TUBERCULOSIS AMONG PATIENT WITH UNILATERAL PLEURAL EFFUSION IN KELANTAN: PREVALENCE AND ASSOCIATED FACTORS

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ABBREVIATION

ADA	Adenosine Deaminase
AFB	Acid Fast Bacilli
BTS	British Thoracic Society
CKD	Chronic Kidney Disease
CT	Computed Tomography
HIV	Human Immunodeficiency Virus
HRPZ	Hospital Raja Perempuan Zainab II
IGRA	Interferon Gamma Release Assay
IQR	Interquartile range
MT	Medical Thoracoscopy
MTB	Mycobcterium Tuberculosis
PCR	Polymerase Chain Reaction
SD	Standard Deviation
TB	Tuberculosis
WHO	World Health Organization

ABSTRAK

Pengenalan

Efusi pleura Tuberkulosis merupakan penyakit yang diketahui umum dalam kalangan pesakit yang mempunyai efusi pleura sebelah. Dengan peningkatan prevalen kes tuberkulosis luar pulmonari di Malaysia khususnya di negeri Kelantan, didapati kebanyakan kes efusi pleura Tuberkulosis masih tidak dikesan dalam kalangan populasi ini. Ini disebabkan oleh kurangnya pengalaman dalam kalangan pegawai perubatan untuk mengenalpasti faktor risiko berkaitan dengan efusi pleura Tuberkulosis. Oleh itu, matlamat kajian ini adalah untuk mengenalpasti jumlah perkadaran efusi pleura Tuberkulosis dan faktor-faktor yang berkaitan dengannya.

Kaedah

Ini adalah kajian rektrospektif yang melibatkan seramai 376 orang pesakit yang mempunyai sebelah lapisan efusi pleura di paru-paru yang masih tidak dikesan menjalani rawatan torakoskopi bermula dari 1 Januari 2011 sehingga 31 Disember 2016 yang telah dikenalpasti daripada buku rekod pendaftaran di Hospital Raja Perempuan Zainab II. Maklumat-maklumat yang berkaitan dengan sosio-demografi (umur, jantina ,bangsa, status merokok, komorbid, persembahan klinikal), ujian makmal efusi pleura (paras protein, LDH, gula, kultur, AFB, kultur MTB, TB PCR, Xpert Gen) dan demonstrasi luka melalui torakoskopi di pleura paru-paru (nodular, ketulan, lekatan, plak, lesi seperti keju dan sagu) telah diambil daripada rekod. Ujian regresi logistic berganda telah digunakan untuk tujuan analisis statistic dan nilai p nominal ditetapkan pada 0.05.

Keputusan

Perkadaran efusi pleura Tuberkulosis dalam kalangan sebelah lapisan efusi pleura yang tidak didiagnosis adalah sebanyak 38.3%. Kemalignanan paru-paru menyumbang diagnosa yang tertinggi sebanyak 44.4% diikuti oleh jangkitan kuman selaput paru-paru sebanyak 10.1% dan penyakit buah pinggang kronik sebanyak 2.7%. Berdasarkan analisis regresi logistic berganda, demostrasi lesi seperti sagu (OR 37.41 (CI: 7.81, 179.24), p value = < 0.001) dan demostrasi lesi seperti keju (OR 30.44 (CI 12.73, 72.80), p value = < 0.001) adalah faktor yang paling berhubungkait dengan efusi pleura Tuberkulosis. Jangkitan kuman Tuberkulosis paru-paru (OR 9.23(CI 1.95, 43.67), p value = 0.005) dan demam (OR 4.70 (CI 2.21, 9.97), p value = <0.001) juga berhubungkait dengan efusi pleura Tuberkulosis. Tambahan juga, positif Kultur MTB (OR 6.80 (CI 2.16, 21.45), p value = 0.001) dan TB PCR juga berhubungkait rapat dengan efusi pleura Tuberkulosis (OR 8.71 (CI 2.54, 29.94), p value = 0.001). Kehadiran ketulan melalui prosedur torakoskopi kurang berhubungkait dengan efusi pleura Tuberkulosis (OR 0.13 (CI 0.02, 0.70), p value = 0.018).

Kesimpulan

Sejarah masa lalu TB pulmonari, demam, kultur MTB yang positif dan PCR TB yang positif dan demonstrasi lesi seperti keju dan sagu dari torakoskopi sangat berkaitan dengan efusi pleura Tuberkulosis. Mengetahui faktor-faktor penting ini akan membantu para doktor untuk mendiagnosis efusi pleura Tuberkulosis supaya rawatan dapat bermula lebih awal untuk mengelakkan morbiditi dan mortalitas.

ABSTRACT

Background

Tuberculosis pleural effusion is a well-known aetiology among patient with unilateral pleural effusion. With the increasing prevalence of Extra-pulmonary TB in Malaysia and particularly in Kelantan state, many TB pleura cases still undiagnosed among this population. This can be due to lack of experience among the clinicians in identifying the potential risk factors for TB pleural effusion. Hence, the aims of this study are to determine the proportion of TB pleural effusion and the factors associated with it.

Methodology

This is a retrospective study which involved a total of 376 patients with unexplained unilateral pleural effusion who underwent medical thoracoscopy from 1st January 2011 until 31th December 2016 was identified from registration book in Respiratory Clinic, Hospital Raja Perempuan Zainab II. Relevant details on sociodemographic data (age, gender, race, smoking history, comorbidity, clinical presentation), laboratory pleural fluid analysis (protein, LDH, glucose, culture, AFB, MTB culture, TB PCR, Gene Xpert) and thoracoscopy findings (nodule, mass, adhesion, plaque, sago-like lesion and cheesy-like lesion) were collected from the medical review. Multiple logistic regression were used to analyses the data.

Results

The proportion of TB pleura amongst unexplained unilateral pleural effusion were 38.3%. Lung malignancy contribute the highest proportion in 44.4% followed by para pneumonic effusion in 10.1% and chronic kidney disease in 2.7%. Based on multiple logistic regression analyses, sago-like lesion is the strongest determinant factors associated with TB pleura (OR 37.41 (CI: 7.81, 179.24), p value = < 0.001) and cheesy-like lesion (OR 30.44 (CI 12.73, 72.80), p value = < 0.001). The present of past history of pulmonary TB is also associated with TB pleura (OR 9.23(CI 1.95, 43.67), p value = 0.005) and fever (OR 4.70 (CI 2.21, 9.97), p value = <0.001). In addition to that, MTB culture is also the strongest determinant for TB pleura with (OR 6.80 (CI 2.16, 21.45), p value 0.001) and TB PCR with (OR 8.71 (CI 2.54, 29.94), p value 0.001). The presence of a mass from thoracoscopy is less likely associated with TB pleura (OR 0.13 (CI 0.02, 0.70), p value 0.018).

Conclusion

Past history of Pulmonary TB, fever, positive MTB culture and positive TB PCR and demonstration of cheesy-like and sago-like lesion from thoracoscopy are strongly associated with TB pleura. Knowing these significant factors will help the clinicians to diagnose TB pleura so that treatment can be start earlier to avoid morbidity and mortality.

CHAPTER 1

INTRODUCTION

1.1 STUDY BACKGROUND

Tuberculosis is a common and potentially serious illness. It is associated with significant morbidity and mortality. According to the World Health Organization, the incidence of tuberculosis (TB) are 9.6 million in 2014 and it is estimated that deaths from TB will increase from 3 million a year to 5 million by the year 2020. Between 2002 and 2020, approximately 1 billion people will be newly infected, 200 million people will get sick, and 36 million will die of TB if proper control measures are not instituted ("World Health Organization. Global Tuberculosis report 2016," 2016).

It is mentioned that among all cases presenting with pleural effusion, 25% are unable to be attributed to a specific diagnosis, even after thoracoscopy and closed pleural biopsy (JM, 2016). As many as 50% of the patients in this undiagnosed pleural effusion will eventually be diagnosed include TB, fungal disease, connective tissue related pleuritis, pulmonary infarction, rib fractures, asbestos-related pleural effusion, and nonspecific pleuritis (Boutin *et al.*, 1981).

Several studies have comprehensively analysed the etiological causes of patient with undiagnosed pleural effusion. A retrospective cross sectional hospital based study conducted a total of 130 cases diagnosed with pleural effusion by chest x-ray without a consent requirement. These patient underwent medical thoracoscopy procedure in the first 24 hours after ultrasonography under aseptic technique. In concern with the etiology, tuberculosis (64.6%) was the most common cause of pleural effusion followed by para pneumonic effusion (14.6%) and malignancy (11.5%) (Srivastava, 2016).

Department of pulmonary medicine at Rohilkhand Medical College and Hospital Bareilly run out of 40 patients of undiagnosed pleural effusion, majority of them were aged between 61-70 years (28.75%) and were males (53.75%). The commonest cause of pleural effusion is tuberculosis (36.2%) followed by para pneumonic effusion (18%) and malignancy (13%). In tuberculosis pleura, 11 out of 14 patients presented with shortness of breath and 12 patients presented with cough (Kumar *et al.*, 2017). From the above studies, they conclude that there were various presenting features for pleural effusion such as shortness of breath, fever, chest pain and there were multiple important diseases contribute to the diagnosis of unilateral pleural effusion such as tuberculosis, para pneumonic effusion and malignancy.

Addition to that, a prospective study conducted at Liaquat National Hospital Karachi, Pakistan during June 2015 to December 2016 among patients with unilateral pleural effusion. Diagnostic pleural tapping was performed in every case and pleural aspiration was sent for laboratory analysis. From this study, majority of cases was exudative pleural effusion (77%) and all of them (48) patients underwent diagnostic medical thoracoscopy. The most common cause of unilateral pleural effusion was Tuberculosis in 26 (54%) cases. Other causes included malignancies in 10 (21%), congestive cardiac failure in 4 (8%), chronic liver disease in 3 (6%), para pneumonic in 2 (4%) and others 3 (6%) (Abbas *et al.*, 2017).

TB pleura is a diagnostic challenge due to its nonspecific clinical presentation, paucibacillary nature of the effusion together with the inefficiency of conventional laboratory methods. (Amer *et al.*, 2016).

Complete history and physical examination should be performed, including an evaluation of disease, employment and medication history. The most usual imaging

technique for identifying pleural effusion is posteroanterior chest X-ray. Thoracic ultrasound should be easily accessible for these patients and it is also more sensitive than X-ray and better than computed tomography (CT) for identifying septa, locating small or encapsulated pleural effusion for puncture or biopsy or providing guidance regarding the entry point for thoracoscopy (Havelock *et al.*, 2010). Chest CT may be useful for modifying the probability of identifying diagnosis in undiagnosed pleural effusion as described from study by Zhen Wang et al where CT imaging revealed pulmonary consolidation or infiltration in 53.5%, pulmonary atelectasis in 43.4%, mediastinal lymphadenopathy in 41.1%, pleural thickening in 33.3% , pulmonary mass or nodules in 21.9% and pleural nodularity in 3.9% (Wang *et al.*, 2015).

Medical thoracoscopy refers to the examination of the pleural space in a nonintubated patients under local anaesthesia, and this procedure has been well documented to be highly sensitive and safe for diagnosing exudative pleural effusions. Thus medical thoracoscopy has a role for further evaluation of the pleura and to see the specific characteristics of tuberculous pleurisy.

Safaa Amer et al conduct a study among 50 patients with unexplained unilateral pleural effusion who underwent medical thoracoscopy at Faculty of Medicine Alexandria University. Twenty-four (48%) of the 50 studied patient were diagnose as malignant effusion and twenty-two were diagnosed as nonspecific fibrinous pleurisy. Three patients (6%) showed caseating granulomas and one of the total patients showed septic pleurisy. Nodules is a common macroscopic appearance from the thoracoscopic finding in 84%, followed by increased vascularity in 40%, adhesions in 38% ,mass in 20% and plaques in 16% among the studied patients(Amer *et al.*, 2016).

1.2 STUDY RATIONALE

Hospital Raja Perempuan Zainab (HRPZ) II is the only tertiary hospital in Kelantan. It has respiratory clinic which provides facilities such as medical thoracoscopy (MT) procedure, thoracic ultrasound machine, sleep study machine, lung function test and record all the patients' file and chest X-ray film including CT film. Since 6 years ago, MT had been used for diagnostic evaluation among patient with undiagnosed unilateral pleural effusion in HRPZ II.

Majority of the patients presented with unspecific clinical presentation and they already treated with multiple course of antibiotics but they still not completely recover very well. So, these group of patients need further assessment by chest physician to determine the final diagnosis of the patients. Bedside ultrasound is useful to see the effusions and to facilitate MT procedure done by chest physician. Almost all the results revealed that they already in advanced state of malignancy either primary lung or secondary lung metastasis as well as TB pleural effusion, unresolved empyema, or others.

Therefore, early detection by complete history, physical examination, imaging, ultrasonography and diagnostic MT should be done earlier to avoid any delay diagnosis. If diagnosis is correct, so the treatment can be initiate as soon as possible and patients can have a better outcome and quality of life.

So, this study intends to determine the proportion of TB pleura among patients presented with unexplained unilateral pleural effusion who underwent MT at HRPZ II. This study also want to determine the associated factors that attribute to the diagnosis of TB pleura. Apart from that, we also can get additional local data regarding cases of unexplained unilateral pleural effusion who underwent MT in HRPZ II since 2011 to 2016.

1.3 OVERVIEW OF TUBERCULOSIS

1.3.1 DEFINITION

Tuberculosis is one of the world's most widespread and deadly illnesses. It is an airborne disease caused by Mycobacterium tuberculosis. Mycobacterium tuberculosis is carried in airborne particles, called droplet nuclei, 1-5 microns in diameter. High lipid content of this pathogen accounts for many of its unique clinical characteristic .This disease is usually affect the lung but also involve other organs in up to one third of cases such as lymph nodes, liver, bones and also brain or meninges. It divides every 16 to 20 hours, an extremely slow rate compare with other bacteria which usually divide in less than an hour (Simon *et al.*, 1980).

MTB can withstand weak disinfectant and survive in a dry state for many weeks. In nature the bacterium can grow only within the cell of a host organism but MTB can be cultured in vitro (Parish and Stoker, 1999).

Since MTB has a cell wall but lacks of phospholipid outer membranes, it is classify as a Gram positive bacterium. However, if a Gram stain is perform, MTB either stain very weakly Gram positive or does not retain dye as a result of the high lipid and myocolic acid content of its cell wall (Madison, 2001).

Majority of drug susceptible tuberculosis is curable if treated properly with anti-TB medication. If untreated, the disease may be fatal within 5 years in 50-65% of cases. This problem has been compounded by the HIV pandemic, neglect towards the disease and international movement. About one-third of the world's population has latent TB, which means people have been infected by TB bacteria but are not yet ill with the disease and cannot transmit the disease. These people with latent TB can get active TB disease if

their immune system is low or if they are taking any treatment that can supress their immune system.

1.3.2 EPIDEMIOLOGY

Globally, the frequency of TB pleura is highly variable and depend on the incidence of tuberculosis in each country, for example in Spain, this entity represents a considerable problem because it is estimated that TB pleura is affected in 23.3% among all patients with tuberculosis (Valdés *et al.,* 2003). TB pleura in the United States and Brazil accounted about 4% while 20% of those in South Africa (Baumann *et al.*, 2007). In Korea, about 2884 new TB pleura cases are reported in 2012, which accounted for 7.3% of a total 39,545 new tuberculosis cases and 34% of all extra pulmonary tuberculosis cases (Park *et al.*, 2013).

Malaysia was classified as a country with an intermediate TB burden. Number of TB cases notified in 2016 are around 24,000 and notification rate for TB about 81 cases per 100,000 populations. Of these cases, 62% are sputum positive, 21% smear negative and 13% extra-pulmonary TB cases. The most common form of extra pulmonary tuberculosis seen in Malaysia are TB lymphadenitis, bone, joint, spine and military TB. Three states with high TB cases are Sabah, Selangor and Sarawak, making up a total of almost 50% of all new cases in Malaysia ("World Health Organization. Global Tuberculosis report 2016," 2016).

One hundred seventy four articles related to tuberculosis are found in a search through a database published in Malaysia between the years 2000 to 2013. About 10 to 11% of tuberculosis at a tertiary level chest clinic were classified as extra pulmonary tuberculosis and 14% of pulmonary tuberculosis also had extra pulmonary involvement

(Jamaluddin *et al.*). TB pleura is the most common cause of exudative pleural effusion about 44%, followed by malignancy in 30%. Conversely, in a smaller study the most common cause for pleural effusion is malignancy in 34%, followed by tuberculosis 23% and para pneumonic effusions in 19% (Liam *et al.*, 2000).

In Kelantan, cases of Tuberculosis that had been notified show gradually increasing in trend from 2000 to 2017. The rate of notification in 2017 are 73.3 per 100000 population have Tuberculosis including case with Extra-pulmonary Tuberculosis (Figure 1).

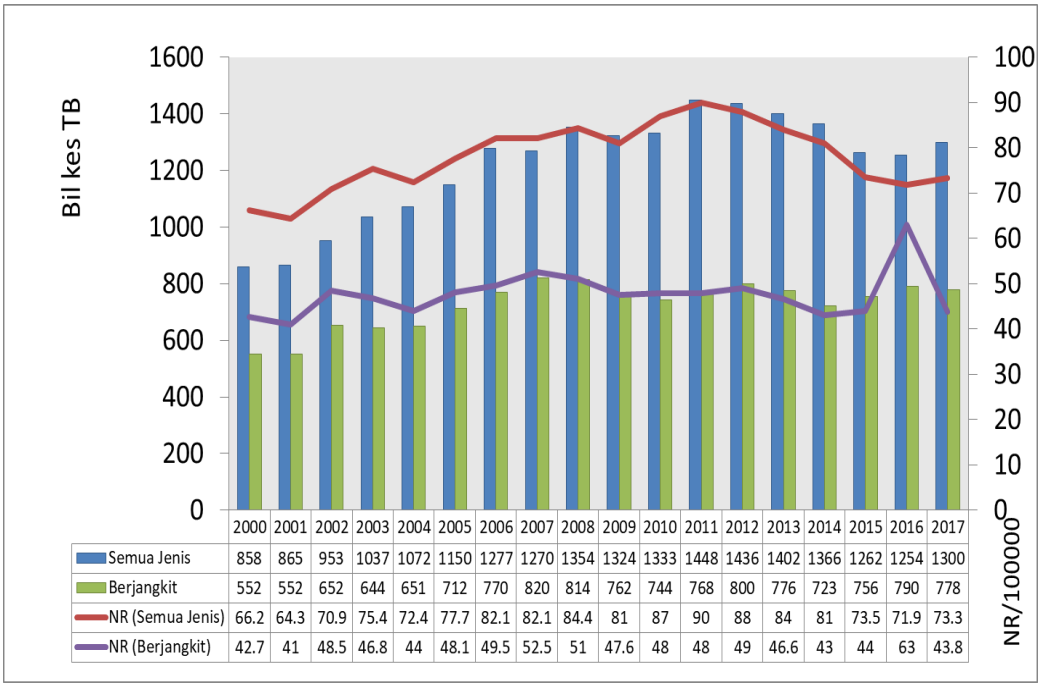
Among those patient who has been diagnosed with tuberculosis, pulmonary TB smear positive still the most commonest cases that has been notified since 2010 to 2017 and shows decreasing in trend for the past one year from 62.1% down to 59.8% as compared to those who has pulmonary TB smear negative, which shows gradually increasing in trend from 22.3% to 25%. Extra pulmonary TB only contribute a minor population and gradually static during this period probably maybe due to lack of notification or undiagnosed cases (Figure 2).

In 2017, TB lymph nodes is the most common among other cases of Extra-Pulmonary TB about 65% followed by TB spine (36%) , TB meningitis (18%) and the rest are rare cases of Extra-Pulmonary TB example gut, miliary, pericardium, ovary and breast. About 30% of TB pleura cases has been notified in 2017 (Figure 3).

Tuberculosis is the largest killer among communicable diseases in 15 to 49 age group, when humans are most productive and it's always high in the elderly with other co morbid diseases like diabetes, smoking and kidney diseases. According to WHO Global TB report, it is estimated that up to 10.4 million Tuberculosis cases reported globally in which 5.9 million (56%) are among men, 3.5 million (34%) among women

and 1.0 million (10%) among children. In 2016 there are 1,696 TB deaths reported (excluding TB/HIV mortality), giving rise to 5.56 TB deaths per 100,000 populations.

In the WHO south-East Asia Region as estimated 4.74 million cases of TB are reported and about 784,000 people died of it. This TB death rate is the highest among all infectious diseases, including dengue, HIV and malaria. People living with HIV accounted for 1.2 million (11%) of all new TB cases. Deaths from TB among HIV-positive people are officially classified as deaths caused by HIV/AIDS in the International classification of diseases. Deaths from TB among HIV-positive people accounted for 37% of deaths classified as caused by HIV/AIDS in 2016 ("World Health Organization. Global Tuberculosis report 2016," 2016).



	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Semua Jenis	858	865	953	1037	1072	1150	1277	1270	1354	1324	1333	1448	1436	1402	1366	1262	1254	1300
Berjangkit	552	552	652	644	651	712	770	820	814	762	744	768	800	776	723	756	790	778
NR (Semua Jenis)	66.2	64.3	70.9	75.4	72.4	77.7	82.1	82.1	84.4	81	87	90	88	84	81	73.5	71.9	73.3
NR (Berjangkit)	42.7	41	48.5	46.8	44	48.1	49.5	52.5	51	47.6	48	48	49	46.6	43	44	63	43.8

Figure 1 Cases of Tuberculosis in Kelantan since 2000 to 2017 in per 100000 population. Adapted from Jabatan Kesihatan Negeri Kelantan.

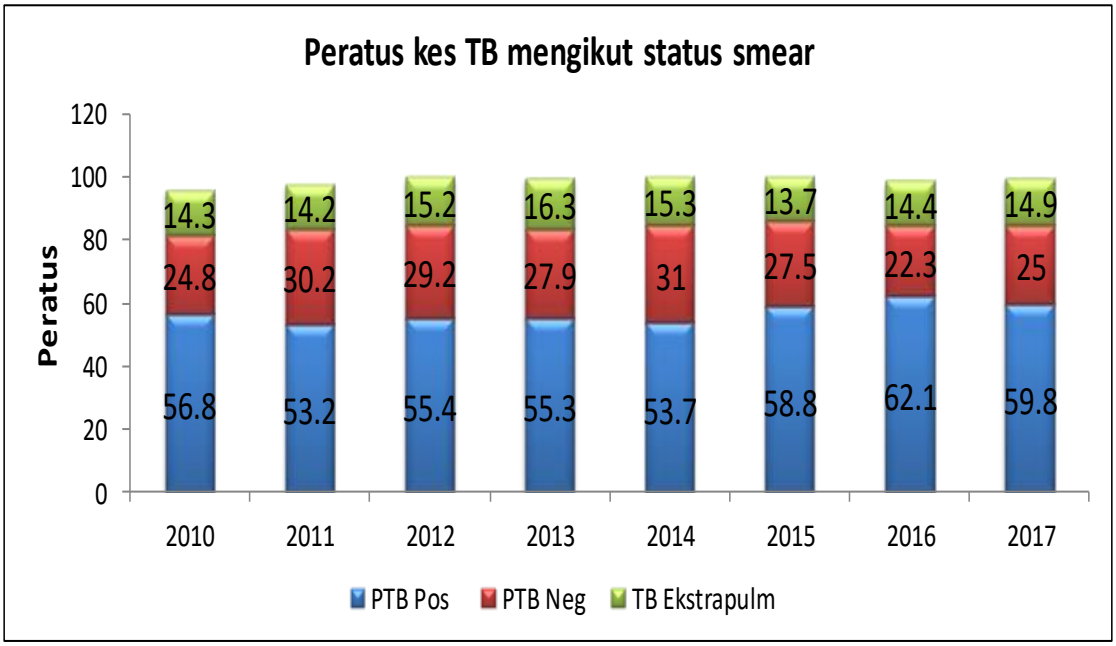


Figure 2 Prevalence of PTB smear positive, smear negative and Extra- Pulmonary TB in Kelantan since 2010 to 2017. Adapted from Jabatan Kesihatan Negeri Kelantan.

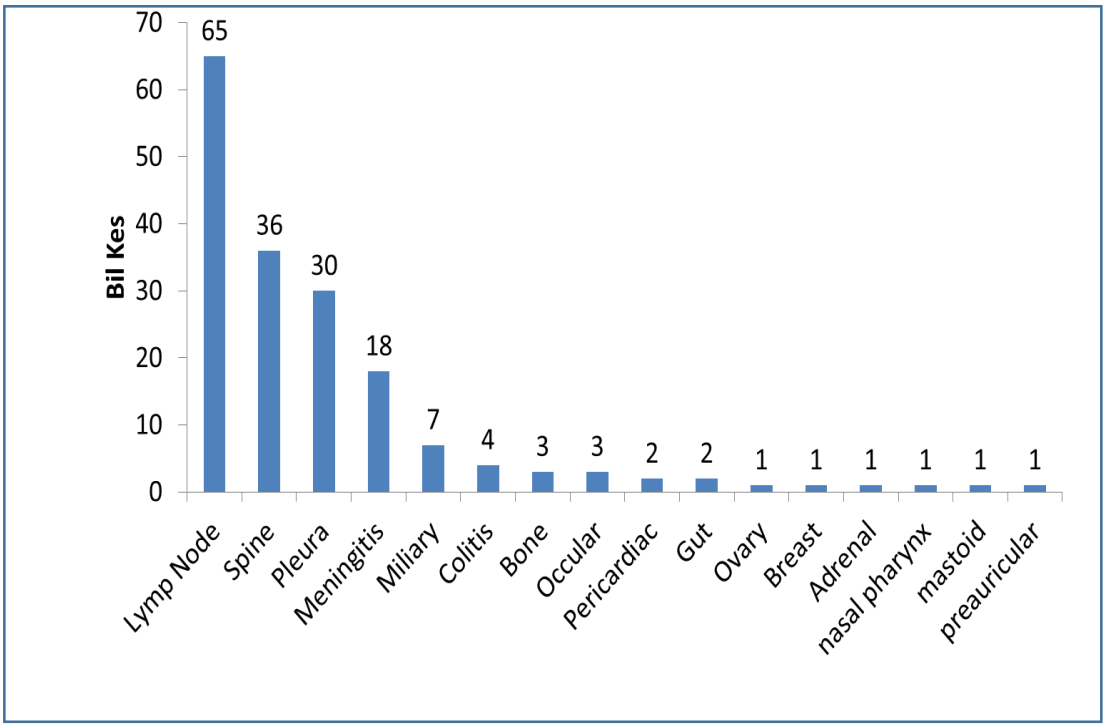


Figure 3 Total of Extra-Pulmonary TB cases in Kelantan in 2017. Adapted from Jabatan Kesihatan Negeri Kelantan.

1.3.3 PATHOGENESIS

Transmission occurs when a person inhales droplet containing Mycobacterium tuberculosis and traverse through the mouth or nasal passages, upper respiratory and bronchi to reach the lungs. Infectious droplet nuclei are generated when person who have pulmonary of laryngeal TB disease. These tiny particles can remain suspended in the air for several hours and is transmitted through the air not by surface contact. Although the majority of inhaled bacilli are trapped in the upper airways and expelled by ciliated mucosal cells, only a fraction (usually < 10%) reach the alveoli.

In the alveoli, macrophages that have not yet activated phagocytose the mycobacterium bacilli and adhesion of mycobacteria to macrophages results largely from binding of the bacterial cell wall to a variety of macrophage cell surface molecules, including complement receptors, the mannose receptor, the immunoglobulin GFcγ receptor, and type A scavenger receptors.

Phagocytosis is enhanced by complement activation leading to opsonisation of bacilli with C3 activation products such as C3b and C3bi. Binding of certain receptors, such as the mannose receptor, regulates post phagocytic events such as phagosome-lysosome fusion and inflammatory cytokine production.

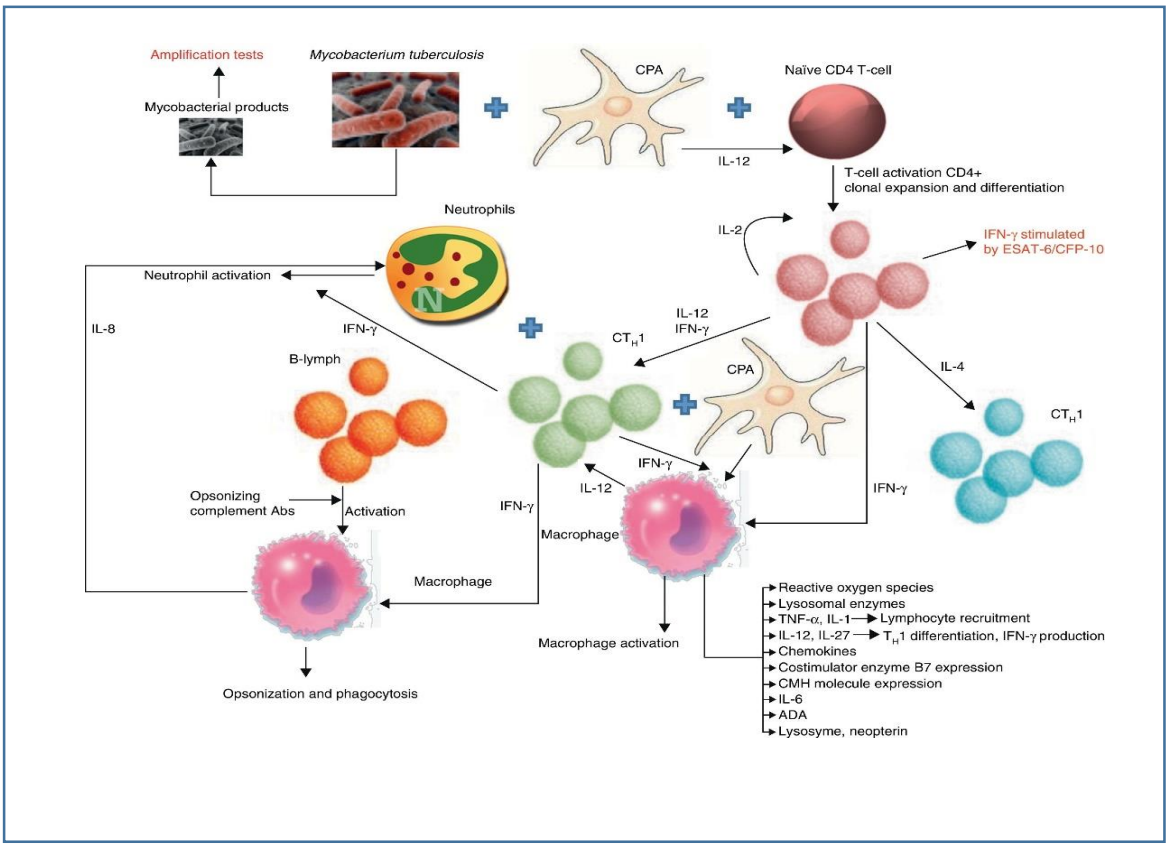
After the phagosome forms, the survival of M. tuberculosis within it seems to depend on reduced of acidification due to lack of assembly of a complete vesicular proton-adenosine triphosphatase. The M. tuberculosis phagosome has been found to inhibit the production of phosphatidylinositol 3- phosphate (PI3P). Normally, PI3P earmarks phagosomes for membrane sorting and maturation, including phagolysosome formation, which would destroy the bacteria.

Bacterial factors have also been found to block the host defense of autophagy, in which the cell sequesters the phagosome in a double-membrane vesicle (auto phagosome) that is destined to fuse the lysosomes. If the bacilli are successful in arresting phagosome maturation, then replication begins and the macrophage eventually ruptures and release its bacillary contents. Other uninfected phagocytic cells are then recruited to continue the infection cycle by ingesting dying macrophages and their bacillary content, thus in turn becoming infected themselves and expanding the infection (Longo and Harrison, 2012)(Longo and Harrison, 2012)(Longo and Harrison, 2012).

Pleural effusion is an abnormal collection of fluid in the pleural space which normally contains 0.1-0.2 ml/kg body weight of fluid. The fluid accumulation is due to increased pleural membrane permeability and pulmonary capillary pressure or decreased negative intra pleural or oncotic pressure and obstruction of lymphatic flow (Miserocchi, 1997).

However, in TB pleura, rupture of a sub pleural caseous focus in the lung into the pleura spaces is thought to be the initial event in the pathogenesis of primary TB pleural effusion. Mycobacterial antigens interact with CD4+ T-lymphocytes, leading to a delayed hypersensitivity reaction in which different cytokines stimulate macrophage anti mycobacterial activity (Figure 4). It is mediated by T-helper type 1(Th1) cells that activate macrophages to switch on mechanisms responsible for the killing of mycobacteria. A strong Th1-like immunity (interferon [IFN]-y˙dominant) is essential for the containment of M tuberculosis, while these protective effects are antagonized by T-helper type 2 cytokines, primarily interleukin (IL)-4. The predominance of Th1 immunity in TB pleural effusions is demonstrated by the significantly higher levels of IFN-y˙ in pleural fluid compared to peripheral blood of the same patient (Sharma *et al.*, 2002)

In industrialized countries, it is though that more pleural effusion are due to reactivation of TB rather than follow a primary infection 6 to 12 weeks previously. This hypothesis is based on the observation by Steal et al that they could demonstrate a caseous focus in the lung contiguous to the diseased pleura in 12 of 15 patients with TB pleural effusion. The three other patient in this study had lung parenchymal disease although they did not have caseous foci adjacent to the pleura. In addition, the lymphocytic pleurisy obstructs the lymphatic in the parietal pleura, which leads to decreased pleural fluid clearance from the parietal spaces. The pleural effusion results from the combination of the increased pleura fluid formation and the decreased pleural fluid removal (Light, 2007).



Biomarkers and pathways involved in the immunological response of tuberculous pleural effusions. B7: proteins expressed by antigen-presenting cells; CFP: culture filtrate protein; MHC: major histocompatibility complex; APC: antigen-presenting cell; CTH1: T-lymphocyte responsible for cell-mediated or delayed immunity; CTH2: T-lymphocyte responsible for humoral immunity; ESAT: early secreted antigenic target; IFN-γ: interferon gamma; IL: interleukin; B-lym: B-lymphocyte

Figure 4 Pathogenesis of TB pleural effusion. Adapted from Tuberculous Pleural effusion, Arch Bronconeumol 2014

1.3.4 CAUSES OF PLEURAL EFFUSION

Causes of pleural effusion are classified into transudate and exudative pleural effusion.

Causes of transudate pleural effusion	**Causes of exudative pleural effusions**
Very common causes	**Very common causes**
Left ventricular failure	Malignancy
Liver cirrhosis	Parapneumonic effusions
Hypoalbuminemia	Tuberculosis
Peritoneal dialysis	**Less Common causes**
Less common causes	Pulmonary infarction
Hypothyroidism	Rheumatoid arthritis
Nephrotic syndrome	Autoimmune disease
Mitral stenosis	Benign asbestos effusion
Pulmonary embolism	Pancreatitis
Rare causes	Post myocardial infarction syndrome
Constrictive pericarditis	**Rare causes**
Superior vena cava obstruction	Yellow nail syndrome
Ovarian hyperstimulation	Drugs
Meigs' syndrome	Fungal infections

Figure 5 Adapted from BTS guideline for the investigation of a unilateral pleural effusion in adults, Thorax, 2013

1.3.5 CLINICAL MANIFESTATION

TB pleural effusion usually presents as an acute illness which is different from pulmonary TB. Approximately one third of patients being symptomatic for < 1 week and two third for < 1 month. Berger et al explain the most common presenting symptoms in TB pleura are pleuritic chest pain (75%) and non-productive cough (70%). Other symptoms included fever, night sweat, weight loss, malaise, and shortness of breath varying in severity according to the size of effusion. Patient with TB pleural effusion tend to be younger and immuno competent than patient with pulmonary tuberculosis (JM, 2016). However, Epstein and colleagues demonstrate a rise in the median age (56 years) at presentation of TB pleural effusions with 19% of patients having reactivation disease (Sharma and Mohan, 2004).

On physical examination, the patients appear cachexia, malnourished and chronically ill. Sometimes they had generalised palpable lymphadenopathy in extra pulmonary tuberculosis. On lung findings, they were present of collapse consolidation at the apical area or pleural effusion in pleural tuberculosis. In immune compromised patients like HIV, they can present with sign of other opportunistic infections such as oral candidiasis, multiple skin lesions and meningeal signs positive.

1.3.6 DIGNOSTIC APPROACH OF TB PLEURAL EFFUSION

The definitive diagnosis of TB pleura depends on the demonstration of MTB in sputum, pleural fluid, or pleural biopsy specimens. Supportive evidence includes demonstration of classical TB granulomas in the pleura and elevated adenosine deaminase (ADA) and IFN-γ levels in the pleural fluid.

The tuberculin skin test is being utilised less and less in patients suspected of having TB pleural effusion. This is primarily because a negative test does not rule out the diagnosis of TB pleural effusion. In a series of 254 patients from Spain, only 66.5% of the patients had a positive skin test while in another case series from Hong Kong, more than half the patients tested have a negative skin test (Valdés *et al.*, 2003). If initial negative tuberculin skin test in patient who suspected TB pleural effusion are repeated after 8 weeks development of symptoms, the skin test will be almost always be positive. However, if the patient is markedly immunosuppressed with HIV infection or is severely malnourished, the skin test may remain negative.

1.3.6.1 PLEURAL FLUID ANALYSES

The initial step in assessing a pleural effusion is to ascertain whether it is a transudate or exudate. A diagnostic pleural fluid sample should be gathered with a fine bore (21G) needle and a 50 ml syringe. The sample should be placed in both sterile vials and blood culture bottles and analyzed for protein, lactate dehydrogenase (LDH), pH, Gram stain, AFB stain, cytology and microbiological culture. After performing pleural effusion, the appearance and odor of the pleural fluid should be noted. The appearance can be divided into serous, hemorrhagic, frankly bloody, or purulent. pH should be performed in all non-purulent effusion.

A pleural fluid pH of < 7.2 represents a substantial accumulation of hydrogen ions as normal pleural pH is about 7.6 because of bicarbonate accumulation in the pleural cavity. In an infected effusion a pH <7.2 indicates the need for tube drainage. The pleural protein should be measured to differentiate between a transudate and exudative pleural effusion. Criteria known as Light's criteria define the exudative or transudate effusion. An exudative effusion will have a ratio of pleural fluid protein to serum protein greater than 0.5, a ratio of pleural fluid lactate dehydrogenase greater than 0.6 or a pleural fluid lactate dehydrogenase greater than two thirds the upper limit of normal serum lactate dehydrogenase (Light, 2002)

Pleural lymphocytosis is common in malignancy and tuberculosis. A pleural fluid glucose level of less than 3.3 mmol/L is found in exudative pleural effusions secondary to empyema, rheumatoid disease, lupus, tuberculosis, malignancy or esophageal rupture. Malignant effusion can be diagnosed by pleural fluid cytology alone in only 60% of cases.

In Malaysia and many other countries, sputum smear microscopy is still used to diagnose TB. Trained laboratory technicians look at sputum samples under a microscope to see if TB bacteria are present. Although inexpensive, AFB microscopy has relatively low sensitivity (40-60%) in culture confirmed cases of pulmonary tuberculosis. Most modern laboratories processing large numbers of diagnostic specimen use auramine – rhodamine staining and fluorescence microscopy which more sensitive than the Ziehl Neelsan method. Microscopy detects only half the number of TB cases and it depends on the quality of the sputum produced by the patients. Up to 40% of active TB cases can be missed if the sputum is used alone to diagnose TB pleura.

Pleural fluid adenosine deaminase (ADA) test is an easy and inexpensive methods for establishing the diagnosis of TB pleural effusion. ADA is a predominant T-

lymphocyte enzyme, catalyzes the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine. A recent meta-analysis of 63 studies including 2796 patients with TB pleura and 5297 with non-TB pleura reported that the sensitivity and specificity of ADA in the diagnosis of pleural TB were 92% and 90%, respectively. ADA levels in pleural fluid are also elevated in HIV patients even with very low CD4 cell counts. The most widely accepted cut-off value for pleural fluid ADA is 40 U/L. The higher the level, the greater the chance of the patient having TB while the lower level the lesser the chance of the patient having TB (Chan *et al.*, 1991). Unfortunately, ADA level is not available in Malaysia.

Although interferon-gamma release assays (IGRAs) were primarily designed to detect latent tuberculosis, it is expected that it might also contribute to the diagnosis of TB pleural effusion. Based on the evidence so far, the IGRAs are not recommended to make a diagnosis of TB pleural effusion. In a meta-analysis from a previous study , the sensitivity and specificity for pleural IGRAs in diagnosing TB pleural effusion were 75% and 82% respectively (Zhou *et al.*, 2011).

Several test systems based on amplification of mycobacterial nucleic acid have some available in the past few years. These test are most useful for the rapid confirmation of TB in persons with AFB - negative pulmonary and extrapulmonary TB. One system that permits rapid diagnosis of TB with high specificity and sensitivity is the fully automated, real time nucleic acid amplification technology known as the Xpert MTB/RIF assay. Xpert MTB/RIF can simultaneously detect TB and rifampicin resistance in < 2 h and has minimal biosafety and training requirement. This test has a sensitivity of 98% among AFB-positive cases and 70% among AFB-negative specimens. The WHO recommends its se worldwide as the initial diagnostic test in adults and children presumed to have MDR-TB or HIV-associated TB. Xpert MTB/RIF should be the initial test applied

to CSF from patients in whom TB meningitis is suspected as well as replacement test for non-pulmonary specimens obtained by gastric lavage, fine needle aspiration, or pleural or other biopsies from patients in whom extra pulmonary TB is suspected (Longo and Harrison, 2012).

Definitive diagnosis depends on the isolation and identification of MTB from a clinical specimen or the identification of specific DNA sequences in a nucleic acid amplification test. Specimen may be inoculated onto egg or agar based medium (e.g Lowenstein Jensen or Middlebrook medium). Because most species of mycobacteria including MTB grow slowly by 4-8 weeks may be required before growth is detected. In modern, well equipped laboratories, liquid culture for isolation and species identification by molecular methods or high pressure liquid chromatography of mycolic acids has replaced isolation on solid media and identification by biochemical tests (Yasinskyi and Solodovnik, 2016).

A widely used technology is the mycobacterial growth indicator tube which uses a fluorescent compound sensitive to the presence of oxygen dissolved in the liquid medium. The appearance of fluorescence detected by fluorometric technology indicates active growth of mycobacteria. A low cost rapid immune chromatographic lateral flow assay based on detection of MTP64 antigen may also be used for species identification of the MTB complex in culture isolates (Shinnick and Good, 1995).

Current methods utilised for routine drug susceptibility testing include the use of commercial liquid medium and the proportion methods on conventional agar medium. Line probe assay is used to detect rifampicin and isoniazid resistance in smear positive sputum specimen or cultures isolates from smear positive and negative specimens. Line probe assay in the routine TB diagnostic algorithm is its rapid turnaround time which has

a direct impact on patient management and ultimately the transmission of TB (Morgan *et al.*, 2005).

Polymerase chain reaction (PCR) is also new method which based on amplification of mycobacterial DNA fragments. TB pleural is a paucibacillary disease, so the sensitivity can be improved by TB PCR and the advantages of TB PCR include rapid diagnosis and high specificity and sensitivity. This can be approved by several study who evaluate the efficacy of PCR in the diagnosis of TB pleura. Based on these previous studies, it shown that a sensitivity ranging from 20 to 90% and specificity from 78 to 100% (Villegas *et al.*, 2000). TB PCR findings are positive in 100% of culture-positive TB pleural fluids and in only 30 to 60% of culture-negative pleural fluids. Causes of false positive results include DNA contamination or presence of nonviable organisms. Pleural biopsy for PCR has 90% sensitivity and 100% specificity, as the overall accuracy being similar to biopsy culture (Hasaneen *et al.*, 2003).

1.3.6.2 CHEST IMAGING

Plain radiography such as PA and lateral view should be performed in the assessment of suspected pleural effusion. The PA chest radiograph is abnormal in the presence of about 200ml pleural fluid. However, only 50ml of pleural fluid can produce detectable posterior costophrenic angle blunting on a lateral chest radiograph. Lateral decubitus films are occasionally useful as free fluid gravities to the most dependent part of the chest wall, differentiating between pleural thickening and free fluid (Blackmore *et al.*, 1996)

Ultrasound is more accurate than plain chest radiography for estimating pleural fluid volume and aids thoracocentesis. After unsuccessful thoracocentesis or in a

loculated pleural effusion, ultrasound guided aspiration yields fluid in 97% of cases. Ultrasound is also useful in demonstrating fibrinous location and readily differentiates between pleural fluid and pleural thickening. Ultrasound also has the added advantages of often being portable, allowing imaging at the bedside with the patient sitting or in the recumbent position.

CT scanning has been shown to be superior to plain radiograph in the differentiation of pleural from parenchymal disease. CT scan can usually differentiate between benign and malignant pleural thickening. It is particularly helpful in the assessment and management of loculated pleural effusion. Loculated effusions on CT scans tend to have lenticular shape with smooth margins and relatively homogeneous attenuation.

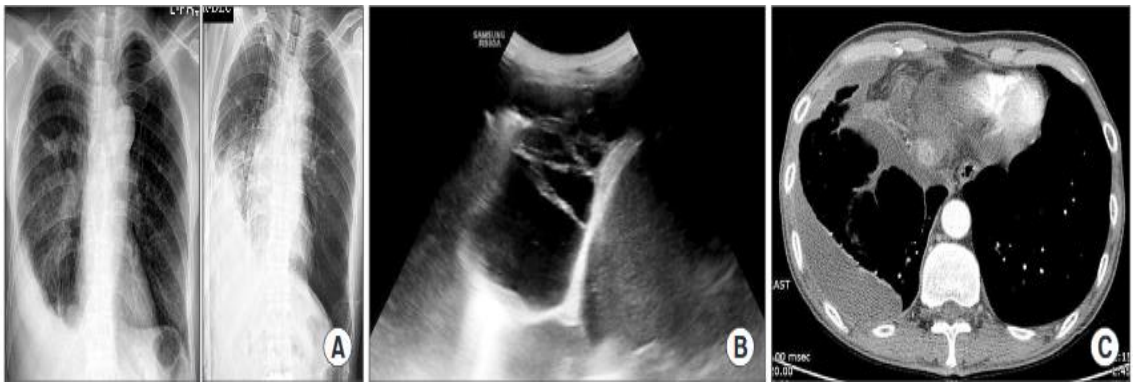


Figure 6 The representative radiographic finding of loculated tuberculous pleural effusion. (A) Chest plain X-ray shows no shifting of pleural fluid on decubitus film, as compared with chest posteroanterior view. (B) Thoracic real-time sonography shows complex septated pleural effusion. (C) Chest computed tomography shows loculated pleural fluid accumulated in nondependent portion

Adapted from Loculated Tuberculous Pleural effusion, Tuberculosis and Respiratory

Diseases, 2017

1.3.6.3 THORACOSCOPY AND PLEURAL BIOPSY

Thoracoscopy should be considered when less invasive test have failed to give a diagnosis especially in undiagnosed pleural effusion. In addition to obtaining a tissue diagnosis, several litres of fluid can be removed during the procedure and the opportunity is also provided for talc pleurodesis. Thoracoscopy may therefore be therapeutic as well as diagnostic. Pleural tissue can also be obtained during thoracoscopy but thoracoscopy is usually not necessary to make the diagnosis of tuberculous pleural effusion.

Thoracoscopy is sometimes indicated when the diagnosis is confusion. A recent meta-analysis revealed that the overall sensitivity 91% and specificity 100%, positive likelihood ratio 4.92 and negative likelihood ratio 0.08 respectively; the area under curve

are 0.93 (Agarwal *et al.*, 2013) Complication of this procedure appear to be few. The most serious, but rare is severe hemorrhage caused by blood vessel trauma.

Pleural biopsy is the most common way to make the diagnosis of tuberculous pleural effusion since 50 years ago by using a blind needle biopsy of the pleura. The presence of granuloma in the parietal pleura suggest tuberculous pleural effusion but caseous necrosis and AFB need not be demonstrated. Although other disorders including fungal diseases, sarcoidosis, rheumatoid pleuritis may produce granulomatous pleuritis, more than 95% of patient with granulomatous pleuritis have TB. The biopsy specimen should be send for AFB and cultured for M. tuberculosis even if no granuloma presence on the biopsy (Meldau *et al.*, 2014)

Sugiyama et al classified tuberculous pleural effusion into 4 stages: Stage 1 where the parietal pleura is swollen, reddened and show tiny white nodules; Stage II where the redness and swelling become more extensive and military white nodules extending diffusely and coalescing together; Stage III white fibrin deposits extend over the pleura in a cord or a membrane-like fashion; Stage IV (chronic stage) the fibrin deposits become fibrous. Part of the pleural effusion becomes encapsulated with a fibrin net, and the parietal pleura become white, thickened, firm, and difficult to biopsy (Sugiyama *et al.*, 2001)

An image guided cutting needle biopsy is indicated in patient who has focal area abnormality from contrast enhanced thoracic CT scan because it has a higher yield than blind needle pleural biopsy in the diagnosis of malignancy. This technique also useful in patient who are unsuitable for thoracoscopy (Maskell and Butland, 2003). Adam et al reported that in a recent prospective study among 33 patients with a pleural effusion with pleural thickening demonstrated on contrast enhanced CT scanning. These patients

underwent percutaneous image pleural biopsy and correct histological diagnosis was made in 21 out of 24 patients, including 13 of 14 patients with mesothelioma (sensitivity 88%, specificity 100%).

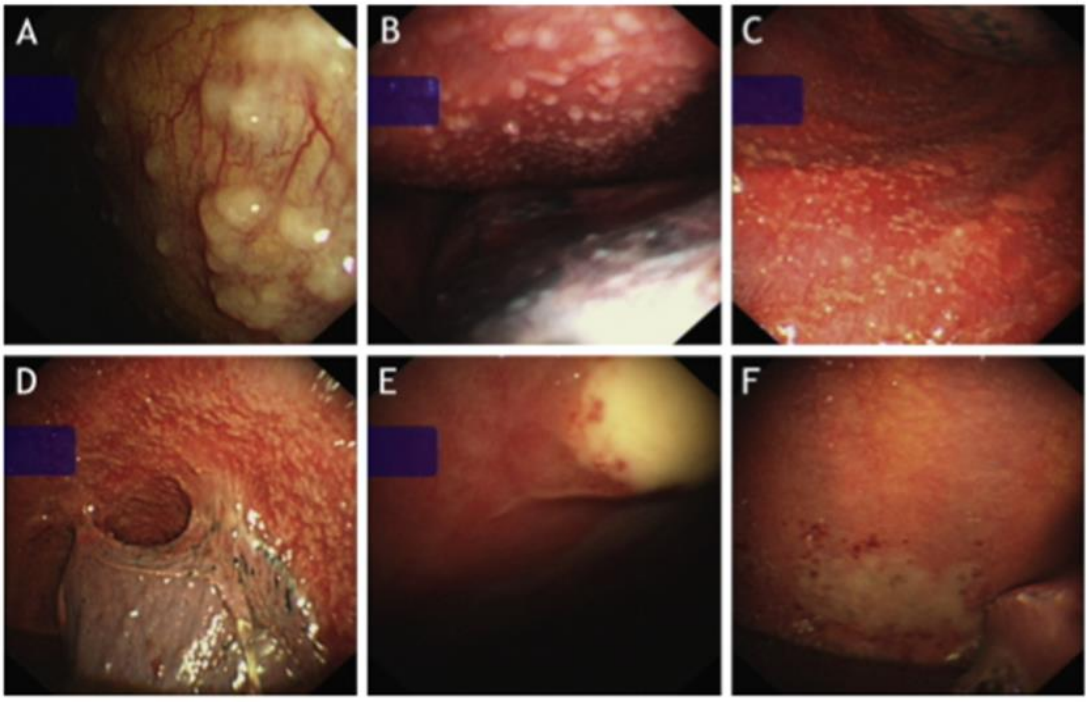


Figure 7 Thoracoscopic images taken from patients with tuberculous pleurisy showing A) tuberculous nodules with irregular distribution on parietal pleura; B) tuberculous nodules on parietal (upper) and visceral (bottom) pleura; C) multiple military tuberculous nodules on parietal pleura; D) diffuse parietal pleura nodules and pleural adhesions; E) parietal pleural hyperemia with necrosis of the pleural node; F) parietal pleural hyperemia with white pleural plaques.

Adapted from Diagnostic value and safety of medical thoracoscopy in tuberculous pleural effusion. Respiratory Medicine, 2015.