor, Department of Respiratory Medicine, Chalmeda Anandarao Medical College, Karimnagar, Telangana, India

Corresponding Author:
Ch. Praveen Kumar
Assistant Professor, Department of Respiratory Medicine, Chalmeda Anandarao Medical College, Karimnagar, Telangana, India

The diagnostic value of ADA, ADA2, and interferon gamma in pleural fluid in tuberculous pleural effusion

Dr. Baburao Eruku and Ch. Praveen Kumar

DOI: https://doi.org/10.22271/27069567.2019.v1.i2b.433

Abstract
Introduction: Mycobacterium tuberculosis causes TB, which has been around for a very long time. Droplets may be inhaled and spread the disease from person to person. It's one of the community's most widespread communicable illnesses.
Methods: 105 patients gave their informed permission to participate in the trial. Informed permission was obtained from patients with pleural effusion who presented to the Department of Respiratory Medicine, Chalmeda Anandarao Medical College, Karimnagar, Telangana, India, between January 2019 to August 2019.
Results: Biomarkers and TB pleural effusion diagnosis were examined. Combining tests increased sensitivity but not specificity. Tuberculous pleural effusion causes pleural fluid lymphocytosis. Pleural fluid lymphocyte neutrophil ratio exhibited 87.5% sensitivity and 16.5% specificity. ADA, ADA1, ADA2, and interferon gamma in pleural fluid are examined in TB, cancer, and lymphoma.
Conclusion: Tuberculous pleural effusion is a frequent yet difficult-to-diagnose cause. Pleural fluid biomarkers, assays, and grading systems can diagnose TB effusion, but they cannot replace the gold standard.

Keywords: Tuberculous, interferon gamma, pleural effusion, biomarkers

Introduction
Mycobacterium tuberculosis is responsible for the transmission of tuberculosis (TB), a chronic bacterial lung infection that has plagued humanity for a very long time. Droplets may be inhaled and spread the disease from person to person. It's one of the community's most widespread communicable illnesses [1, 2].
For those with HIV, tuberculosis is the most prevalent opportunistic illness. People who are HIV-positive have a 50% greater lifetime chance of contracting tuberculosis than those who are not affected. Multidrug-resistant TB presents a further difficulty in treatment. The tuberculosis bacilli in a patient with multidrug-resistant TB cannot be killed by the standard first-line treatment regimen of isoniazid and rifampicin. XDR-TB, or Multidrug-Resistant Tuberculosis, is MDR-TB that is also immune to fluoroquinolones and injectable [3, 4].
Tuberculosis is one of the illnesses that has been around since prehistoric times. During the 18th and 19th centuries, it spread like wildfire, earning the deadly captain moniker as it did so. The "white epidemic" of tuberculosis ravaged Europe in the late Seventeenth and early Eighteenth Centuries. The tubercle bacilli were identified by Dr. Robert Koch in 1882. Clemens Freiherr von Pirquuet pioneered the use of tuberculin protein injections and tuberculin sensitivity testing in 1907. As early as 1907, people were using sanatoriums and other forms of outdoor therapy. A child whose mother was dying of pulmonary TB was given the first BCG vaccination and lived through it in 1921. The United Nations Children's Fund and the Danish Red Cross vaccinated thousands of people throughout Europe against a strain of the BCG virus. The World Health Organization's first official disease control initiative [5, 6].
These medications were the first to be used in the fight against tuberculosis. Despite their 1940s discovery, bacteriostatic medicines like para-amino salicylic acid (PAS) and thiosemicarbazone were ineffective. In 1944, streptomycin was developed and successfully used to treat tuberculosis (TB). Then, in 1952 and 1957, scientists found the effective medications isoniazid (INH) and rifamycin. Domiciliary tuberculosis therapy became possible with the development of more powerful chemotherapeutic medicines.

The bacteria Mycobacterium tuberculosis is responsible for tuberculosis (MTB). The name "Koch's bacilli" is also often used to refer to this organism. Mycobacterium tuberculosis is the most well-known member of the Mycobacterium genus. Mycobacterium tuberculosis is an aerobe that can't move and doesn't make spores, according to the scientific definition of the bacteria. Its resistance to antibiotics and lack of permeability are due to the lipid-rich composition of its cell wall. Middlebrook's medium, which is made of agar, and Lowenstein-media, Jensen's made of eggs, are used to cultivate the MTB. Normal gramme staining will not work on these microorganisms. Staining with Zeil-Neelsen staining using carbol fuchsin reveals that these organisms are acid fast [7-10].

Methodology

After gaining informed permission from each participant, a total of 105 patients were enrolled in the research. After receiving their informed permission, patients who visited the Department of Respiratory Medicine, Chalmeda Anandarao Medical College, Karimnagar, Telangana, India, complaining of pleural effusion were invited to take part in the research. This study was done between the period between January 2019 to August 2019.

Inclusion criteria

- Patients who are undergoing evaluation and have all the pleural effusions and are willing to participate in the study

Exclusion criteria

- Patients with imaging- or biopsy-based diagnoses of cancer
- The patient is hesitant to sign the permission form.

Method of evaluation

Aspiration of pleural fluid and a pleural biopsy were performed on all patients after recruitment (closed or ultrasound guided). Cell and protein analysis, as well as LDH and mycobacterial culture, were performed on the pleural fluid samples. Pleural biopsies were taken using a true cut biopsy needle, and the tissue was sent out for mycobacterial culture and histology. The clinical biochemistry lab received a 5mL sample of pleural fluid in a red tube for analysis. The specimen is frozen at a temperature of 70 degrees Celsius. The samples collected for the research are pooled, frozen, and analysed later.

Results

The research included participation from a total of one hundred different patients. Patients who had an independent diagnosis of cancer or radiological indications of cancer were not allowed to participate in the study. The patients who were recruited in the trial were given a pleural aspiration and a pleural biopsy. Additionally, the biomarkers ADA, ADA1, and ADA2 as well as interferon gamma were examined in the pleural fluid sample.

Demographic Details

Table 1: Displaying the patient demographics for those who have pleural effusion

Patient characteristics	Total patients= 105
Age, (years)	45
Male	14
Female	26
Unilateral	15
Bilateral	3
HIV infection	1
Sputum positive for TB	1

The gender breakdown of the 105 patients with pleural effusion is shown in Table 1 and Figure 1. The average patient's age was 42 when first seen. Unilateral pleural effusion was the most common presenting symptom. Sputum analysis confirmed HIV infection in 1 patient and found pulmonary TB in 1 patient.

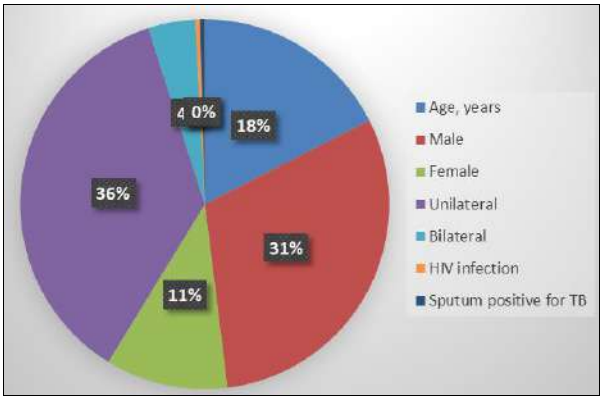

Fig 1: Displaying the patient demographics for those who have pleural effusion

Age distribution of diseases

A breakdown of the patients' ages according to their participation in the trial. The patients who have been diagnosed with tuberculous pleural effusion have an average age of 42 years. It was often determined that the older patients who presented with pleural effusion had a malignant origin. Whereas lymphoma patients were 48 years old. There were 58 patients diagnosed with tuberculous pleural effusion.

Pleural fluid cellular characteristics

All patients exhibited transudative and pleural effusions according to Light's criteria, which examined the composition of the pleural fluid. Tuberculosis and cancer were the most common underlying illnesses in the patients who had lymphocytic pleural effusions. Para-pneumonic effusions were the aetiology of neutrophil-predominant effusions in 24 cases. Only around 6 individuals showed evidence of eosinophilic effusion.

Table 2: Displays the cellular features of pleural fluid

Cell counts	Number of patients & percentages N
Lymphocyte	80
Neutrophil	19
Eosinophil	06

The pleural fluid biochemical parameters were analyzed with the etiology. Tuberculosis had the highest mean pleural

fluid protein content, followed by malignancy and lymphoma. The pleural fluid lactate dehydrogenase showed highest levels in malignancy, followed by lymphoma and Tuberculosis had the least.

Aetiology of pleural effusion

Seventy of the study's 105 participants had TB, while the other 30 had various types of cancer (mostly lung cancer but also cancers from other locations). A pleural biopsy confirmed the presence of lymphoma in seven cases. Two patients with benign mesothelial proliferations and two patients with mesothelioma each exhibited effusion related to mesothelial tumour. Eosinophilic effusion related to parasite infection (H. nana and Churg Strauss syndrome) was found in two patients. The pleural effusion in one patient was due to a fibrous tumour. Despite pleural fluid investigations and pleural histology, 36 individuals remained undiagnosed.

Biomarkers and sensitivity and specificity

There was an investigation on the sensitivity and specificity of biomarker combinations. With the addition of the ADA1/ADA ratio of 0.42, the sensitivity increased to 78.4% from 71.5%, while the specificity dropped to 39.2%.

Table 3: ADA, ADA2, and Interferon gamma biomarker sensitivity, specificity, AUC, and cut-off values

Biomarkers	Sensitivity %	Specificity %	AUC
ADA	71.5	71.5	0.75
ADA2	18.5	18.5	0.701
Interferon gamma	15.0	15.0	0.631

The combination of ADA, ADA1/ADA ratio, and interferon gamma increased the sensitivity from 70.4% to 93.8%, but it did not enhance the specificity, which remained at 35%. A combination of ADA and ADA2 had a greater sensitivity of 80% but lacked specificity by 62.7%. This was due to the fact that ADA2 was a newer version. The lymphocyte neutrophil ratio demonstrated a sensitivity of 87.5% but fell short in terms of sensitivity, reaching just 16.5%. The combination of tests did not succeed in improving the specificity, albeit increasing the sensitivity.

Pleural fluid biomarkers in disease conditions

Comparative analyses of the pleural fluid biomarkers were performed for TB, lymphoma, and malignancy. The mean levels of ADA in TB were high, whereas those in malignancy were low and those in lymphoma were quite high. ADA2 levels followed a trajectory that was quite similar. Interferon gamma was found in high levels in individuals with tuberculosis, but it was found in low levels in lymphoma and malignancy.

Table 4: Revealing TB, cancer, and lymphoma pleural fluid biomarkers

Biomarkers	ADA U/L	ADA1 U/L	ADA2 U/L	Interferon Gamma IU
Tuberculosis	50.1	21.1	40.1	19.1
Malignancy	14.9	53.9	40.0	59.9
Lymphoma	40.0	30.0	24.9	20.0

Discussion

One hundred individuals with pleural effusion were included in the research; 74 were men and 26 were women. The average patient's age was 42 when first seen. Unilateral pleural effusion was the most common presenting symptom. Sputum examination confirmed HIV infection in 1 patient and pulmonary TB. Eight individuals had signs of bilateral pleural effusion. Further investigation into the cause of the bilateral pleural effusion revealed that TB was present in 3 of the patients, adenocarcinoma in 1, Sjogren's disease in 1, and in 3 cases the cause was not determined. Likewise, bilateral pleural effusion due to tuberculosis is possible. In a cohort of 105 patients, 6% of those with bilateral pleural effusion had TB, according to a 1945 report by E. Montushi [11, 12].

HIV infection was found in one patient who had pleural effusion. Only two of the hundred patients with pleural effusion tested positive for acid fast bacilli (AFB), and 38 of the patients didn't even have their sputum examined. If a pleural effusion is present and it is thought that it is due to tuberculosis, a sputum sample should be taken and examined. Sputum examination in tuberculous pleural effusion had a detection rate of 22.22%, according to a research by Chaudhuri *et al*. Induced sputum had a 52% sputum culture success rate for detecting tuberculous pleural effusion, according to a separate investigation. Fifty-three males and fifteen women were diagnosed with tuberculous effusions. With males outnumbering females by a ratio of 3.50 to 1. A Spanish research found a similar trend, with a male to female ratio of 1.6:1. Male gender was included as a variable in a scoring system developed for the diagnosis of tuberculous pleural effusion, suggesting that males are more likely to have the condition [13, 14].

Patients diagnosed with tuberculous pleural effusion had a median age of 39.13 years. Those diagnosed with TB effusion ranged in age from 17 to 85. Malignant tumours and lymphoma both often strike in their 47th year of life. According to the aforementioned statistics, tuberculous pleural effusion is more prevalent in younger people, whereas malignant pleural effusion is more common in the elderly. According to research by Valdes *et al*., pleural effusion is caused most often by TB, followed by malignancy and heart failure [15].

About 80% of patients had lymphocyte predominate pleural effusion, whereas less than 10% had neutrophilic effusion and 1% had eosinophilic pleural effusions based on the pleural fluid characteristics. Parapneumonic effusion is the most prevalent cause of neutrophilic pleural effusion, whereas TB and malignancy are the most common causes of lymphocytic pleural effusion. Ten people developed eosinophilic pleural effusion. We found that adenocarcinoma was responsible for two cases of eosinophilic pleural effusion, T-cell lymphoma was responsible for one case, tuberculosis, Churg-Strauss syndrome, Sjogren's syndrome, and H.nana infection was responsible for one case each, and in three cases the reason was unknown. In his research, Krenke *et al*. found that cancer is the most prevalent cause of eosinophilic effusion, followed by infections, the unknown, post-traumatic conditions, and other reasons [16, 17].

Biochemical markers, such as protein and lactate dehydrogenase (LDH), in pleural fluid were evaluated across diseases. Our research showed that tuberculous pleural effusion had the highest protein levels, at a mean of 5.4mg/dL, followed by malignancy and lymphoma, at 4.9mg/dL and 4.1mg/dL, respectively [18]. In 94.34 percent of cases with tuberculous pleural effusion, the protein

concentration was more than 3 grammes, according to an Indian research. Using a combination of three markers (pleural fluid protein 5g/dl, lymphocytes >80%, and ADA > 45U/l) exhibited 100% specificity and 34.9% sensitivity in a different research of pleural fluid analysis for the diagnosis of TB pleural effusion. Lymphoma and other malignancies are characterised by elevated levels of lactate dehydrogenase (LDH). Pleural effusion caused by tuberculosis had a mean LDH of 1209. Ernam *et al*. found that the highest levels of lysosomal dehydrogenase (LDH) in pleural fluid were seen in parapneumonic effusions, followed by malignant effusions and tuberculous effusions. Pleural fluid LDH is a non-specific diagnostic for establishing a particular diagnosis since it may be increased in numerous illnesses, including malignancy, lymphoma, TB, and others [19, 20].

One hundred individuals were analyzed to determine what caused pleural effusion in them. 50 patients had pleural tuberculosis, 36 had pleural effusion of unknown cause, and 31 had malignant pleural effusion. Lymphoma affected 7 individuals, whereas mesothelial neoplasms (2 benign mesothelial proliferations and 2 mesothelioma) caused effusion in 4 others. In a tiny percentage of people, effusion was caused by unusual conditions such Sjogren's disease, Churg Strauss syndrome, parasite infection, or a fibrous tumour. Based on the information provided, it seems that TB is the leading cause of pleural effusion, followed by malignancy. According to research by Valdes *et al*., pleural effusion is most often caused by TB in high-tuberculosis areas, followed by malignancy and heart failure [4, 5, 20].

As Trajman *et al*. shown in their study, the yield of pleural fluid microscopy for AFB is less than 5%, and the sensitivity of pleural fluid cultures is only 24-58%. As a whole, pleural biopsy culture was more successful than pleural fluid culture. Malignant pleural effusion was diagnosed by pleural fluid cytology in 57.6% of a retrospective study of 78 individuals. The yield of pleural fluid cytology was calculated to be 65% in a separate investigation. Depending on the cytologist's expertise and the specific tumour being examined, pleural fluid cytology might have varying levels of diagnostic value [21, 22]. The very small size of the study's sample is a major caveat. A total of 105 individuals were enlisted, and 68 of them were diagnosed with TB. To further understand the significance of biomarkers in tuberculous pleural effusion, larger-scale studies are needed. The biopsy findings for 36 individuals were deemed inconclusive. To determine the root of the effusion and establish a link with the biomarkers, further testing is necessary.

Conclusion

The presence of tuberculous pleural effusion is one of the major reasons of pleural effusion that might be difficult to diagnose. Although the biomarkers in pleural fluid, test combinations, and scoring system all play an essential part in the diagnosis of TB effusion, they are not adequate enough to fully replace the traditional procedures that are considered to be the gold standard for diagnosis.

Conflict of Interest

None

Funding Support

Nil

References

1. Li M, Wang H, Wang X, Huang J, Wang J, Xi X. Diagnostic accuracy of tumor necrosis factor-alpha, interferon-gamma, interlukine-10 and adenosine deaminase 2 in differential diagnosis between tuberculous pleural effusion and malignant pleural effusion. Journal of Cardiothoracic Surgery. 2014 Dec;9(1):1-6.
2. Valdés L, San José E, Dobano JA, Golpe A, Valle JM, Penela P. Diagnostic value of interleukin-12 p40 in tuberculous pleural effusions. European Respiratory Journal. 2009 Apr 1;33(4):816-20.
3. Keng LT, Shu CC, Chen JY, Liang SK, Lin CK, Chang LY. Evaluating pleural ADA, ADA2, IFN-γ and IGRA for diagnosing tuberculous pleurisy. Journal of Infection. 2013 Oct 1;67(4):294-302.
4. Ahire ED, Kshirsagar SJ. Efflux Pump Inhibitors: New Hope in Microbial Multidrug Resistance: Role of Efflux Pump Inhibitors in multidrug resistance protein. Community Acquired Infection. 2022 May 10;9.
5. Krenke R, Korczynski P. Use of pleural fluid levels of adenosine deaminase and interferon gamma in the diagnosis of tuberculous pleuritis. Current opinion in pulmonary medicine. 2010 Jul 1;16(4):367-75.
6. Valdes L, Pose A, San José E, Vázquez JM. Tuberculous pleural effusions. European journal of internal medicine. 2003 Mar 1;14(2):77-88.
7. Tian RX, Gao ZC. Clinical investigation of the diagnostic value of interferon-gamma, interleukin-12 and adenosine deaminase isoenzyme in tuberculous pleurisy. Zhonghua jie he he hu xi za zhi= Zhonghua jiehe he huxi zazhi= Chinese journal of tuberculosis and respiratory diseases. 2004 Jul 1;27(7):435-8.
8. Valdés L, San José E, Alvarez D, Sarandeses A, Pose A, Chomón B. Diagnosis of tuberculous pleurisy using the biologic parameters adenosine deaminase, lysozyme, and interferon gamma. Chest. 1993 Feb 1;103(2):458-65.
9. Skouras VS, Kalomenidis I. Pleural fluid tests to diagnose tuberculous pleuritis. Current opinion in pulmonary medicine. 2016 Jul 1;22(4):367-77.
10. Valdés L, San José E, Ferreiro L, Golpe A, Gude F, Álvarez-Dobaño JM. Interleukin 27 could be useful in the diagnosis of tuberculous pleural effusions. Respiratory care. 2014 Mar 1;59(3):399-405.
11. Krenke R, Safianowska A, Paplinska M, Nasilowski J, Dmowska-Sobstyl B, Bogacka-Zatorska E. Pleural fluid adenosine deaminase and interferon gamma as diagnostic tools in tuberculosis pleurisy. J physiol Pharmacol. 2008 Dec 1;59(Suppl 6):349-60.
12. Gopi A, Madhavan SM, Sharma SK, Sahn SA. Diagnosis and treatment of tuberculous pleural effusion in 2006. Chest. 2007 Mar 1;131(3):880-9.
13. McGrath EE, Warriner D, Anderson PB. Pleural fluid characteristics of tuberculous pleural effusions. Heart & Lung. 2010 Nov 1;39(6):540-3.
14. Adilistya T, Astrawinata DA, Nasir UZ. Use of pleural fluid interferon-gamma enzyme-linked immunospot assay in the diagnosis of pleural tuberculosis. Acta Medica Indonesiana. 2016 Sep 19;48(1):41.
15. Poyraz B, Kaya A, Ciledag A, Oktem A, Gonullu U. Diagnostic significance of gamma-interferon in tuberculous pleurisy. Tuberk Toraks. 2004 Jan 1;52(3):211-7.

16. Zhai K, Lu Y, Shi HZ. Tuberculous pleural effusion. Journal of thoracic disease. 2016 Jul;8(7):E486.
17. Ferreiro L, San José E, Valdés L. Tuberculous pleural effusion. Archivos de Bronconeumología (English Edition). 2014 Oct 1;50(10):435-43.
18. Zhang X, Meng Q, Miao R, Huang P. The diagnostic value of T cell spot test and adenosine deaminase in pleural effusion for tuberculous pleurisy: A systematic review and meta-analysis. Tuberculosis. 2022 Jun 22:102223.
19. Mohammadtaheri Z, Mashayekhpour S, Mohammadi F, Mansoori D, Masjedi MR. Diagnostic value of adenosine deaminase isoenzyme (ADA2) and total ADA in tuberculous pleural effusion. TANAFFOS (Respiration). 2005 Oct 1;4(3 (summer)):37-42.
20. Ferreiro L, Toubes ME, Valdés L. Contribution of pleural fluid analysis to the diagnosis of pleural effusion. Medicina Clínica (English Edition). 2015 Aug 21;145(4):171-7.
21. Kataria YP, Khurshid I. Adenosine deaminase in the diagnosis of tuberculous pleural effusion. Chest. 2001 Aug 1;120(2):334-6.
22. Porcel JM. Tuberculous pleural effusion. Lung. 2009 Oct;187(5):263-70.

How to Cite This Article
Eruku B, Ch. Kumar P. The diagnostic value of ADA, ADA2, and interferon gamma in pleural fluid in tuberculous pleural effusion. International Journal of Advanced Research in Medicine. 2019;1(2):168-172.