

Role of CBNAAT in Suspected Cases of Tubercular Pleural Effusion

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Abstract Summary

Introduction:

Tuberculosis (TB) remains a major health concern worldwide. Extra pulmonary tuberculosis (EPTB) in India is up to 20% of all tuberculosis cases. EPTB often remains undetected due to variable clinical presentation and lack of diagnostic means. Early detection of EPTB and drug resistance is important in the management of EPTB.

Methods:

This prospective study was conducted from NOV 2017 to OCT 2019 with an objective to find the role of cartridge based nucleic acid amplification test (CBNAAT) in tubercular pleural effusion. We also observed comparison of CBNAAT i.e. MTB DNA PCR with other conventional diagnostic techniques like pleural fluid biochemistry, ADA (adenosine deaminase level) and cytology. It included 100 cases with signs, symptoms, history and radiological features suggestive of tubercular pleural effusion. All the cases were subjected to pleural fluid analysis, smear for AFB, ADA, cytology & CBNAAT using GX4Gene Xpert MTB/Rif test system. Statistical analysis was done.

Results:

Among a total of 100 patients of tubercular pleural effusion (male 80 and female 20) with the mean age 46.57 ± 17.81 years, 06% cases were sputum positive for AFB, 03% pleural fluid sample positive for AFB, 27% mantoux positive, 72% cases had ADA >40 units/liter, 21% were CBNAAT positive, 87% fulfilled the lights criteria that is pleural protein and serum protein ratio is >0.5 . Sensitivity of CBNAAT is 95.24%, specificity 36.70%, PPV 28.57%, NPV 96.67%.

Conclusion:

Cartridge based nucleic acid amplification test is very useful in confirming tuberculosis as a cause for pleural effusion. Results of CBNAATs in this situation are very useful, sensitive, less time consuming and comparable to pleural fluid culture. It is more specific and sensitive than pleural fluid ADA estimation.

Keywords: CBNAAT, ADA, AFB, EPTB.

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I. Introduction

Pleural effusion has been recognised for more than 2000 years in clinical medicine. Pleural effusion is either a manifestation or complication of respiratory or non respiratory disease and heralds a serious prognosis if not diagnosed or treated properly.

Tuberculosis is a contagious and air borne disease, caused by *Mycobacterium tuberculosis*. According to WHO 2018, TB is one of the most leading causes of death worldwide.⁽¹⁾ In 2017 new cases of TB were 10 million worldwide, out of which 5.8 million were men, 3.2 million were women, and 1 million were children.⁽¹⁾ India accounts for 27% of global burden with estimated 27.5 lakh patients as per Global TB report 2018⁽²⁾. India has highest burden of both TB and MDR TB according to TB REPORT 2018.²

Tuberculosis can potentially involve any system or organ of the body. Pulmonary tuberculosis is the most common presentation; extrapulmonary tuberculosis is also an important clinical problem^{3, 4}. Pleural effusion is one of the common complications of primary tuberculosis or in conjunction with pulmonary infiltrate typical of post primary tuberculosis.

The obvious explanation for the development of the tuberculous pleural effusion is that the delayed hypersensitivity reaction increases the permeability of the pleural capillaries to protein, intense inflammatory reaction in the parietal pleura impedes the lymphatic drainage from the pleural space and leads to the accumulation of pleural fluid⁵.

Extra-pulmonary TB in India accounts for 15-20% that remains undetected and untreated not only due to diverse presentations but also due to lack of diagnostic means⁴. In 2013 WHO has endorsed the use of gene Xpert MTB assay (CBNAAT) a fully automated rapid cartridge based nucleic acid amplification test for rapid diagnosis of EPTB⁴. The role of (NAAT) in Mycobacterium tuberculosis DNA PCR in the diagnosis of tubercular pleural effusion has been evaluated extensively as an additional diagnostic tool⁵. It has yielded variable results with sensitivity ranging between 42 to 100% and specificities ranging between 85 to 100% using various PCR targets such as 156110, 65KDA, TRC4, GCR5 etc⁵. Thus CBNAAT of pleural fluid can be a rapid, reliable alternative to conventional diagnostic tool for TB pleural effusion in our institute where the case burden is high.

Culture is the gold standard for detection of Mycobacterium Tuberculosis, but it is slow and may take up to 2-8 weeks. Microscopic examination for (AFB) is rapid and inexpensive. But it has poor sensitivity and specificity and is unable to differentiate between tuberculosis and non tuberculosis mycobacterium. Histological examination has its limitations as it cannot differentiate between TB and other related diseases like sarcoidosis or Non Tuberculous Mycobacteria infection. Other tests like serological assays (both antigen and antibody detection), and Mantoux test have variable sensitivity and specificity. Thus rapid diagnosis by CBNAAT is essential for early initiation of management.

II. Aims & Objectives

GENERAL OBJECTIVE:

To study the role of cartridge based nucleic acid amplification test (CBNAAT) in suspected cases of Tubercular pleural effusion.

SPECIFIC OBJECTIVE:

To find out the percentage of positivity of CBNAAT in suspected cases of tubercular pleural effusion.

SECONDARY OBJECTIVE:

To correlate with other investigations like pleural fluid biochemistry, ADA, and cytology.

III. Review of Literature

ANATOMY

Pleura is a double layered mesothelial membrane that covers the lungs (visceral pleura), the rib cage, mediastinum, and the diaphragm (parietal pleura). Visceral pleura covers the lungs at the hilus where the mediastinal (parietal) pleura covers the lungs roots and forms a pleural fold posterior to the lung roots (pulmonary ligament). The virtual space between the parietal and visceral pleura is called pleural cavity. This space is filled by a thin layer (5 micron in human) of fluid- pleural fluid, which lubricates the pleural surfaces and allows the sliding of the lungs on the chest wall during respiration. The volume of pleural fluid remains constant due to a balance between filtration from the surrounding tissues and absorption into the pleural lymphatics. The right and left pleural sacs are distinct from each other, even though they are in contact for just a short distance behind the upper half of the body of the sternum, and are separated only by a narrow interval behind the esophagus in the mid thoracic region.

Parietal pleura

The parietal pleura is divided into costal, diaphragmatic, cervical (dome of the pleura) and mediastinal according to the regions they cover. The diaphragmatic pleura covers part of the upper surface of the corresponding diaphragm. The outer part of its circumference is continuous with the costal pleura, while medially it is continuous with the mediastinal pleura. The cervical pleura (dome of the pleura) is the continuation of the costal pleura over the apex of the lungs.

The mediastinal pleura forms the lateral boundary of the interpleural space or mediastinum. Above the root of the lung it is continuous between the sternum and vertebral column. On the right side it is in contact with the right brachiocephalic vein, upper part of superior venacava, terminal part of azygous vein, right phrenic nerve, right vagus, trachea, and esophagus. On the left side it is in relation with the arch of aorta, left phrenic, left vagus N., left brachiocephalic and intercostal veins, the left common carotid and sub-clavian arteries, the thoracic duct and oesophagus. At the root of the lung the mediastinal pleura runs laterally as a tube of serous membrane enclosing the structures of lung root and continues with the visceral pleura. This double layer is known as pulmonary ligament.

The costal and diaphragmatic pleura comes in contact to form a narrow slit known as costodiaphragmatic recess. In quiet respiration the lower limit of the lung is about 5 cm. above lower limit of the pleura.

The pleura is composed of flat mesothelial cells of few micron thick and about 30 microns in diameter. The mesothelial cells also contain microvilli in rabbit and probably also in humans⁶.

Visceral pleura is composed of :

- a. Mesothelial layer
- b. Basement membrane (50-60 Micrometer Thick)
- c. Elastic lamina (0.3—0.7 mm thick)

Most of the collagen fiber and interstitial cells are located between the elastic lamina and basement membrane.

Beneath the mesothelial layer of the parietal pleura are found:-

- a. Continues basement membrane
- b. Collagen fibre
- c. A discontinuous elastic lamina
- d. Thick and densely packed collagen fibres or bundles and
- e. A second discontinuous elastic lamina separating the sub-mesothelial space from the muscular layer.

Development

The parietal and visceral pleura develop from the somatopleural and splanchno-pleural layers of the lateral plate of mesoderm respectively.

Blood supply and venous Drainage

The blood supply of the parietal pleura is provided by branches of intercostal arteries. Mediastinal pleura is supplied by pericardiophrenic artery and the diaphragmatic pleura is supplied by the superior phrenic artery and musculophrenic artery. The venous drainage of the parietal pleura is mainly into the azygous, hemiazygous and internal mammary veins. Arterial supply of visceral pleura comes from pulmonary artery and bronchial artery (Miller, 1970)⁷ and the veins drain into the pulmonary veins (Agostoni, 1972)⁸

The pleural veins connect with the bronchial veins in the vicinity of the hilus.

Lymphatics

The visceral pleural lymphatics are located in the connective tissue layer beneath the pleura and have anastomosis with parenchymal lymphatics, which drain into the hilar lymph nodes. In the parietal pleura the lymphatics are located in the immediate submesothelial layer. Lymphatics of the costal portion drain into intercostal lymphatics and end in the parasternal and paravertebral node, while the lymphatics of mediastinal surface drain into the tracheobronchial lymphnodes.

The lymph from these regional nodes returns to the circulation through the right lymphatic duct. The diaphragmatic pleura drains into the efferent lymphatics of diaphragm.

Nerve supply

The costal pleura and the pleura on the peripheral part of the diaphragm are supplied by the intercostal nerves. The mediastinal pleura and the pleura on the central part of the diaphragm are supplied by phrenic nerve. Irritation of the former part of the pleura results in the pain being referred along the intercostal nerves to the thoracic or abdominal wall, whereas irritation of the central part of diaphragm results in pain being referred to lower part of the neck and over the shoulder. The visceral pleura is supplied by autonomic nerves innervating the lung. Visceral pleura has no pain fibres.

PHYSIOLOGY

The visceral and parietal pleura form a smooth membrane that facilitates the movement of the lungs within the pleural space. The membrane secretes and absorbs pleural fluid, which acts as a lubricant.

Intrapleural Pressure

Pressure within the pleural cavity represents the difference between the opposing elastic forces of the chest wall and that of the lungs. The mean pressure within the pleural cavity is sub-atmospheric. This is because of the retractive force of the lung which consists of :

1. The elastic tissue throughout the interstitium of the lung and in the bronchial wall.
2. The geodesic pattern of bronchial muscle which tends to shorten the airways.
3. The surface tension of the alveolar lining film.

The intrapleural pressure can be measured by the induction of a small pneumothorax, but this potentially dangerous procedure is not feasible for routine investigation and it is unnecessary since it has been

shown in many studies that there is a close relation between intra-oesophageal and intrapleural pressure. The interpleural pressure can also be measured by Lilington Pearsons apparatus. But the pleural pressure is not uniform throughout the pleural cavity (Millic –Emili et al, 1966).⁹

Pleural pressure is more negative at the apex than the base and gradient is about 0.2cm of H₂O per centimeter vertical height although a steeper pressure gradient has been suggested by Rutishauser et al., 1966.¹⁰ The gradient is not uniform. It is greater over the upper than the lower zones of the lung. It is gravity dependent, being reversed in the head down position. The pleural pressure also varies in accordance with lung volume i.e.

1. In functional residual capacity it is 8 cm H₂O in the upper part and -2 cm H₂O in the lower part. The average is -5 cm H₂O.
2. In total lung capacity it is -30 cm of water in the upper part and -24 cm H₂O in lower part
3. In residual volume it is -4 cm H₂O in upper part, +2cm, H₂O in lower part.

Pleural Fluid Dynamics

According to Yamada (1993)¹¹ the amount of pleural fluid in normal man ranges from 1 ml to 20 ml. The volume of liquid in a single pleural space is approximately 2 ml. (Pare & Freser, 1985)¹²

In normal man transudation and absorption of fluid within the pleural cavity follows the Starling equation and depends upon a combination of hydrostatic, colloid osmotic and tissue pressures. The tissue pressures are not known, but the knowledge of the magnitude of the first two forces suggests that fluid is formed at the parietal pleura and absorbed by the visceral pleura. The rich vascularity and increased number of microvilli of the visceral pleura favour the liquid absorption.

A net hydrostatic pressure of 35 cm water (30 cm water Hydrostatic pressure of the systemic capillaries plus -5cm. water of pleural pressure) tends to force fluid from the parietal pleural capillaries, into pleural space (Landis et al., 1963).¹³ The colloid osmotic pressure in the systemic capillaries is 34 cm water minus that of the fluid in the pleural cavity is 8 cm water, resulting in a net opposing force of 26 cm water colloid osmotic pressure. The difference between these forces (35cm – 26cm) is 9 cm water. This tends to force fluid from the parietal pleura into the pleural cavity.

The visceral pleura is supplied by pulmonary artery capillaries that have hydrostatic pressure of approximately 11 cm water. The net hydrostatic pressure difference between the visceral pleura and the pleural cavity therefore is 16 cm water (11 cm + 5 cm). The colloid osmotic pressure in the visceral pleural capillaries is 34 cm water minus colloid osmotic pressure of 8 cm H₂O in the pleural fluid which comes to be 26 cm H₂O which creates a net effect of 10 cm water pressure (26cm -16cm) and tends to force fluid towards the visceral pleural capillaries. Clause, Yacobiuan and Barker (1957) showed that collections of pleural fluid are not stagnant pools, but constantly changing. About 30-75 percent of water volume is exchanged each hour. Water and electrolytes (Crystalloids) reach the blood directly through the pleural membrane whereas the protein molecules (colloids) are absorbed from the pleural space only through the lymphatics, so that their turnover rate is much slower. The lymphatic absorption takes place almost entirely into the lymphatics of lower mediastinal folds. These lymphatics mostly drain into the right lymphatic duct (75 percent) and to a lesser extent into the thoracic duct. The parietal pleura overlaying the intercostal spaces plays a major role in lymphatic absorption, while the whole of visceral pleura and diaphragmatic pleura have little capacity to absorb protein (colloids) (Courtice and Simonds, 1954).¹⁴ The absorption of pleural proteins is increased by chest wall and diaphragmatic movement (Courtice & Morris, 1953).¹⁵ The amount of fluid that traverses the pleural space for 24 hours is high. Agostoni (1972) has shown that the surface area of the visceral pleura is 5000 sq. cm. and between 5000 to 10,000 ml of protein-free fluid pass through the pleural space in 24 hour.

Normally there are small amounts of fluid with a protein content of approximately 1.5 gm. per 100 ml, present in the pleural space. This protein leaks from the pleural capillaries into the pleural space. If the protein accumulates, colloid osmotic pressure of the pleural fluid would increase and the pressure gradients would favour fluid movement from both the visceral and the parietal pleura to the pleural space. It should be expected that the pleural fluid would continue to accumulate until the protein is diluted and or the pleural pressure increases to restore conditions favourable for fluid absorption. Since the serum protein concentration is greater than the pleural fluid protein concentration, the pleural fluid protein would never be removed by simple diffusion and pleural effusions would never resolve spontaneously. Nevertheless, some pleural effusion with high protein content resolve spontaneously.

How is the protein removed from the pleural space ? Courtice and Simmonds (1949) ligated the thoracic duct and the right lymphatic ducts in cats with pleural effusion. When labelled protein was placed in the effusion, more of it reached the systemic circulation. Stewart (1963) subsequently demonstrated that in patients with transudative pleural effusion, the lymphatic flow from the pleural space averaged 0.2 ml per kg. per hour at night and nearly double during the day. Therefore in a 60 kg individual the lymphatic clearance of the pleural space is in the range of 250-500ml. per 24 hours, this is less than 10 percent of the clearance of protein-free

fluid. Red blood cells and white blood cells are also removed from the pleural space by the lymphatic (Steward and Berger, 1958).

The protein-free fluid is constantly entering the pleural space from the parietal pleura and leaving through the visceral pleura. Protein enters the pleural space from both pleural surfaces and leaves via the lymphatics. Pleural fluid accumulates when the normal dynamic equilibrium is upset and continues to accumulate until another equilibrium is reached.

In health there is a constant movement of fluid into the pleural space which is balanced by absorption. Any deviation in the balanced rate of capillary filtration and lymphatic drainage gives rise to pathological collection of fluid into pleural space (Hinshaw, 1980).¹⁶

The pleural fluid of normal humans contains 4500/cu.ml. (corresponding to 54 percent) monocytes, 29.5 percent degenerative mesothelial cells, 10 percent lymphocytes and 3.5 percent granulocytes. According to Wang (1974) and Yamada (1933) the cell counts ranged from 1700 to 6200/cu. ml.¹⁷

The fluid also contains 12 gm protein. The pleural fluid has the same electrolytes as that of plasma although the bicarbonate concentration is about 25% higher and the chloride concentration is about 8% lower than plasma.

The factors which contribute to the development of pleural effusion are:

1. Increased pulmonary capillary pressure
2. Increased pleural capillary permeability.
3. Decreased pleural lymphatic absorption.
4. Decreased plasma oncotic pressure.

ETIOLOGIC CLASSIFICATION OF PLEURAL EFFUSIONS

The etiological classification of pleural effusions depends upon whether the pleural fluid is an exudate or transudate. The differentiating features of transudates and exudates are described later on.

A transudate occurs when the systemic factors influencing the formation or absorption of fluid are altered. Decreased plasma osmotic pressure or elevated hydrostatic pressure in the systemic or pulmonary circulation are alterations that produce transudates. The pleural surfaces are not involved by the primary pathologic process when there is a transudative pleural effusion.

An exudate results from disease of the pleural surface. The two main mechanisms by which pleural disease leads to pleural fluid accumulation are:

1. Increased permeability of the capillaries for protein, such as occurs with tuberculosis, pneumonia and pulmonary embolisation.
2. Lymphatic obstruction such as occurs with lymphoma.

The first question that must be answered when a pleural effusion is discovered is whether that effusion is a transudate or an exudate. If the effusion is a transudate no further diagnostic procedures are necessary and therapy is directed towards the underlying cause. On the other hand, if the effusion proves to be an exudate, more extensive diagnostic procedures are required in order to pinpoint the cause of the pleural diseases.

The various causes of exudative pleural effusions are –

1. INFECTIVE

a. Bacterial infections

Mycobacterium tuberculosis	Nocardiasis
Pneumococcus	Streptococcus
Staphylococcus	Salmonella
Tularaemia	Brucellosis
Anthrax	Plague
Actinomycosis	

b. Viral

Coxsackie Virus – A
Coxsackie Virus –B
Influenza Virus

c. Mycoplasma

Mycoplasma pneumoniae

d. Rickettsia

R. Prowazeki

e. Parasites

Protozoa
Pneumocystis carini

f. Mycotic infection

Histoplasmosis
Coccidioidomycosis
Blastomycosis
Cryptococcosis
Aspergillosis

Entamoeba histolytica
Metazoa

Filariasis	Paragonimus westermani
Echinococcus granulosus	Strongyloidosis
Liver flukes	
2. NEOPLASTIC	
Primary tumours	
Mesothelioma of pleura	
Secondary tumours of	
Lungs	Breasts
Ovary	Kidney
Stomach	Pancreas
Uterus	Bladder
Prostate	Thyroid
Bone	Chest wall
Lymphoma	Leukemia
Hodgkins	Acute/Chronic Leukemia
Non-Hodgkins	
3. CIRCULATORY	
Pulmonary embolism	
Pulmonary infarction	
4. LYMPHATIC OBSTRUCTION	
Lymphoma	Mediastinal tumour
Metastatic chylothorax	Malignant chylothorax
5. COLLAGEN DISEASES	
Systemic Lupus Erythematosus	Rheumatoid disease
Rheumatic fever	Polyarteritis nodosa
Hamman Rich Syndrome	Scleroderma
Dermatomyositis	
6. TRAUMA	
Haemothorax	Chylothorax
Esophageal rupture	
7. INTRA ABDOMINAL	
Abscess (subdiaphragmatic, hepatic, splenic, Perinephric)	
Pancreatitis	
Pancreatic pseudocyst	
Peritoneal Dialysis	
8. ALLERGIC	
Drugs	
Nitrofurantoin	Methysergide
Practolol	Hydralazine
Diphenylhydrazine	Isoniazide (Huchon et al., 1986)
Methotrexate	5 F.U.
Bleomycin	Nitrogen mustard
9. MISCELLANEOUS	
Chronic Haemodialysis	Myxoedema
Sarcoidosis	Familial Mediterranean fever
Asbestosis & Silicosis	Infectious hepatitis (Richard et al., 1980)
Meig's syndrome	Postmyocardial infarction syndrome
Post-radiation therapy	Yellow nail syndrome

TRANSUDATIVE VERSUS EXUDATIVE PLEURAL EFFUSION

Separation of Transudates from Exudates

Classically a pleural fluid has been classified as exudates when the protein level exceeds 3.0gm per 100 ml or its specific gravity is above 1016. Unfortunately the use of either of these criteria results in wrongly classifying over 10 percent of effusions (Currance Power, 1958, Leualler and Car, 1955, Light et al, 1972)

It has subsequently been shown that the simultaneous use of protein and lactic acid dehydrogenase (LDH) level for pleural fluid and serum effectively separates transudative from exudative effusion. In a prospective study of 150 pleural effusion (light et al, 1972)⁷⁶ 102 of 103 exudates had at least one of the following characteristics, while only one of 47 transudates had any of these characteristics.

1. Pleural fluid protein divided by serum protein > 0.5
2. Pleural fluid LDH divided by serum > 0.6.
3. Pleural fluid LDH greater than 2/3rd the upper limit for the serum LDH.
4. Pleural fluid LDH > 200

If the fluid has none of the above characteristics, it is transudate and therapy need only be directed towards the underlying causes.

However if the fluid has one or more of the above characteristics, it is an exudate and attention must be focused on methods to elucidate the aetiology of the pleural disease.

Specific Gravity

A specific gravity of 1016 corresponds to a protein content of 3.0 gm per 100 ml and each deviation of +0.003 in specific gravity represents approximately 1.0 gm. per 100 ml protein. For example a specific gravity of 1022 corresponds to a protein level of 5.0 gm per 100 ml (Puddack, 1941)

AETIOLOGICAL REVIEW

Among all pleural effusions 60-70% of pleural effusions are tubercular in origin. So every case of pleural effusion is presumed to be tuberculous in origin unless proved otherwise. Tuberculosis is an ancient disease. Man may have been affected by it since he evolved as a species on this planet. Evidence of existence of tuberculosis has been found in the bones of pre-historic man, found in Germany. These remains date back to about 8000BC. Typical tuberculosis changes have been found in the spines of skeleton of ancient Egyptians dating about 2500-1000BC.

Hippocrates (460-377BC) also devoted some attention to tuberculosis. However his interest in tuberculosis remained for a very short duration of time.

Aristotle first thought of tuberculosis as an infectious disease before 2000 years of the discovery of tuberculosis bacillus. It was also discovered that close contacts also developed the disease. Galen also confirmed these views, but little progress was made till 16th century. 400-1400AD has been termed the dark ages, as here all knowledge of tuberculosis was lost. The touching of kings' feet for cure of tuberculosis was popular in England.

Fracastorius in 16th century postulated that phthisis and other maladies were caused by small particles, the contagion vivum, which could possibly have air-borne spread. He was the first to anticipate the germ theory of disease. Some people also believed in alternative theory, that hereditary was a causative factor.

Villemin in 1865 proved by a series of classical experiments that a specific agent causes tuberculosis and it can be transmitted from man to man by inoculation of infectious material.

Laennec, himself a patient of tuberculosis, invented the stethoscope in 1819 and described auscultation. He and Bayle first described the tubercles.

Rudolf Virchow described the pathology in tuberculosis tissue, development of caseation and believed that susceptibility to the disease is inherited and not due to the disease itself.

Roentgen discovered X- rays in 1895 and it was put to clinical use by 1904. Radiological, clinical and bacteriological advances, all three together helped in further development of the knowledge of tuberculosis.

Calmette and Guerin produced attenuated Bovine Bacillus after sub-culturing about 230 times from 1908-1921.

Robert Koch (1843-1910) in 24th March 1882 announced the aetiological agent of Tuberculosis and discovered the Tubercle bacillus. Till today the world celebrates this day as World Tuberculosis day. In 1890 he developed Tuberculin, which he used for curing Tuberculosis. But this caused many adverse reactions and was far from being the magical cure, for which he was criticized. Nevertheless it remained an important diagnostic tool.

Lenec (1840) was perhaps the first to suggest that pleurisy was a manifestation of tuberculosis.¹⁸ Tuberculous pleurisy with effusion sometimes occurs as a complication of established pulmonary or extra pulmonary tuberculosis. Pleural effusion occurs both in primary and post primary stage. Wallgren (1948)¹⁹ showed that it mostly occurs during 3-6 months of the primary infection. 25 percent of the effusion occurs

within 3 months and the rest 75 percent of cases occurs within months. When pleurisy in an young adult is observed it is likely that primary infection has recently occurred. On the other hand tuberculous pleurisy in persons over 45 is probably due to recrudescence of an old primary lesion or apical focus (Pagel, 1964).²⁰ In many cases the primary focus may be unrecognised. However, in some cases evidence of previous infection is found. Usually neither history nor the evidence of previous primary infection is available (Robson and Imerson, 1963).²¹

The response of the host depends upon his/her level of sensitivity to tubercular proteins. When this is high there is massive outpouring of fluid. When the sensitivity is low as in young infants and malnourished older children, the results are much less dramatic (Miller, 1982).²² The frequency of pleural effusion in pulmonary tuberculosis is about 20 percent and this usually appears 3-7 months following the primary infection. Most effusions due to primary infection are common on the right than the left. But on the whole in children pleural effusion is much less common, the incidence being 3.3 percent in the age group of 2-5 years (Hardy and Kending, 1945).²³ Between ages of five and puberty it is about 7 percent of patients with primary tuberculosis.²⁴ The incidence of tuberculous pleural effusion is higher in males than in females.

Pathophysiology and natural history

Immunological and microbiological factors

MTB may affect the pleura at different stages of pulmonary or systemic disease and by a number of different mechanisms. Thus pleural involvement occurs in primary, postprimary and reactivated TB alike and is basically believed to arise directly from contiguous macroscopic or microscopic lung lesions or else lymphatic or hematogenous spread, but probably also via immunogenic mechanisms. Pleuritis exudativa tuberculosa is by far the most common clinical variety and has been classically interpreted as an early delayed-hypersensitivity-type phenomenon rather than direct organ involvement^{25,26}. Many clinical observations and experimental findings are in favour of this hypothesis such as:

- Its frequent association with known primary infection and a typical 6-12 weeks latency
- An often striking absence of significant pulmonary or systemic TB-lesions
- An often culturally negative or paucibacillary effusion²⁷,
- The sometimes abundant isolation of specifically purified protein derivative (PPD)- protein sensitized T-lymphocytes from pleural fluid²⁸
- More recently the inducible pleurisy in previously PPD-sensitized animals when exposed to intra-pleural mycobacterial protein.

Also the vigorous expression of inflammatory mediators interleukins (IL) like interferon (IFN) γ , IL-1 and IL-8 observed in this model (or conversely their suppression by antilymphocyte serum) support this view^{29,30}.

On the other hand there is also strong evidence that infectious invasion of the pleural space actually occurs at a substantial, albeit variable degree. In thoracoscopy, even with negative fluids studies, extensive inflammatory granuloma formation and fibrin deposits with unexpected abundant mycobacteria recovery are a common finding (see also section on invasive endoscopic-biopic studies)³¹. The increasingly emerging evidence of a preferred association of TB-pleurisy with reactivated TB in Western populations clearly points to infectious as well as immunological mechanisms being interrelated and operative in a complex manner. Direct infectious invasion however clearly prevails in chronic tuberculous involvement of the pleura as in specific empyema.

According to present views and based on experimental evidence the sequence of immunological processes involved in TB-pleuritis appears to follow a three stage pattern of cellular reactions and granuloma formation as a topic variant of general interaction mechanisms between MTB and the human immune system. A schematic representation is given in figure 1^{32,33}.

Any trigger-mechanism that allows access of mycobacterial protein to the pleura will set off a rapid mesothelial cell initiated and IL-8 mediated polymorphonuclear neutrophil (PMN) influx within a few hours³⁴. In addition macrophages and blood-borne monocytes determine this IL-1, IL-6 and tumor necrosis factor (TNF)- α -orchestrated *early stage* reaction.

Within roughly 3 days, in the following intermediate stage, lymphocyte subpopulations, mainly of CD4+ helper cells but also a substantial CD8+ cytotoxic (natural killer cells) fraction dominate the scene resulting in a CD4+/CD8+-ratio of ~ 4.3 ³⁵. A minor contribution includes so-called T-cell receptor double negative (DN) $\alpha\beta$ -T-cells and $\gamma\delta$ -T-cells which appear to have regulatory functions. More recently in tuberculous pleural fluid another unique CD4+CD25+ T-cell-class could be demonstrated being specifically involved in the down-regulation of autoreactive IFN- γ -producing T-cells, thus preventing inflammatory overshoot [36]. IFN- γ , a strong promoter of macrophage activation and granuloma formation (together with

TNF- α) is the predominant interleukin in this stage. IFN- γ -producing cells have been phenotypically identified as CDW29+ subpopulation and make up a substantial portion of the granuloma core structure [37].

The late phase is characterised by an equilibrated and sustained CD4+/CD8+ cell-based response with continued IFN- γ release and prolonged granuloma formation. Several modulating interleukins are involved in this process such as T-helper-cells (CD4+)-supporting IL-12 and counter-regulatory anti-inflammatory cytokines like IL-10 and transforming growth factor(TGF- β).

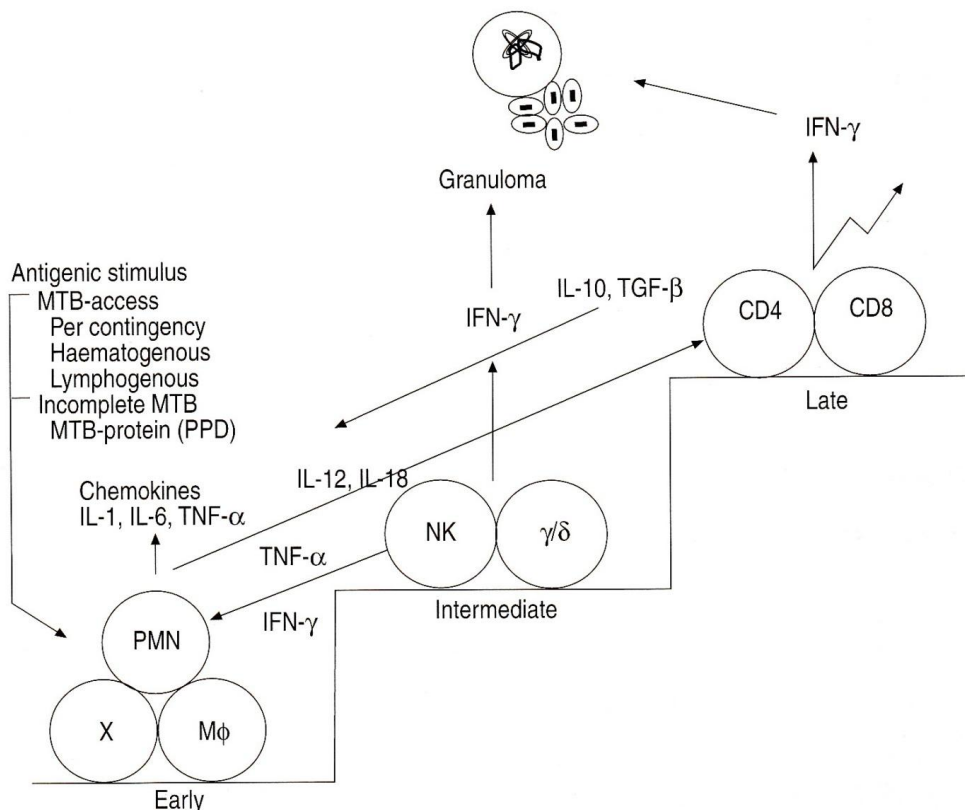


Figure 1. Mechanisms and immunogenesis of tuberculous pleurisy: the three stages of protective immune response.

IFN: interferon; TNF: tumor necrosis factor; IL: interleukin; PMN: polymorphonuclear granulocyte; X: undefined cell;

M: macrophage, MTB: mycobacterium tuberculosis; PPD purified protein derivative.

Results of HIV and AIDS research also emphasize the importance of T-cell response. Several working groups have shown that the prevalence of tuberculous pleurisy in HIV-infected patients with TB is strikingly correlated with their CD4+ blood lymphocyte count. In one series pleurisy prevalence in individuals with a count of > 200 cells/ml was 27 % as compared to 10 % in those with a count of < 200 cells/ml [38]. The data support the view, that the clinical expression of exudative pleural effusion requires a largely intact cellular immune system and features pleurisy as a high activity response in a still immune competent individual. In epidemiologic terms one would conclude that pleural effusion should be more frequent in the still immune competent host than in patients with AIDS. In reality however in most HIV-high-prevalence countries like South Africa, Uganda and Zimbabwe the percentage of thoracic TB-patients with pleural effusion is reportedly higher in HIV+ patients [39]. As an explanation the situation is probably blurred by a variable and poorly defined immune status in HIV+ individuals.

Other factors

The mechanisms of fluid accumulation and of abundant protein leakage to the pleura with often extensive fibrin deposits in tuberculous pleurisy have so far not been fully elucidated. In actual fact pleuritis exudativa tuberculosa generally presents with the highest protein levels commonly seen in exudative conditions. The intensity of inflammation and a proportionately increased vascular permeability would provide a satisfying explanation [38,40] although at least in animal models, no such significantly altered vascular permeability could be demonstrated [25]. Current opinion holds that grossly impeded lymphatic protein clearance from the pleura due to altered parietal lymphatic channels is probably of tantamount importance.

Again the entry mechanism of mycobacteria to the pleura has remained unclear. It is usually assumed that the release of infectious material from a ruptured subpleural TB-lesion is the most common mechanism. While this is likely to occur in more or less extensive pulmonary TB, it would not explain the frequent association of tuberculous pleuritis with an – at least radiographically – unaffected lung. There are also no convincing data yet to quantify the contribution of a purported hematogenous or lymphogenous contribution. One might reasonably speculate that different patterns of pleural tuberculous involvement are operative which might correspond to the different clinical settings of primary, post-primary and reactivated TB.

Caseous tuberculous pleuritis or specific empyema is nowadays a rare condition which is believed to be the result of longstanding or chronic infection of the pleura, when either caseous material gains access to the pleura or chronic pleuritis develops on the background of impaired local defence such as pre-existing fibrous damage of the pleura or as a sequel and complication of artificial pneumothorax, oleothorax or other TB-specific surgery dating back to the prechemotherapy era. Correspondingly there is usually an extremely long history often with a remarkable paucity or even absence of symptoms. Penetration to deeper chest wall structures (specific abscess) and ultimately transcutaneous discharge (empyema necessitans) or creation of a specific bronchopleural fistula, as not infrequently seen in the pre-chemotherapeutic era, may complicate this condition [40]. Putrid discharge from a thoracic mass or putrid expectoration with or without haemoptysis may ultimately advert to the condition.

DIAGNOSIS

Clinical Manifestations

Patients with pleural effusion usually present with dyspnea, chest pain, dry cough, fever and symptoms related to the underlying cause. As the fluid accumulated chest pain usually subsides, dyspnea develops depending upon the amount of the fluid in the pleural cavity and rapidity with which it accumulates, which is the most common symptoms related to the effusion. Patients may have a heavy sensation or a dull pain on the affected side (Ingram, 1986). General constitutional disturbances like loss of appetite, loss of weight, general malaise and febrile reactions are present. If there is associated parenchyma disease of the lungs there occurs cough with or without expectoration. Particularly immediately above the stony dull area, the percussion note is moderately dull due to the underlying relaxed lung and this is called Garland’s triangle. In massive effusion there may be a triangular area of dullness on the contralateral side, the apex of which is near the vertebral column, while the base is about 2.5cm away from midline. This is called the Grocco’s triangle and is due to prolapse of pleural cavity to healthy side. Skodaic resonance may be noted above the fluid level anteriorly over the intraclavicular region.

The breath sound over the area of dullness is diminished or absent. At the upper level of dullness, sometimes bronchial breathing is heard, which is due to relaxed lung or due to collapsed lung with patent bronchus. Sometimes bronchial breath sound is heard over the fluid due to underlying pathology. Bronchial breath sound may be heard over a wide area in case of massive or tense pleural effusions as the underlying lung is completely collapsed and bronchial breath sound from healthy bronchi is conducted through the fluid to the chest wall. Adventitious sounds are usually absent but fine crepitations may be audible immediately above the fluid from the relaxed lung. Sometimes pleural rub is heard above the fluid. The vocal resonance is diminished or absent. Aegophony may be present, fine crepitations may be heard over opposite lung due to passive congestion. Examination of heart may show soft systolic murmur due to displacement. Liver or spleen may be palpable due to downward displacement by the fluid.

Stephen (1986)⁴¹ correlated the chief findings with the volume of effusion which is given in a tabular form below.

Amount of effusion (ml)

Parameter	Small (300ml)	Moderate (300-1500ml)	Large (>1500ml)
Respiratory distress	+/-	+,++	++
Respiratory rate	Normal/Increased	Normal/Increased	Increased
Chest expansion	Normal	Normal/decreased	Decreased
Fremitus	Normal	Normal/decreased	Decreased
Breath sound	Normal	Normal/decreased	Decreased
Tracheal shift	-	+/-	+
Apical shift	-	+/-	+

INVESTIGATIONS

Non-invasive Pleural Imaging

Imaging of the chest using standard radiologic techniques, taking different views like postero-anterior, lateral, and lateral decubitus are most valuable. Posterior anterior chest roentgenogram detects 100ml of fluid in the pleural space (Crofton,1990). The most common finding of pleural effusion on chest roentgenogram is

obliteration of costophrenic angle, while in moderate and massive effusion, the costophrenic and cardiophrenic angles, diaphragmatic outline and cardiac border are obliterated and the fluid may rise upto 4th intercostal space laterally. A massive effusion obliterates most or all of the lung field and causes gross mediastinal displacement. But ultrasonic examination is simple and it can be done at bed side, without any radiation hazard. The results of examination are known immediately. The examination is also comfortable to the patient and it is also harmless. C.T. scan can detect fluid up to 3-5 ml in the pleural space, but it is expensive.

Thoracentesis

Confirmation of pleural effusion is achieved only when fluid is aspirated by thoracentesis. Hence all cases of smaller or larger effusions should be subjected to aspiration and the physical, biochemical, cytological and bacteriological examination of aspirated fluid should be undertaken to find out the aetiology of the effusion.

EXAMINATION OF THE PLEURAL FLUID

Physical Examination

Under physical examination, colour, turbidity, viscosity and odour of the pleural fluid are noted. Croften (1990) noted that transudates are clear or faint yellow whereas exudates are dark yellow or amber. Pagel et al.(1964) opined that haemorrhagic fluid favours the diagnosis of malignancy, trauma, tuberculosis and pulmonary infarction. In malignancy, effusions are massive in volume and tends to recur rapidly (Leahy et al., 1986).⁴² Tuberculosis is thought to be the commonest cause of either serous or turbid effusion but in 20% of cases, haemorrhagic effusion in tuberculosis is possible.

Perry & Holmes sellers (1963)⁴³ stated that pleural effusion is haemorrhagic in 80 percent of malignant effusion and in 18 percent of tuberculous effusion. Desmukh and Verdi(1972)⁴⁴ found that the colour of the pleural fluid in tubercular effusion is straw yellow in 88%, pus-like in 8 percent and haemorrhagic in 2 percent. Jain and Gupta (1975)⁴⁵ found straw coloured fluid in 82.8 percent of tubercular effusion, rest being haemorrhagic. A white milky appearance indicates chylothorax, or chyliform effusion. The pyothorax can be distinguished from the chylothorax and chyliform effusion in that after centrifugation there is a clear supernatant only with pyothorax.

A feculent odour is highly suggestive of an empyema secondary to anaerobic organism. A clear or bloody fluid that is quite viscous is highly suggestive of malignant mesothelioma, the high viscosity being secondary to elevated pleural fluid hyaluronic acid levels (Rasmussen & Feber, 1967). The fluid from a frank empyema is viscid and opaque.

Lactic Dehydrogenase (LDH)

Simultaneous measurement of serum and pleural fluid L.D.H are helpful in differentiating transudate from exudates. In a study of 150 patients Light et al.(1972)⁴⁶ found that no transudate had an L.D.H level more than 200 I.U. whereas most exudates (71%) had higher than 200 I.U. Light et al. suggested three biochemical characteristics for the differentiation of exudates from transudate as follows:

1. Pleural fluid to serum protein ratio greater than 0.5.
2. Pleural fluid L.D.H. greater than 200 I.U.
3. Pleural fluid to serum L.D.H. ratio greater than 0.6.

In congestive heart failure the LDH level is same both in serum and pleural fluid. A high level of L.D.H. is also observed in most malignant effusion. Sometimes in lymphoma and also in small cell lung cancer L.D.H. may be low, presumably due to metastatic liver lesion, as opined by Vergon et al (1984).⁴⁷

Protein and Lactic Dehydrogenase

In general measurement of the pleural fluid protein and lactic dehydrogenase are not useful in differentiating various types of exudative pleural effusion (Light et al. 1972). All exudates tend to contain increased amounts of both. However if the lactic dehydrogenase is elevated and the protein is not, the effusion is probably due to malignancy. Conversely most patients with pleural fluid proteins above 6.0gm per 100ml have tuberculous or parapneumonic effusions.

Pleural Fluid Sugar

Hirsan et al. (1979)⁴⁸ proposed that pleural fluid sugar must always be compared to a blood specimen obtained simultaneously in fasting state. As has been mentioned by Rao (1981)⁴⁹ the range of pleural fluid sugar in tubercular effusion is 30-60mg/100ml and often less than 30mg% when the effusion yields tubercle bacilli on smear or culture. However Lilington et al (1971)⁵⁰ opined that if the pleural fluid sugar is less than 30mg/100ml, rheumatoid pleuritis should be strongly suspected. Huchon and Chretien (1986) observed that an abnormally low concentration of glucose in pleural fluid can result from (I) Selective block for glucose transfer in rheumatoid pleurisy due to inhibition by mediators, released from the inflammatory pleural rheumatoid reaction.

(2) Fibrous pleural thickening and (3) excess glycolysis in cell (Leucocyte or tumour cells) metabolism or bacterial mycobacterial metabolism.

But the ratio of the pleural fluid glucose to blood glucose is more than 0.5 in transudate and a decrease in the ratio suggests the diagnosis of tuberculosis, malignancy, rheumatoid disease or parapneumonic effusion.

Pleural Fluid pH

Sahn (1986)⁵¹ stated that the pH of normal pleural fluid collected by thoracentesis from normal volunteers was 7.64, this value being 0.23 pH units higher than in simultaneously drawn blood sample. Light & Associates (1973)⁵² found that pH below 7.30 was highly suggestive of tuberculosis, whereas values greater than 7.40 usually indicates malignancy. Funahashi (1973)⁵³ found that the pH value varied from 6.0 to 7.47. However in all effusions secondary to congestive heart failure, hepatic cirrhosis and malignancy the pH was 7.29 or higher. By contrast most of non-malignant inflammatory effusions showed pH value 7.28 or less. In clinical practice measurement of pH is most important in parapneumonic effusion and empyema. A low pleural fluid pH indicates empyema or bacterial pleurisy. The value of pH, pCO₂, and pO₂, depends upon the concentration of the leucocytes within the pleural fluid, the bacterial activity and block of transfer for CO₂ and H⁺ (Hydrogen ion) which is related to the change in membrane and the failure of buffer mechanisms. When pleural fluid pH is less than 7.20 it will not resolve without chest tube (Light, Macgregor and Ball, 1973).⁵²

CEA (Carcino-Embryonic Antigen)

Rittgers et al (1978) stated that carcino-embryonic antigen (CEA) is a glycoprotein secreted by different tumours. Its concentration in pleural effusion may be higher than that in blood. In a study by Sourenson (1984) of 62 cases with malignant pleural effusion, he could find CEA in only 73% of the fluid in known malignant cases.⁵⁵

A malignant effusion is associated with CEA level ranging from 5-9.0 ng/ml (Nystrum et al 1977). The pleural fluid level is greater than twice the plasma CEA titre.

A preliminary report, Kinand Hirata (1976) on 6 patients has shown the pleural fluid from some patient with malignancy has a high level of CEA like substance (CEA-LS) while the CEA-LS is not elevated in benign effusion.

Complement

Low levels of complement in pleural fluid are found in effusions caused by either rheumatoid pleuritis or lupus erythematous. Hunder et al.(1972) measured the pleural fluid complement levels of 50 patients with pleural effusion of various aetiologies. The pleural fluid complement was low i.e. about 40 units/ml in 11 out of 12 patients with S.L.E. and Rheumatoid pleuritis, but exceeded this level in 37 out of 38 patients with other collagen vascular diseases. The pleural fluid complement levels are decreased substantially more than the serum complement levels. If collagen vascular disease is suspected, pleural fluid complement levels should be obtained.

Cytokine Content in Pleural Effusion

Kaorushimomata et al. (1991) studied interleukin-1 (IL-1) Interleukin-2 (IL-2) and interferon gamma (IFN- γ) levels in pleural fluid and found that tuberculous pleural fluid had higher level of IL-1, IL-2 and IFN- γ . IL-1, IL-2 and IFN- γ are key mediators of the host response to various infections, inflammatory and immunologic challenges. The median value of IFN- γ levels in 20 patients with tuberculous pleurisy were 73units/ml, while in carcinomatous pleurisy it was less than 1.0 units/ml. The result of this study shows that IL-1, IL-2 and IFN- γ values are significantly higher in tuberculous effusion than in malignant effusion.

Rheumatoid factor

Although the rheumatoid factor is high in effusion secondary to rheumatoid arthritis, it is also frequently elevated in exudates secondary to pneumonia, tuberculosis, and carcinoma. Therefore it is not used diagnostically (Levine et al., 1968).

Lupus Erythematosus (LE) Cells

Pleural fluid LE preparations should be obtained in all patients who have puzzling exudative effusion when the effusion is secondary to S.L.E. The pleural fluid will usually contain LE cells sometimes even when there are no LE cells in the peripheral blood. LE cells in the pleural fluid are thought to be pathogenic of SLE (Winslow, Plass and Loitman, 1958)

Amylase Activity

Chretien et al. stated that increase of amylase activity in pleural fluid has been considered as a good marker for pleural effusion related to a pancreatic cyst or to acute or chronic pancreatitis when pleural fluid amylase is more than 160 S.U./dl or more than twice the serum amylase level. Increase in amylase activity have been found in other conditions like ovarian tumour and gastrointestinal tumour.⁵⁶

The origin of amylase in oesophageal rupture is salivary rather than pancreatic in this situation (Sherr et al, 1972). The saliva enters the pleural space through the tear in the oesophagus. The pleural fluid amylase is elevated in approximately 10 percent of patients with malignant pleural effusion, as opined by Light and Ball (1973). The primary tumour in such instances is usually not in the pancreas.

Adenosine deaminase activity in pleural fluid

Nevertheless pleural tuberculosis is often difficult to diagnose since mycobacterium in pleural fluid is scanty and rarely observed on direct examination. In addition pleural biopsy and culture are positive in less than 50 percent of cases. In this context attempts have been made to identify markers which allow more rapid diagnosis of the disease. One such marker is adenosine deaminase (ADA), which has been proposed to be a useful diagnostic tool for tuberculous disease in pleura, pericardium and peritoneum. Adenosine deaminase is an enzyme of purine metabolism. The level of this enzyme is 10 times higher in lymphocytes than in R.B.C. and activity is more in T-cells than B-cells. The activity of ADA is related to lymphocytic proliferation and differentiation i.e. it is more in rapidly proliferating and immature lymphocytes. Therefore wherever there is cell-mediated immune response to an antigenic stimuli, the ADA levels are the highest. ADA is measured by the calorimetric method of Giusti.

On 1978 Piras et al found a high activity of ADA enzyme in pleural effusion of tubercular origin.⁵⁷ They also found high activity in pericardial and articular effusions and in few samples of ascitic fluid.

Piras et al (1973) were the first to report that this enzymatic activity was consistently high in the cerebrospinal fluid of subject with tuberculous meningitis and high activity of the same enzyme in pleural effusions of tubercular origin. They also found that there is high ADA activity in pericardial and articular effusions and ascitic fluid that originated from tubercular disease. Subsequently Piras et al. (1975), Blake & Burman (1982), Martiz, Malan and L. Roax (1982), Ocana et al(1986), Pattersson, Ojala Weber (1984), Strakinga et al. (1987), Fonten et al. (1988) Segur et al(1988) and may others have confirmed the diagnostic utility of ADA activity in tuberculous pleural effusions with high level of sensitivity and specificity. Strakinga et al.(1987)⁵⁸ found that ADA activity in the tuberculous pleural effusion was significantly higher than other effusion with specificity of 87% and sensitivity of 100% when the reference limit of ADA was 53U/L. ADA concentration was measured in effusion from 86 patients. Other laboratory test like total protein, LDH, cytology, and bacteriological examination including smear and culture for M. tuberculosis were performed. The diagnostic criteria of tuberculous pleurisy were a positive culture for M. Tuberculosis or a pleural biopsy showing typical epitheloid cell granuloma. The patients were divided into 9 groups according to the final clinical diagnosis. The results were as follows.

Diagnosis	No. of Patients	ADA Activity U/L		
		Mean	Median	Range
Tuberculosis	10	116.5	104.5	79-175
Empyema	10	94.0	73.0	16-222
Malignant Neoplastic disease	32	20.2	19.0	05-40
Non-Specific				
Pleural Effusion	17	22.2	18.0	00-71
Pneumonia	06	26.7	27.5	13.44
Congestive failure	07	16.0	27.5	13.44
Leukemia	03	19.7	22.0	12.25
Rheumatoid A	02	76.5	76.5	46-107
Non-Hodgkins Lymphoma	02	44.5	44.5	24-65

The ADA is synthesized by cells within the pleural cavity. Although the activity of ADA is high in T. lymphocytes and tuberculous pleurisy shows a marked lymphocytic predominance, their number does not correlate with ADA activity. This study does not differentiate between tuberculous pleurisy, empyema, and rheumatoid pleurisy. However this distinction can be made on the basis of bacteriological culture and by measuring concentration of complement in the pleural fluid. Inma Ocana et al. (1986) studied that the lowest value of ADA in tuberculous pleural effusion is 50 U/L. This test had sensitivity of 100% (no false negative) and a specificity of 97% (3% false positive). The mean enzyme value was clearly higher for tuberculous pleural fluids (93.81±29.65U/L) than that obtained in neoplastic effusions (13.02±9.66 U/L), lymphoma effusions (36.65 ± 30.83 U/L) and miscellaneous conditions (5.91±4.70 U/L).⁵⁹

Bansal et al (1991) studied the ADA activity in 218 patients with pleural effusions. In their series all but one patient displayed ADA activity higher than 70 IU/L with 99% sensitivity and 89% specificity. On the other hand there were 5.7% false positive result, which included lung cancer, lymphoma, empyema, mesothelioma, rheumatoid arthritis etc. In this situation it is therefore wiser not to rely on the ADA activity for diagnosis but to search for other evidence such as extrapulmonary manifestations in malignancy, pleural fluid pH in empyema or serum analysis for rheumatoid factors. Fontes Baganha et al. (1990) reported that there is higher percentage of CD4 T cells in the group of patients of tuberculosis and higher percentage of CD8 T-cells in the group of patients with neoplasm in both blood and pleural exudates. They were impressed by the conspicuously increased percentage of CD4-T cells in tubercular pleural effusions which showed a positive correlation with pleural fluid ADA level. They proposed that ADA constitutes a marker of cell mediated immune activity.

According to Hirschore et al. (1980) activity originates from the action of two principal isoenzymes ADA-1 and ADA-2 which have different pH, Michaelis constant (Km) and relative substrate specificity pattern. ADA-1 has low Km and an optimal pH of 7.0 to 7.5. It is present in all tissues and is essential for an efficient immune response. Congenital absence of ADA-1 in lymphocytes and erythrocytes causes severe combined immune deficiency syndrome. It has similar affinity for both adenosine and 2-deoxyadenosine. ADA-2 has high Km and an optimal pH of 6.5. It is not related to severe combined immune deficiency syndrome. It is found only in the monocyte macrophages and not in other cells and released by the presence of live micro-organisms in their interior. It has poor affinity for 2-deoxyadenosine. Incubating identical quantities of sample in two test tubes, one containing 20mmol of adenosine substrate and other 20 mmol of 2-deoxyadenosine substrate both at pH of 6.5, the enzymatic activity is measured in each tube. Then the 2-deoxyadenosine-ADA ratio is calculated. When there is high ADA-2 activity in presence of 2-deoxyadenosine substrate the ratio is below 0.45 and when it is due to ADA-1 activity the ratio is higher than 0.45. Low 2-deoxyadenosine-ADA ratio in the sample with an increase in ADA activity suggests tuberculosis. In the same fluid a ratio higher than 0.45 coupled with high or moderate ADA activity is found in subjects who have empyema or systemic malignant pathology. An enzymatic profile similar to that just described is also found in serum of patients with lymphoma and leukemia.

ADA-2 activity is more specific for tuberculous disease. But because of the technical difficulties involved in determining ADA-1 and ADA-2 activities separately most others have confirmed their studies in determining ADA activity as a whole and that policy has been followed in the present work also.

Hyaluronic Acid

Determination of level of Hyaluronic Acid in Pleural fluid may be useful in the diagnosis of pleural mesothelioma.

Interferon-Gamma^{70,73}

Interferon- γ is produced by the CD4+ T-lymphocytes in patients with TB pleural effusion in response to mycobacterial antigens. In fact, the concentration of Mycobacterium tuberculosis in pleural liquid correlates with the amount of IFN- γ . Patients with pleural effusion may have up to 25- fold higher IFN- γ level in the pleural fluid as compared to their peripheral blood, suggesting homing of Th1 cells in the pleural fluid from the peripheral blood and enrichment of pleural space with Th1 cytokines. The IFN- γ can be estimated either by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA). Estimation of pleural fluid IFN- γ levels is reported to be useful in differentiating TB from other pleural fluids. A number of reports have demonstrated that IFN- γ levels in patients with TB pleurisy are high, with sensitivity and specificity ranging from 90-100 percent. However proper comparison of results from different studies is not possible due to differences in methods of estimation and units used for quantification. Although the test is promising, it is expensive and still not widely available. The cost of performing a single IFN- γ test in India is, in fact, equivalent to the cost of a complete course of anti tuberculosis treatment for such patients, and therefore, does not appear to be a cost-effective investigation for differentiating TB from non-TB pleural effusion. Recently, meta-analyses have demonstrated the utility of estimation of pleural fluid IFN- γ in the diagnosis of TB pleurisy.

SERODIAGNOSIS⁷³

Till date only a few studies are available on the immune diagnosis of TB in pleural effusion. Both mycobacterial antigens and their antibodies have been estimated in pleural fluid and / or serum using ELISA based techniques to assess their utility in the diagnosis. The sensitivity reported in most studies is much less than desirable. The problem of false positive results has also been troublesome in other studies. The kaolin agglutination test, which detects antituberculo-phospholipid antibodies, may also provide equivalent sensitivity and specificity, while being much simpler. The origin of antibodies to mycobacterial antigens in pleural fluid of these patients is not clear. Levy and co-workers found close correlation between pleural fluid and serum levels, reflecting passive diffusion. Other investigators have demonstrated higher titres of antibodies in pleural fluid,

indicating local accumulation. Assays based on detection of tuberculostearic acid (TSA) in pleural aspirates have not yielded encouraging results. Further work needs to be done before immunodiagnostic techniques can be recommended for routine use in the diagnosis of TB pleural effusions.

Lysozyme⁷³

Lysozyme is a low molecular weight bacteriolytic protein distributed extensively in organic fluids. Its estimation in pleural fluid has been proposed as a useful test in the diagnosis of TB pleural effusion. Mean lysozyme levels in tubercular pleural fluid have been reported to be higher than in other exudative effusions. However, there is so much overlap that the levels themselves are not diagnostic. Pleural fluid to serum lysozyme ratio of more than 1 or 1.2 can differentiate better between TB and non-TB pleural fluids. A more recent report has, however, not reproduced these good results.

Nucleic Acid Amplification Techniques^{71,73}

Various nucleic acid amplification methods that have been used in the diagnosis of mycobacterial infection include target amplification techniques such as polymerase chain reaction (PCR), strand displacement amplification, and transcription mediated amplification, as well as probe, and primer amplification techniques such as ligand chain reaction and Q-Beta replicase amplification. Polymerase chain reaction, the most widely used of these techniques, is based on the amplification of mycobacterial deoxyribonucleic acid (DNA). In respiratory specimens, the PCR can be performed rapidly and has diagnostic yield comparable to that of culture. This procedure has also been used to detect mycobacterial DNA in pleural fluid. Its sensitivity in the diagnosis of TB pleural effusion ranges from as low as 17 percent to as high as 100 percent, depending on the patients selected, genomic sequence amplified, and the procedure used in the extraction of DNA. Specificity ranges from 61 to 100 percent. The parameter that determines the sensitivity of PCR is probably the number of bacilli in the sample of pleural fluid analysed. Series with a pleural fluid culture positivity of as high as 69 percent report more than 80 percent sensitivity of PCR. The PCR may be positive in 100 percent of culture-positive TB pleural fluids and only in 30 to 60 percent of culture negative pleural fluids. The lower sensitivity is most likely attributable to the inefficient recovery of genomic DNA from the characteristically low number of mycobacteria in patients with pleural TB. Genomic sequences present in multiple copies in mycobacteria give better results than sequences present in only a single copy. Contamination of samples by mycobacterial DNA in the laboratory environment is partly responsible for the low specificity.

Although these techniques are promising, the high cost and the technology involved in the procedure do not permit the routine diagnostic use of PCR at present.

CYTOLOGY

Light et al (1972) have shown that more than 80% transudative effusions but less than 20% exudative effusions have pleural fluid WBC count less than 1000/cu.mm. In effusion due to pneumonia, WBCs are more than 10,000/cu.mm, while in 50% of cases the WBC count may be increased up to 50,000/cu.mm. In case of malignancy and tuberculosis WBCs may be more than 10,000/cu.mm.

Differential White Cell Count

In all cases of pleural effusions, particularly in exudative effusions a detailed cytological examination of pleural fluid is essential in arriving at a diagnosis as to the underlying aetiology.

White Cell Count

About 20 percent of exudative effusion have a pleural fluid white cell count of less than 1000/cc and about 20 percent of transudative effusions have a pleural fluid white cell count of more than 1000/cc. Further in patients with transudative effusions who are on diuretic therapy, the pleural fluid cellular count will be more than 1000/cc. A large number of neutrophils in pleural fluid indicates bacterial pneumonia but may also be found in pancreatitis, pulmonary infarction and occasionally in malignant neoplasm and tuberculosis. Hinshaw (1980) pointed that higher percentage of neutrophils excludes the chance of tuberculosis, although neutrophilic predominance may be found during the early stages of tuberculous effusion. Some neutrophils are found in almost every pleural effusion. They appear in empyema. In this case the neutrophils degenerate and their nuclei become blurred and lose their normal purple staining characteristics. Their cytoplasm shows toxic granulation and fat granulation, and their normal granulation is lost. In all other effusions, the neutrophils appear much the same as they do in the peripheral blood. If neutrophils predominate in an effusion attributed to congestive heart failure, the possibility of pulmonary emboli should be investigated.

Lymphocytes

Mistitz and Pollard (1959) and Light et al, (1972) said that an effusion containing 50% or more lymphocytes is almost certainly of tuberculous or neoplastic origin. Reddy and Indira (1963) proposed that round cells are invariably encountered in all effusions, other than due to pyogenic infections. Ingram (1986) has also stated in the effusions of tuberculous and neoplastic disease the percentage of lymphocytes is usually more than 50%. The discovery that more than 50% of the white cells in the pleural fluid are small lymphocytes is important diagnostically in two series (Light et al., 1973; Yan 1967). It was found that 96 of 211 exudative pleural effusion had more than 50% small lymphocytes. Of these 96 effusions, 90 were due to tuberculosis or malignancy. Since these are the two diseases that can be diagnosed with pleural biopsy, the finding of predominantly small lymphocytes in an exudative pleural effusion is a strong indication for pleural biopsy. When the same two series are combined almost all effusions secondary to tuberculosis (43 out of 46) but only about half of the effusion secondary to malignancy (47 out of 90) had predominantly small lymphocytes.

Eosinophils

The presence of pleural fluid eosinophilia is of very little use in the differential diagnosis of pleural effusion (Springs and Beddington, 1968)). Most effusions with significant eosinophilia are either bloody or associated with pneumothorax. If the effusion is not bloody and there is no accompanying pneumothorax, the most likely aetiology is a viral pleuritis or resolving parapneumonic effusions. The presence of pleural fluid eosinophilia in a parapneumonic effusion is a good prognostic sign as the effusion virtually never becomes purulent. Pleural fluid eosinophilia may be found in pulmonary infarction, polyarteritis nodosa, parasitic disease, benign asbestos or pleural effusion.

Mesothelial Cells

A large number of mesothelial cells in pleural fluid excludes the diagnosis of tuberculosis. Sprigg (1960) found that in pulmonary infarction the percentage of mesothelial cells increases up to 20-30%. Mesothelial cells may be confused with malignant cells and their presence or absence is often useful diagnostically. Mesothelial cells are uncommon in tuberculous effusion (Spriggs and Beddington, 1968).

Red Blood Cells

It requires only 5000 to 10,000 red blood cells /cu.mm to impart a red colour to a pleural effusion. Assuming that a given pleural effusion has total volume of 500 cc and the red blood cells count in the peripheral blood is 5 million/cu.mm, a leak of only 1 cc of blood into the pleural space at the time of thoracentesis will result in a blood tinged pleural effusion. For this reason, the mere fact that a pleural effusion is blood tinged has limited diagnostic implications. Over 15 percent of transudate and over 40 percent of all types of exudative pleural effusion will be blood tinged i.e. pleural fluid red cell counts between 5000 to 10,000/cu.mm (Light et al., 1973)

The question is to whether the blood has been introduced via the thoracentesis or had been there previously present can frequently be resolved with Wright's stain of the sediment. If there has been blood on the pleural space for a matter of hours, the macrophages in the pleural fluid will contain haemoglobin inclusion bodies that will stain pink.

Grossly bloody pleural effusions have pleural fluid red cell counts above 100,000/cu.mm. This finding is very suggestive of one of three diseases such as trauma, malignancy, pulmonary embolism.

Malignant Cells

Repeated examination of pleural fluid is done to get the positive result. Springs (1957) found malignant cells in less than 50% of effusion, due to bronchogenic carcinoma and 71% of all malignant effusion. Positive findings in exfoliative cytology of pleural fluid ranges from 33 to 66 percent (Chretine, 1963; Bernard et al., 1964, Light et al., 1972) observed that when three separate specimens of pleural are tested, diagnosis is positive in about 80% of patients with malignant pleural effusion. Dekkar Buppa (1978) observed that false positive results are very common. Activated mesothelial cells may be confused with the mesothelial tumour cells (Bernaudin et al, 1983)

BACTERIOLOGICAL STUDY OF PLEURAL FLUID

The centrifuged deposit of pleural fluid is stained by Gram's stain and Zeihl Neelsen Stain and fungal stain. Since pleural space is normally sterile and presence of aerobic, anaerobic, tuberculous or fungal, cultures provide definite causes of pleural effusion. Close (1946), Sahn(1975), Ropper and Waring(1955), Meztitz and pollard (1959), Sahn (1975) and Ingram (1986) demonstrated AFB by culture of pleural fluid in 70%, 45%, 36.2%, 28.6%, 75-80% and 20 respectively.

CULTURE OF PLEURAL FLUID⁷³

Culture methods provide definitive diagnosis by establishing the viability and identity of the organisms. Further in order to distinguish between different mycobacterial species as well as to perform drug susceptibility tests, culture examinations becomes a necessary.

1. Conventional method of culture (Culture in L.J. Medium).

It is still considered a gold standard for diagnosis of tuberculosis. It can detect as few as 10 to 100 viable organisms per ml of specimen. It also detects nontuberculous mycobacteria in addition to mycobacterium tuberculosis.

The growth appears in about two weeks but may be delayed upto six to eight weeks. Optimum temperature for growth is 37° C and optimum PH for growth is 6.4 to 7.0. Increased Co₂ tension (5 to 10%) enhances growth. Human strains grow more luxuriantly in culture (eugonic) than do bovine strains (dysgonic). The human type of tubercle bacilli give rise to discrete, raised, irregular, dry and wrinkled colonies which are creamy white to begin with and then develop buff colour.

2. Rapid culture methods

Radiometric detection of growth of mycobacteria was first developed by Middlebrook. BACTEC radiometric system uses fatty acid substrates like palmitic acid or formic acid labeled with radioactive carbon (14C). As mycobacteria metabolises fatty acids, radioactive carbon dioxide (14Co₂) is released which is measured as a marker of bacterial growth.

Non tuberculous mycobacteria can be distinguished from MTB by testing for NAP (P-nitro α - Actyl Amino β hydroxy propiophenol) sensitivity.

BACTED – 460 utilizes liquid media & cuts down the detection time and is a rapid culture method which can detect MTB as early as 7 to 14 days on the basis of release of radio labeled Co₂ from growth of mycobacterium in a selective liquid media.

Average time for detection by BACTEC 460 system was found to be 13 days and 15 days as against 31 days and 35 days by L.J. media method for pulmonary and extrapulmonary specimen respectively.

Rapid liquid tuberculosis culture is known as Mycobacteria Growth Indicator Tube. (MGIT). The culture is done using manual or automated systems in which tubes contain enriched Middlebrook 7 H 9 broth and an oxygen sensitive fluorescent sensor embedded in silicone on the bottom of the tube. The presence of oxygen dissolved in broth quenches emissions from the compound. As the activity growing and respiring mycobacteria consume the dissolved oxygen, the sensor glows indicating mycobacterial growth. This can be observed by using an ultraviolet lamp with a wave length of 365nm.

SPUTUM EXAMINATION¹⁰³

Only a minority of patients with TB pleural effusion are sputum smear-positive for acid-fast bacilli (AFB). Sometime, AFB can be demonstrated even in patients with no radiographic evidence of pulmonary involvement. Sputum cultures grow mycobacteria in 30 to 50 percent of patients having both pulmonary and pleural TB. However, cultures are positive in less than five per cent of patients with isolated TB pleural effusion.

TUBERCULIN SKIN TEST⁷³

In populations with a low prevalence of TB infection, a positive tuberculin skin test (TST) in a patient with exudative pleural effusion strongly suggests the diagnosis of TB, whereas the diagnostic value of a positive test in countries with a high prevalence of TB is lower. The TST is positive in majority of patients, but a negative test does not rule out the diagnosis. About 30 to 50 percent patients can demonstrate a negative skin test at initial evaluation. Such negative test may be even more common in HIV-infected patients. This energy to PPD appears to be due to an antigen –specific extrapleural immune suppression. Although pleuritis is considered to be related to a delayed hypersensitivity, circulating adherent cells in the acute phase of the disease may suppress the specifically sensitized T-lymphocytes in the peripheral blood and in the skin (but not in the pleural fluid), accounting for the negative results in these patients. If the patient is not anergic or immunosuppressed, the skin test will almost always become positive within eight weeks of the development of the symptoms.

IV. Materials and Methods

The study comprised of 100 patients of pleural effusion admitted in the Department of general medicine and pulmonary medicine VIMSAR, Burla during November 2017 to October 2019.

Selection of cases

The diagnosis of pleural effusion was made in each case by clinical and radiological examination. Detailed history was taken and thorough clinical examination was done in each of the cases.

1. Routine laboratory investigations of blood and urine were done.
2. Sputum for AFB examination was done.
3. Chest X ray PA and lateral view were done.
4. USG Thorax was done.
5. Mantoux test were done in each case.
6. Pleural fluid examination was done after pleural aspiration under the following headings.

Physical:

Colour, turbidity, nature of fluid such as purulent, haemorrhagic, chylous.

Biochemical: Glucose, Protein, ADA

Cytological: DC, TLC, other cells

Microbiological : ZN stain, Gram stain, CBNAAT

Inclusion criteria:

- Patients of age of ≥ 14 years
 - Clinically compatible with tubercular pleural effusion
- Pleural effusion diagnosed by chest X-ray and USG&CT thorax

Exclusion criteria:

- Patient's refusal for pleural fluid aspiration.
- Trauma induced haemothorax and empyema.
- Any contraindication to thoracentesis like bleeding diathesis, on anticoagulation therapy, on mechanical ventilator, local causes like herpes zoster and pyoderma .

Methods

Full history taking with special emphasis on family history and personal history of Tuberculosis was done. Thorough clinical examination including general physical examination and local examination was performed. Radiological examinations including plain chest x-ray posteroanterior (PA) and lateral view were performed whenever necessary.

Other investigations like complete blood counts, sputum for AFB (acid fast bacilli), and tuberculin testing were done. Written informed consent for thoracentesis was taken as protocol. Pleural aspiration/ Thoracentesis was done in 6th intercostals space in midaxillary or posterior axillary line after clinical localization of pleural effusion on respective side of thoracic cavity. In selected cases we used USG guidance for thoracentesis, especially in loculated and minimal pleural effusion.

Pleural fluid analysis including gross and microscopic examination was performed. Pleural fluid for nucleic acid amplification tests, DNA PCR (deoxyribonucleic acid polymerase chain reaction) were performed on all the study samples. Other pleural fluid tests like microbiology (ZN and Gram's stain), biochemistry, ADA, cytology & pleural fluid AFB culture were performed.

Laboratory distribution of pleural fluid obtained with diagnostic thoracentesis

1. Biochemistry- 5 ml – Glucose, Protein, pH, ADA levels
2. Hematology- 5 ml – White Blood cell count
3. Bacteriology- 10 ml for M. tuberculosis Culture
4. Pathology – 5 ml for M. tuberculosis DNA PCR

For CBNAAT examination the sample reagent were added at 3:1 ratio to clinical specimens. The closed specimen container was manually agitated twice during a 15 minute period at room temperature, before 2ml of the inactivated material (equivalent to 0.5 ml of decontaminated pellet) was transferred to the test cartridge.

DNA extraction and PCR protocols (Nucleic acid amplification test)

DNA extraction was performed in an identical manner for all patients' samples using High Pure PCR Template preparation kit (provided from Roche Co.). The kit is designed to purify nucleic acids from different requested specimens for PCR test. It contains a primary step as a pre-lysis for some specific specimens such as tissue or even embedded tissue. The main steps are started of applying proteinase K and binding buffer on samples, and then use of inhibitor removal, washing and elution buffers respectively. At each step reagents were added to the filter tube. Supernatant was passed through collection tube after centrifugation. This procedure will

highly reduce the risk of contamination and increase the efficiency of the recovery rate of the nucleic acids as much as possible. PCR was carried out on the prepared purified nucleic acid using M.tuberculosis PCR kit. It contained specific primers to target transposable element (IS6110) for amplification. 5 µL of template, 10 µL PCR buffer and 10 µL mixture (containing specific primers and dNTP, 2.5U taq polymerase) were mixed and amplified with the recommended program. The applied PCR kit was constructed in a format of competitive PCR with internal control. Provided specific primers were used to amplify a product from fragment encoding 900 base pair as internal control to ensure proper extraction and removal of any expected inhibitors. This fragment was added before commencing extraction procedure.

The kit also contained specific labelled probes for specific and internal products to enable detection of the amplified products by the fluorescence detector, called Fluorescent Amplification-based Specific. The PCR depends on the ability to alternately denature (melt) double-stranded DNA molecules and renature (anneal) complementary single strands in a controlled fashion. As in the membrane-hybridization assay described earlier, the presence of non complementary strands in a mixture has little effect on the base pairing of complementary single DNA strands or complementary regions of strands. The second requirement for PCR is the ability to synthesize oligonucleotides at least 18–20 nucleotides long with a defined sequence. Such synthetic nucleotides can be readily produced with automated instruments based on the standard reaction scheme.

Pleural fluid ADA

In our study, we considered ADA level of 40 units/liter as a cut off for interpretation, values above 40 units/liter being suggestive of tuberculous pleural effusion.

Pleural fluid ZN (Ziehl-Neelsen) staining

The pleural fluid which was collected in sterile container was centrifuged, the supernatant was discarded, and 0.5 ml of the sediment deposit was used to prepare slides. The slides were then covered with strong carbolfuchsin, heated till steaming and left to stand for 5 minutes. The cycle was repeated three times before the excess stain was washed off with running tap water. The slides were then decolorized by flooding with 20% sulphuric acid for 3 to 5 minutes and then washed with water before they were counter stained with 0.3% methylene blue for 2 minutes. The slides were again washed. The slides were examined under the 100X oil immersion objective and 10X eye piece. A minimum of 100 fields were examined per slide before declaring it positive or negative. A slide was considered positive if it had at least one bacillus, which appeared as red, beaded rods.

Sputum for AFB

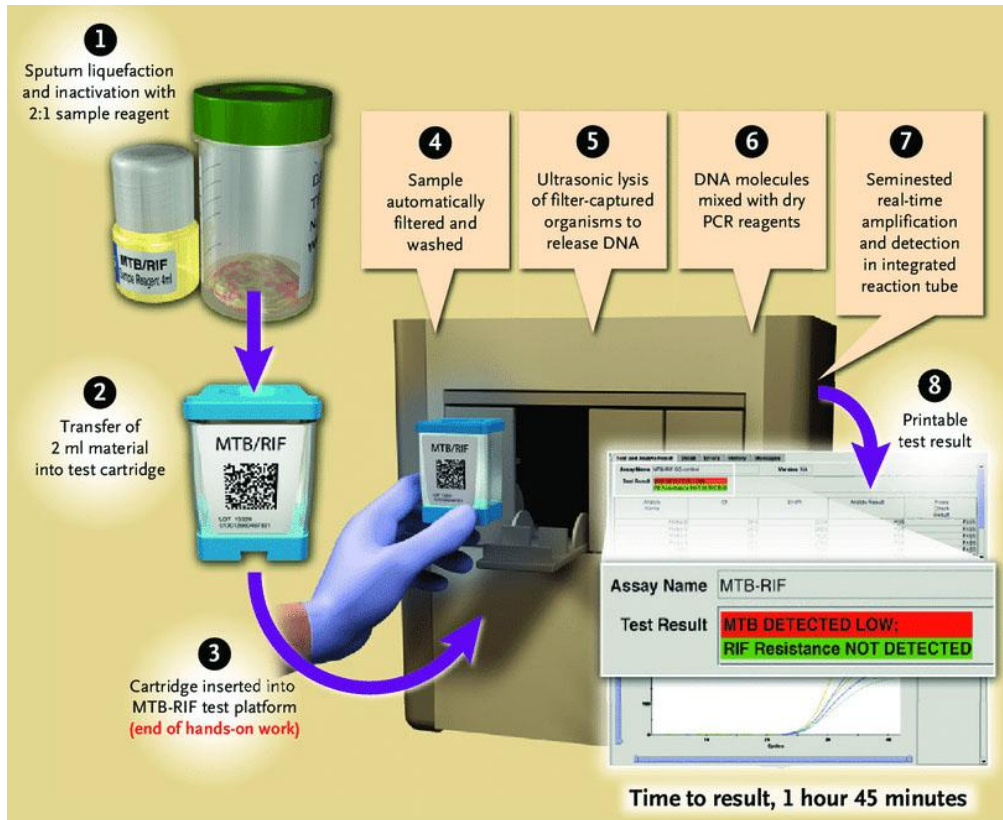
Same technique for processing, staining and interpretation of sputum samples used as in for pleural fluid.

SAMPLE SIZE

I have studied 100 cases of suspected tubercular pleural effusion patients admitted in the department of general medicine and pulmonary medicine.

STATISTICAL ANALYSIS

Statistical analyses were performed using the statistical package for the social sciences (SPSS) version 22 windows. Continuous variables were presented as mean ± standard deviation and categorical variables as percentages. Chi-square test was used to determine the association between categorical variables.



Procedure of CBNAAT Assay



Xray showing Massive pleural effusion

V. Observation

TABLE- 1 AGE AND SEX DISTRIBUTION OF STUDY GROUP

AGE	MALE	FEMALE	TOTAL	PERCENTAGE
15-19	6	1	7	7%
20-29	8	6	14	14%
30-39	10	4	14	14%
40-49	12	1	13	13%
50-59	15	4	19	19%
>60	29	4	33	33%
TOTAL	80	20	100	100%

In the present study, out of 100 patients 80 % are male and 20% are female with a male to female ratio of 4 :1. Majority (33%) of cases were >60 years of age.

GRAPH -1 : AGE AND SEX DISTRIBUTION OF CASES

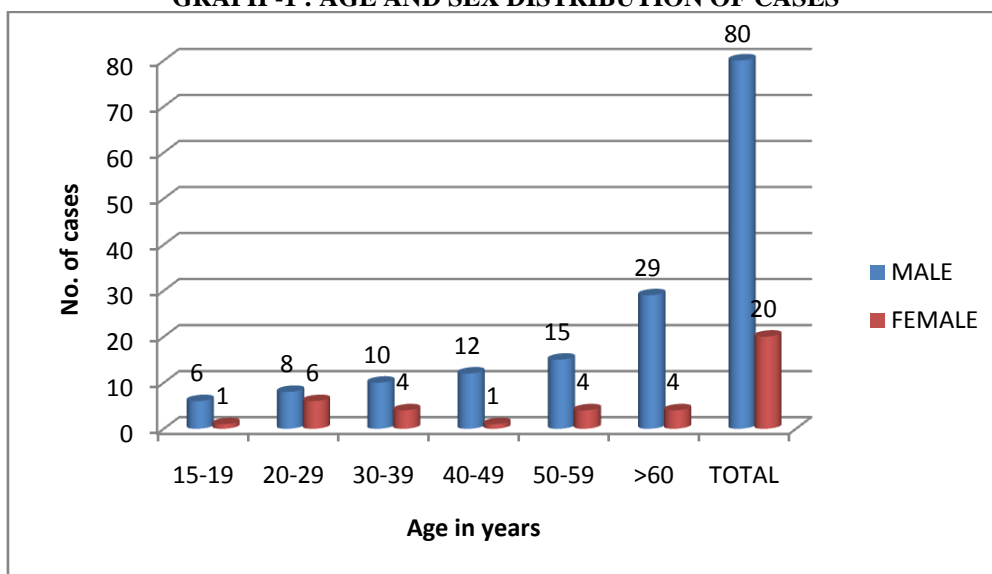


TABLE-2 PRESENTION OF VARIOUS SYMPTOMS IN PLEURAL EFFUSION CASES (n=100)

SYMPTOMS	NO. OF CASES	PERCENTAGE
FEVER	75	75%
COUGH	67	67%
CHEST PAIN	33	33%
ANOREXIA	76	76%
LOSS OF WEIGHT	58	58%
HAEMOPTYSIS	5	5%
BREATHLESSNESS	71	71%

Fever, cough, chest pain, anorexia loss of weight, breathlessness and haemoptysis were common symptomatology in our study group. Anorexia was present in maximum (76%) cases and minimum number of cases(5%) had haemoptysis.

GRAPH -2 : CLINICAL PROFILE OF PATIENTS SHOWING SYMPTOMS

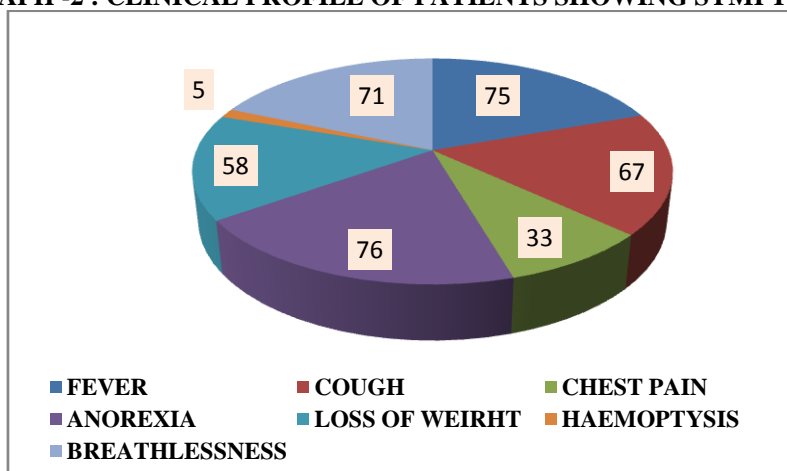


TABLE 3: BODY MASS INDEX IN STUDY POPULATION

BMI	MALE	FEMALE	TOTAL
<18.5	24	5	29%
18.5-24.9	53	12	65%
≥25	3	3	6%

Our study shows 29% patients of study population having BMI <18.5 (underweight), 65% patients having BMI of 18.5-24.9 (normal weight) and 6% patients having BMI ≥25 (overweight).

GRAPH 3 : BODY MASS INDEX IN STUDY POPULATION

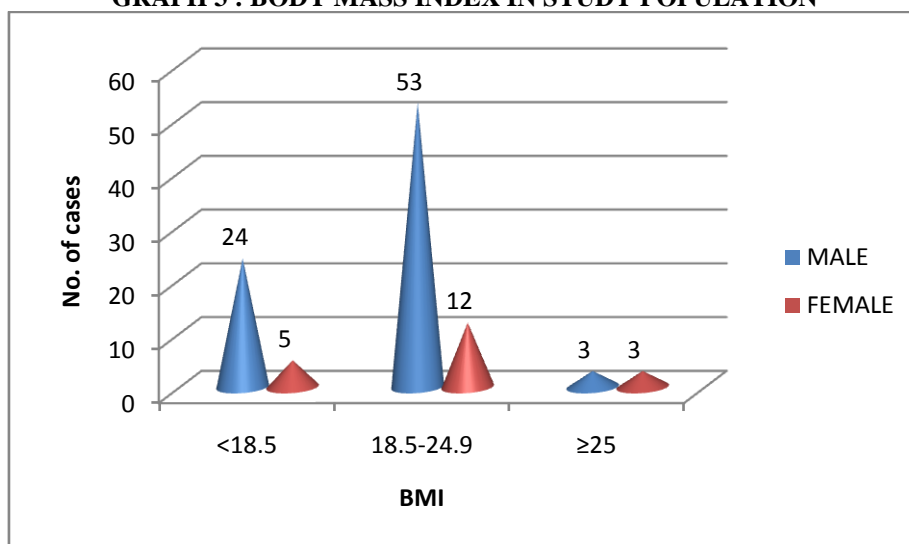


TABLE 4: VARIOUS PHYSICAL SIGNS PRESENT IN CASES (n=100)

SIGNS	NO. OF CASES	PERCENTAGE
MEDIASTINAL SHIFTING	16	16%
PLEURAL RUB	9	9%
FIBROSIS	8	8%
CAVITY	12	12%
CONSOLIDATION	10	10%
WHEEZE	11	11%
LYMPHADENOPATHY	21	21%
ASCITES	10	10%

The above table shows that among the respiratory findings mediastinal shifting to opposite side, consolidation, lung cavity, pleural rub and fibrosis were present in 16%,12%,10%9%and8% of cases respectively.

Among the non respiratory signs lymphadenopathy was the most prominent being present in 21% patients, followed ascites 10% of patient.

GRAPH 4: VARIOUS PHYSICAL SIGNS PRESENT IN CASES

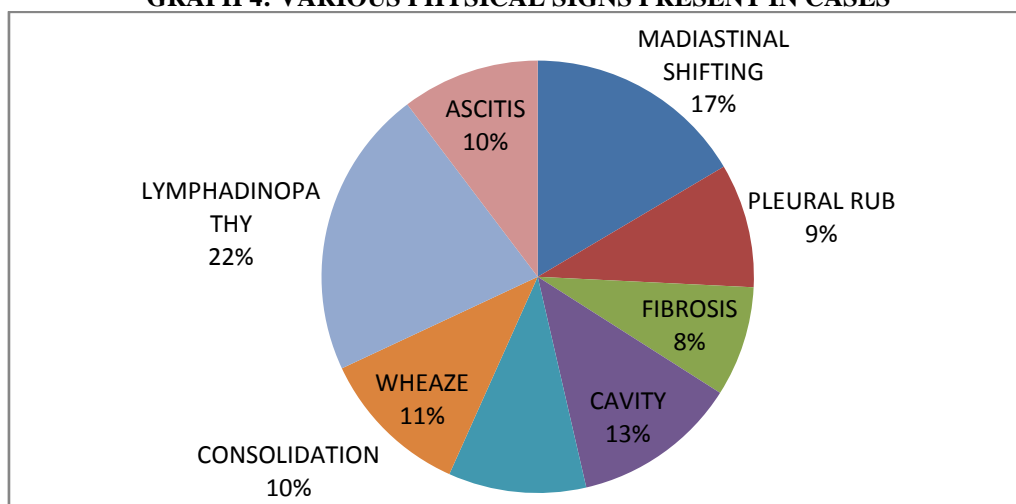


TABLE -5: HAEMATOLOGICAL INVESTIGATION IN STUDY POPULATION

INVESTIGATION	MEAN	RANGE
Hb(gm%)	9.6	3-15
TLC(cmm)	11400	3500-30000
ESR(mm in 1 st hr)	70.57	5-142

The mean Hb%, TLC&ESR was 9.6gm/dl,11400/cmm &70.57mm(in 1st h)r respectively.

TABLE – 6 SERUM PROTEIN ESTIMATION OF STUDY GROUP(n=100)

INVESTIGATION	RANGE	CASES
SERUM PROTEIN (gm%)	<3	2
	>3	1
	5-7	69
	>7	28

From this table it was observed that 69 cases had serum protein ranging between 5 to 7 gm/dl and 28 cases had serum protein >7gm/dl.

TABLE -7 MANTOUX TEST AND SPUTUM FOR AFB IN CASES OF PLEURAL EFFUSION(n=100)

INVESTIGATION	NUMBER OF CASES	PERCENTAGE
MANTOUX +VE	27	27%
SPUTUM FOR AFB +VE	6	6%

According to Table-7,out of 100 cases 27 % cases were mantoux positive & 6 % cases were sputum AFB positive.

TABLE -8: RADIOLOGICAL FINDINGS ACCORDING TO SEVERITY OF PLEURAL EFFUSION IN STUDY GROUP

PLEURAL EFFUSION	NO. OF CASES	PERCENTAGE
MINIMAL	32	32(%)
MODERATE	49	49(%)
MASSIVE	19	19(%)

Above table shows that maximum number of cases 49% had moderate pleural effusion,32% had minimal effusion and 19% had massive pleural effusion.

GRAPH 5 : RADIOLOGICAL FINDINGS ACCORDING TO SEVERITY OF PLEURAL EFFUSION IN STUDY GROUP

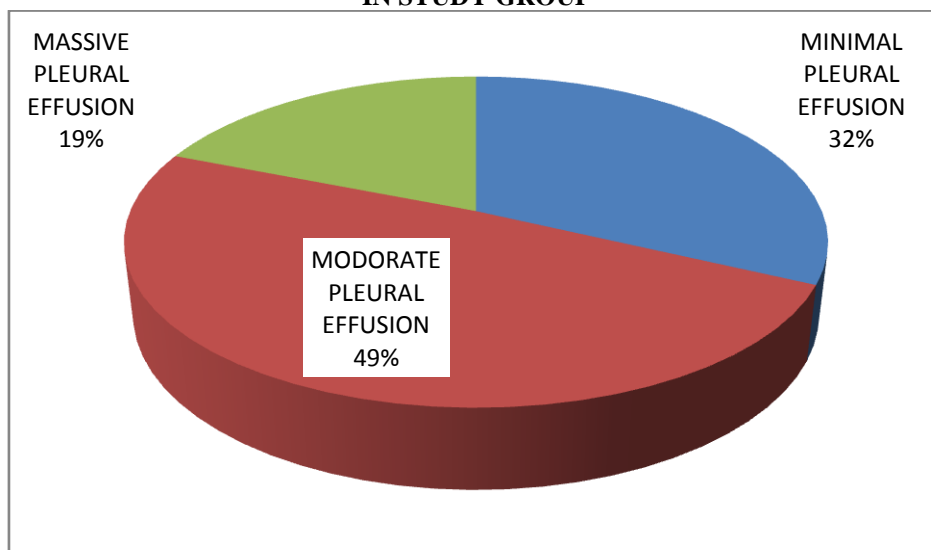


TABLE-9 RADIOLOGICAL PICTURE OF PARENCHYMAL LESION ASSOCIATED WITH PLEURAL EFFUSION IN STUDY GROUP

FIBROSIS	6	6(%)
CAVITY	3	3(%)
INFILTRATION	4	4(%)
CONSOLIDATION AND OTHER	1	1(%)

Present case study shows that out of 100 cases of pleural effusion Parenchymal lesions like fibrosis, cavity, infiltration and consolidation were present in 6%,3%,4%,and 1% of patients respectively.

GRAPH- 6 : RADIOLOGICAL PICTURE OF PARENCHYMAL LESION ASSOCIATED WITH PLEURAL EFFUSION IN STUDY GROUP

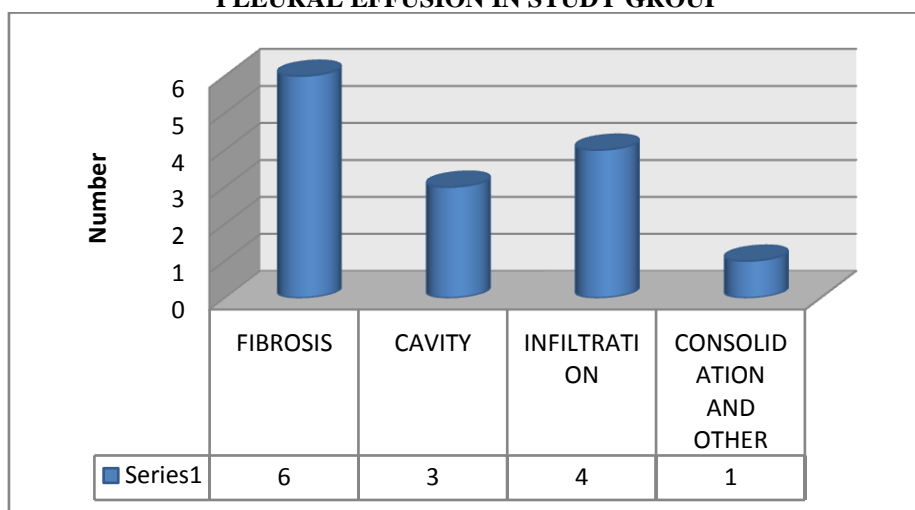


TABLE-10 RADIOLOGICAL PICTURES SHOWING SITE OF EFFUSION IN STUDY GROUP (n=100)

RIGHTSIDE PLEURAL EFFUSION	58	58(%)
LEFT SIDE PLEURAL EFFUSION	33	33%
BILATERAL PLEURAL EFFUSION	9	9%

Radiological picture shows 58% patients presented with right side pleural effusion 33% cases with left sided pleural effusion and 9% patients presented with bilateral pleural effusion .

GRAPH-7 : RADIOLOGICAL PICTURES SHOWING SITE OF EFFUSION IN STUDY GROUP

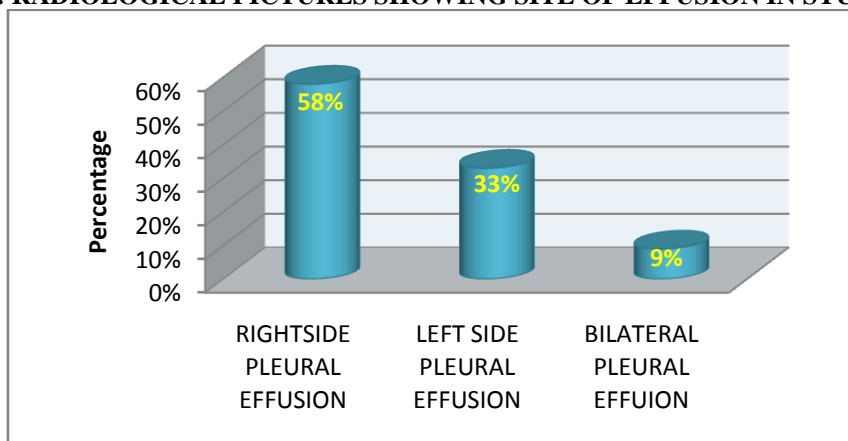


TABLE-11 OBSERVATION OF PLEURAL FLUID ANALYSIS IN CASES

PLEURAL FLUID EXAMINATION	PARAMETER	CASES	(%)
PROTEIN LEVEL(gm/dl)	<3	17	17%
	>3	83	83%
PLEURAL FLUID/SERUM PROTEIN RATIO	<0.5	13	13%
	>0.5	87	87%
PLEURAL FLUID/SERUM LDH RATIO	<0.6	20	20%
	>0.6	80	80%
GLUCOSE	>50	82	82%
	<50	18	18%
CELLS	LYMPHOCYTIC	69	69%
	POLYMORPHONUCLEAR	31	31%
AFB STAINING OF PLEURAL FLUID	+VE	3	3%

Gross examination of pleural fluid aspirate revealed straw coloured fluid in 70% of cases, where as 30% cases were hemorrhagic in nature.

The protein content of the pleural fluid was exudative (>3gm/dl) in 83% cases and pleural fluid protein and serum protein ratio was more than 0.5 in 87% cases.

Transudative nature was found in 17% cases and ratio was below 0.5 in 13% of cases.

Pleural fluid glucose was <50mg/dl in 18% of cases while 82% of cases had a pleural fluid glucose of >50mg/dl.

Pleural fluid cytological examination showed lymphocytic predominance in (69%) patients and polymorphonuclear cells were predominant in 31% of cases.

AFB was positive in (3%) cases on Zeil-Nelson staining of pleural fluid.

TABLE 12: ADA ACTIVITY PLEURAL FLUID IN STUDY POPULATION

ADA ACTIVITY	NO OF CASES	PERCENTAGE
≥40	72	72%
<40	28	28%

From the above table it was found that 72% patients had ADA activity more than 40 and 28% patients had ADA activity less than 40. The mean ADA activity of the study population was 59.38±48.20 (range 3-404).

GRAPH: 8 ADA ACTIVITY PLEURAL FLUID IN STUDY POPULATION

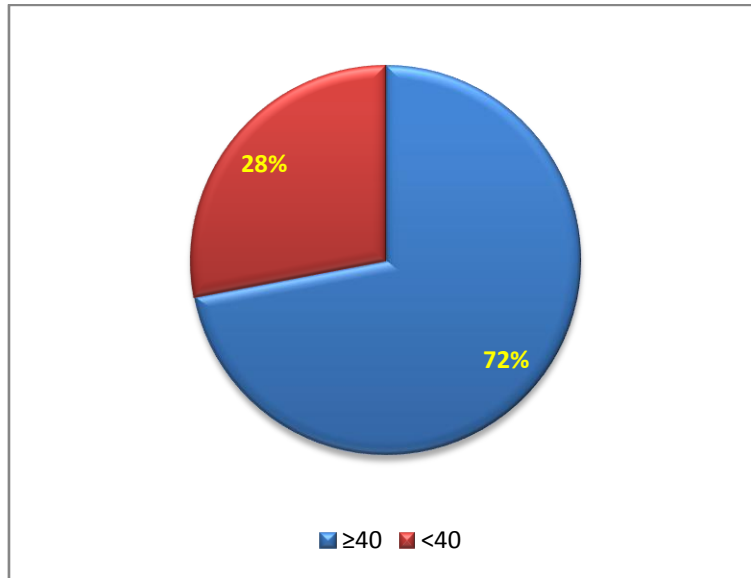


TABLE 13 PLEURAL FLUID CBNAAT RESULT IN SUSPECTED TUBERCULAR PLEURAL EFFUSION CASES

CBNAAT FINDING	NO OF CASES	PERCENTAGE
MTB DETECTED	21	21%
MTB NOT DETECTED	79	79%
TOTAL	100	100%

From above table it was observed that pleural fluid CBNAAT was positive in 21% cases and 79% cases were CBNAAT negative.

GRAPH : 9 PLEURAL FLUID CBNAAT RESULT IN SUSPECTED TUBERCULAR PLEURAL EFFUSION CASES

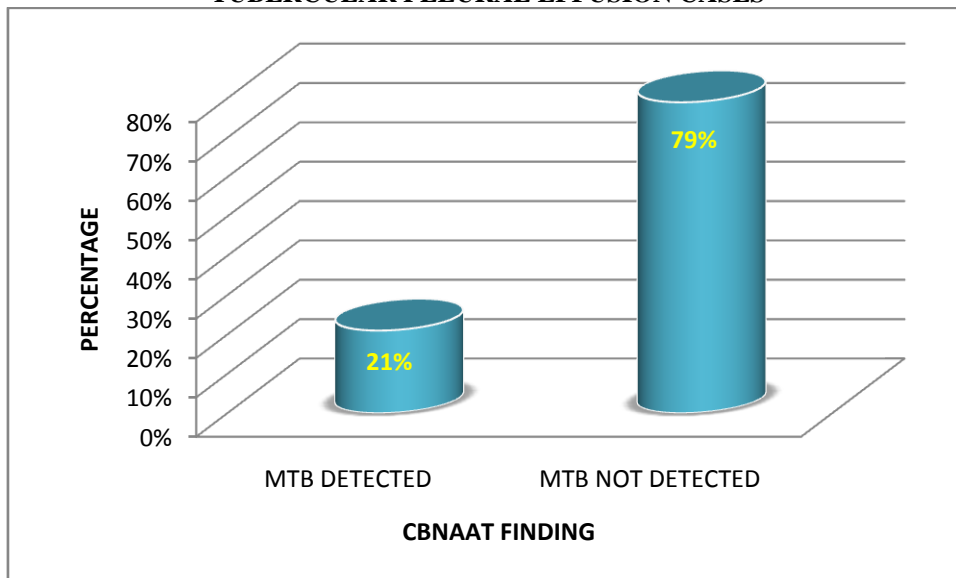
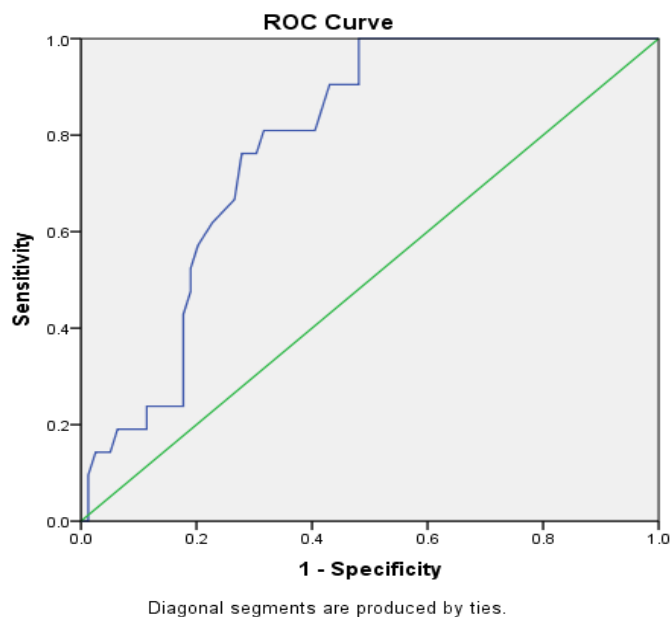


TABLE 14 COMPARISON BETWEEN CBNAAT RESULT AND ADA

	CBNAAT +VE	CBNAAT -VE	TOTAL
ADA >40	20	50	70
ADA <40	1	29	30
	21	79	100

Table -14 shows that CBNAAT results had a Sensitivity of 95.24%, with a Specificity of 36.70%, positive predictive value was 28.57% while negative predictive value was 96.67% ,and odds ratio was calculated to be 11.6 (1.48-90.99) with a Positive likelihood ratio of 1.50 and negative likelihood ratio of 0.13.

GRAPH : 10 COMPARISON BETWEEN CBNAAT RESULT AND ADA



VI. Discussion

AGE AND SEX DISTRIBUTION

In the present study, out of 100 patients, 80% were male and 20% were females. Male and female ratio was 4:1. Male dominance had also been previously observed in various studies like Reddy et al (1963), Desmukh et al(1972),Tondon et al(1975) and Dingley et al in (1983).

In the present series the patients were distributed in all age groups, but maximum number of patients were >60years of age. In contrast to our study, the certain observers like Reddy et al and Deshmukh et al had found that middle age group patients were more in number. Another two authors Tondon et al and Dingley et al had noted that pleural effusion cases were predominant in a younger age group. Above references indicate that pleural effusion is well distributed in all age groups.

CLINICAL FEATURES

Symptoms

Our patients had presented with the symptoms of anorexia, fever, breathlessness, dry cough, loss of weight and chest pain in 76%, 75%, 71%, 67%, 58% and 33% of cases respectively. This type of presentation had been previously observed in Livene et al. (1962), Bhaduda et al.(1957),Tondon et al.(1957) and Mathur et al.(1982).

Physical signs

In our study population, unilateral pleural effusion with contra lateral mediastinal shifting had been observed in 16% cases, and pleural effusion was associated with cavitory lesion in 12% cases and infiltration in 14% cases. Pleural effusion with thickened pleura was found on the opposite side in 6% cases and pleurisy in 9% cases as evidenced by pleural rub. Dissemination of tuberculosis was present in 11% cases in the form of ascites and pericardial effusion.

Routine investigations are usually unhelpful in diagnosing tubercular pleural effusion with conviction. But in our study tubercular pleural effusion group showed relatively higher TLC count and higher ESR, this finding corroborates well with other studies. Out of 100 patients 27 patients of the suspected tubercular pleural effusion were mantoux positive and 6 patients were sputum positive for AFB which was diagnostic for tubercular aetiology.

CHEST X- RAY

Radiological finding of pleural effusion

In this study, pleural effusion was moderate in (49%) cases , massive in (19%) cases and minimal effusion was seen in (32%) cases. More or less similar result was observed by Singh et al. (1965).

Underlying parenchymal pathology

In present study pleural effusion was associated with underlying parenchymal lesions like fibrosis, cavity, and infiltration in other part of chest.

PLEURAL FLUID EXAMINATION

Physical appearance

In this present study (70%) patients had straw yellow coloured pleural fluid. (30%) patients had haemorrhagic effusion. In their series Brijkishore et al.(1970) had found straw colour fluid in 79.54% and haemorrhagic fluid in 20.45% of their cases.

Pleural fluid protein and ratio of pleural fluid protein and serum protein

According to light's criteria in this study 83% of case in pleural fluid protein was >3gm/dl and 17% had <3gm/dl. The ratio of pleural fluid and serum protein was >0.5 in 87% of cases in our study. The ratio of pleural fluid LDH and serum LDH was >0.6 in 80% cases.

Reddy et al in 1963 observed that 94.73% cases of exudates had a pleural fluid protein >3gm/dl.

PLEURAL FLUID GLUCOSE

In the present work 82% cases had pleural fluid glucose content >50mg/dl and 18% cases had <50mg/dl. Reddy et al in 1963 had found that pleural fluid glucose content varied from 40-80mg% cases, where as in 1962 Glenert had opined that glucose contained of the pleural fluid had no relevance to aetiological diagnosis

CYTOLOGY

In the present series 69% of patients had a lymphocytic predominate picture in pleural fluid analysis and 31% had polymorph nuclear cells . This study is in agreement with other studies like Mestitz et al in (1959), Reddy et al in (1963)and Light et al.in (1973)where lymphocytic predominante was found in pleural fluid cytology. There were no malignant cells found from pleural fluid in present study.

MICROBIOLOGY

AFB staining of pleural fluid for Mycobacterium Tuberculosis was positive in 3% cases in our study

ROLE OF PLEURAL FLUID ADENOSINE DEAMINASE ACTIVITY

In the present study 72 (72%) patients had ADA levels >40 units/liter and the mean ADA was 59.38 ± 48.20. Swamy et al in (2011) and Basu et al in 2012 had reported a mean ADA of 100±19.48 and 100.5 respectively. The findings obtained by different authors are given below.

Authors	year	Cut off value	Mean ADA
Swamy et al	2011	>40	100±19.48
Basu et al	2012	>40	100.05
Soe et al	2010	42.5	73.90±33.95
Maldhure et al	1994	>40	77.20±32.63
Krenke et al	2008	40.3	75.1±39.1

ROLE OF PLEURAL FLUID CBNAAT

We subjected all the pleural fluid samples for CBNAAT assay,and observed CBNAAT positivity in 21% cases. Out of all study population the Sensitivity of CBNAAT was 95.24% and specificity was 36.70%. A positive predictive value of 28.57% and negative predictive value of 96.67% was found in our case analysis.

In contrast to other studies done by Reechaipichitkul et al in 2000, Bahador et al (2005) and Chakarvarthy et al in 2005, our study had low specificity as we had compared CBNAAT with ADA and they had compared CBNAAT with pleural fluid culture .A second possible cause may be due to cross contamination during the procedure, which is a common problem in laboratories using in house protocol. The findings obtained by different authors are given below.

Authors	Sensitivity	Specificity	PPV	NPV
Reechaipichitkul et al	50%	61%		
Bahador et al	85%			
Chakarvarthy et al	75%	93.8%	97%	53.35%
Handojo et al	53.35%	93.75%	96.15%	41.67%

Khusboo et al	68.6%	66%		
Rhoda Lynn et al	61%	75%		

VII. Summary & Conclusion

The commonest cause of pleural effusion in India is tuberculosis (TB) yet it poses a diagnostic difficulty because of the low sensitivity of culture technique and many cases are diagnosed on the basis of pleural fluid ADA >40 units/liter and cytology analysis showing lymphocyte predominance with lymphocyte/neutrophil (L/N) ratio >0.75.

In cases of exudative pleural effusion with Lymphocytes in pleural fluid >50% and L/N ratio >0.75 with ADA <40 units, MTB DNA PCR (CBNAATs) will be very useful in confirming tuberculosis as a cause for pleural effusion. Results of CBNAATs in this situation are very useful, sensitive, less time consuming and comparable to pleural fluid culture. Though pleural fluid ADA estimation is less time consuming and sensitive but specificity is low.

We observed complete therapeutic response to antituberculosis chemotherapy in all the study cases. Hence we recommend MTB DNA PCR (CBNAAT) in all cases of suspected tubercular pleural effusion. Additionally Pleural fluid NAATs will have good place in routine evaluation of Tuberculous pleural effusion where resources of pleural fluid culture are limited, case burden is high and is time consuming. The result of CBNAAT is also available on the same day with acceptable sensitivity of these tests.

Acknowledgement

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