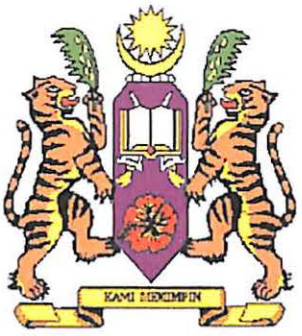

A Pilot Study On Sensitivity And Specificity Of QuantiFERON-TB GOLD Test On Newly Diagnosed Mycobacterium Tuberculosis Infection In Kelantanese Population

By
Dr Azreen Syazril B. Adnan
Department of Medicine USM

Supervised By:

Assoc. Prof (Dr) Mustaffa Musa
Dr. Che Wan Aminud-din Hashim

2006

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF MEDICINE (INTERNAL MEDICINE)

Acknowledgements

I wish to thank my beloved wife Dr. Fauziah Jummaat for her patience and faithfulness and continuous support to me through the hardships and difficulties. In memory of my late father Adnan Omar, whom has been and always given me the strength to be a good physician, May Allah bless you. Al-Fatihah. To my mother for trying her best to support me in every way and for her patience and continuous support.

To my greatest supervisor, Associate Prof Dr. Mustaffa Musa, thanks for your support, encouragements, time, financial support, patience and continuous motivation. Associate. Prof Dr. Syed Hatim Noor for all the guidance, patience and teachings since my undergraduates time until now. To Pn. Malisa of Immunology department, for being very helpful and encouraging towards completion of this study. Owi of Microbiology dept, currently in INFORMM for his help in explaining Lowenstein Jensen culture for AFB method.

TABLE OF CONTENTS

LIST OF FIGURES **Page**

LIST OF TABLES

LIST OF ABBREVIATIONS

AFB	Acid Fast Bacilli
BCG	Bacille Calmette-Guérin
CFP-10	Control Filtrate Protein 10-kDa
EHRZ	Ethambutol: Isoniazid: Rifampicin: Pyrazinamide
ESAT-6	Early Secreted Antigen 6-kDa
ESR	Erythrocyte Sedimentation Rate
IFN-γ	Interferon-Gamma
SHRZ	Streptomycin: Isoniazid: Rifampicin: Pyrazinamide
TB	Tuberculosis

ABSTRACT

Diagnosis of tuberculosis infection has not been simple, commonly diagnosis been made after reviewing several investigation results. Unfortunately delay in diagnosis and hence treatment has made tuberculosis infection not treated early. Therefore a new test with reliability and rapidity is required. This study was carried out to compare between QuantiFERON-TB GOLD test and sputum culture for the detection of active Mycobacterium tuberculosis infection. Twenty four suspected active tuberculosis infected patients enrolled in this pilot cross sectional study and each patient was required to provide sputum and 5 mls of blood. The T-cells from the patient's serum were stimulated in-vitro with antigens specific for M tuberculosis (ESAT -6 and CFP-10). Hence, interferon gamma, (a cytokine that is released during tuberculosis infection) was detected after the in-vitro stimulation and using the QuantiFERON-TB GOLD kit, the level was quantified via ELISA method. It was analyzed using a computer software provided by the Cellestis (Manufacturer of QuantiFERON-TB GOLD test). Simultaneously sputum samples were obtained from the same patient and cultured for M tuberculosis. The results showed that when compared to sputum Acid Fast Bacillii culture, QuantiFERON-TB GOLD assay is 94.7 % sensitive and 80% specific, positive and negative predicitive values of 94% and 80%. While the likelihood ratios were 4.73 for positive cases and 16.7 for negative cases for M tuberculosis infection. Our study suggests that QuantiFERON-TB GOLD assay is a useful diagnostic kit for diagnosis of active tuberculosis infection in our country which is endemic for M tuberculosis infection and in which most of the population had been vaccinated with BCG. The sensitivity 94.7% obtained in this study is high in comparison to other study carried out in Japan,

USA and Australia in which each represents 89.5%, 91.3% and 83.3% (in pulmonary TB cases). While the specificity of 80% in this study is considered low as compared to other studies, 98.1% (Japan), 97.8% (Australia) and 99.8% (USA). The increasing numbers of reported cases and delay in diagnosis due to delay in obtaining culture results are reasons for carrying out this study. Delay in diagnosis has made tuberculosis difficult to eradicate in our country. Therefore this study will provide evidence on the usefulness of QuantiFERON-TB GOLD assay as a rapid diagnostic tool kit in diagnosing new tuberculosis cases in our country. It will provide a useful supporting diagnostic instrument to the clinician for fast and accurate result.

ABSTRAK

Diagnosis jangkitan tuberkulosis sering menjadi permasalahan, kebiasaannya diagnosis dibuat setelah semua keputusan pemeriksaan telah diperolehi. Malangnya ini telah menyebabkan kelewatan dalam diagnosis dan rawatan kepada pesakit. Oleh itu suatu kaedah diagnostik yang bermutu dan cepat sangat diperlukan. Perbandingan antara Esei 'QuantiFERON-TB GOLD' dan kultur kahak untuk mengenalpasti jangkitan aktif Mycobacterium tuberculosis telah dilakukan. 24 pesakit tuberkulosis telah menyertai kajian rentas ini. Setiap pesakit diperlukan untuk memberikan kahak dan sample darah sebanyak 5 cc. Sel-sel di dalam darah dirangsang secara 'in-vitro' dengan bahan antigen yang spesifik untuk Mycobacterium tuberculosis (ESAT-6, CFP-10). Dengan menggunakan kit 'QuantiFERON-TB GOLD', Interferon gamma dirembeskan di dalam supernatant yang kemudiannya dikesan menggunakan kaedah ELISA yang terdapat dalam kit tersebut. Kultur kahak turut dilakukan pada masa yang sama. Keputusan yang terhasil dibandingkan dengan keputusan kultur kahak dan 'QuantiFERON-TB GOLD'. 'QuantiFERON-TB GOLD' mempunyai sensitiviti sebanyak 94.7% dan spesifikasi 80% untuk mendiagnos jangkitan oleh Mycobacterium tuberculosis. Manakala nilai positif dan negatif prediktifnya adalah 94% dan 80% masing-masing. Bagi kes-kes positif "likelihood ratios" adalah 4.73 dan 16.7 untuk kes-kes yang negative untuk jangkitan Mycobacterium tuberculosis. Kajian kami menunjukkan essei 'QuantiFERON-TB GOLD' adalah kit yang berguna untuk mendiagnos jangkitan tuberkulosis aktif di negara kita yang endemik untuk jangkitan Mycobacterium tuberkulosis dan yang mana majoriti penduduk telah diimunisasi dengan BCG. Sensitiviti sebanyak 94.7% yang didapati daripada kajian ini adalah lebih tinggi berbanding keputusan kajian lain yang didapati

daripada Jepun, USA dan Australia, di mana masing-masing mewakili sensitiviti sebanyak 89.5%, 91.3% dan 83.3% (dalam kes TB pulmonari) Manakala spesifisiti bagi kajian ini adalah 80% dan adalah rendah berbanding kajian lain iaitu 98.1% (Jepun), 97.8% (Australia), dan 99.8% (USA). Peningkatan jumlah pesakit TB yang dilaporkan dan kelewatan dalam melakukan diagnosis telah menyebabkan jangkitan Mycobacterium tuberculosis sukar untuk dihapuskan di negara kita. Oleh itu kajian ini yang merupakan yang pertama di negara ini akan memberikan bukti tentang keberkesanan penggunaan essei 'QuantiFERON-TB GOLD' sebagai alat untuk mendiagnos jangkitan kes-kes TB yang baru di negara kita. Ia akan menjadi perlatan yang sangat berguna untuk membantu pakar klinikal untuk mendiagnos TB dengan keputusan yang cepat dan tepat.

INTRODUCTION AND LITERATURE REVIEW

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Tuberculosis Infection: The global and local burden

Since the reemergence of tuberculosis (TB) in Malaysia following the epidemic of HIV infection, health professionals and scientists became aware of the need to respond immediately to the threat. A M Aziah in 1998 described that the problem of tuberculosis in Malaysia had declined significantly between 1970 and 1990. However recently in Malaysia there has been an increase in the reported cases particularly due to HIV infection. Factors that have been credited with this reduction in tuberculosis incidence include improvements in nutrition and housing, better ventilation of homes and work sites, improved health set up, introduction of the National Tuberculosis Control Program in 1961 and isolation of highly infectious tuberculosis cases.

Raviglione *et al,*. in 1977 described that about one third of the world's population has latent tuberculosis, caused by *Mycobacterium tuberculosis* infection. While Dollin *et al,*. informed that globally according to the WHO, the number of new cases in 1990 and 1995 were 7.5 million and 8.8 million respectively and the numbers are predicted to rise to 10.2 million by the year 2000, a 37% increase from the 1990 estimate.

As shown in *Figure 1.1*, in Malaysia, there was a documented increased in the number of reported tuberculosis cases from 1977 to 1999 (Kementerian Kesihatan Malaysia 2000: Laporan Tahunan Unit TB dan Kusta). In the year 2000, a total of 15,057 cases of all forms of TB were notified (Fig)

Figure 1.2, revealed the number of reported cases in 2000 involving mainly patients from the productive age group, ranging between 15-54 years old (67%). In the year 2002, local statistics indicate that in West Malaysia, 9122 cases of tuberculous infection of all types and East Malaysia 5250 cases were reported; and there was 1292 mortalities in that year alone. (Kementerian Kesihatan Malaysia 2002: Laporan Tahunan Unit TB dan Kusta).

The incidence rate was 64.7 per 100,000 population. Of these 8154 (i.e. 54.2%) were smear positive. The incidence of smear positive cases is 34.7 per 100,000 population (Kuppusamy Iyawoo, 2004). In 1999, as shown in table 1, among the states and federal territories, Sabah and Kuala Lumpur have recorded incidence (notification) rates for TB above 100 per 100,000 persons. Four other states in the country have recorded incidence rates of 50 to 100 cases per 100,000 persons, namely Sarawak, Kelantan, Perlis, and Pulau Pinang. (Sources: Laporan TB Tahunan 1999). The distribution of these rates shows that there is still a strong association between TB and rural poverty (in Perlis, Kelantan, Sarawak, and Sabah), size and mobility of migrants/migrant worker

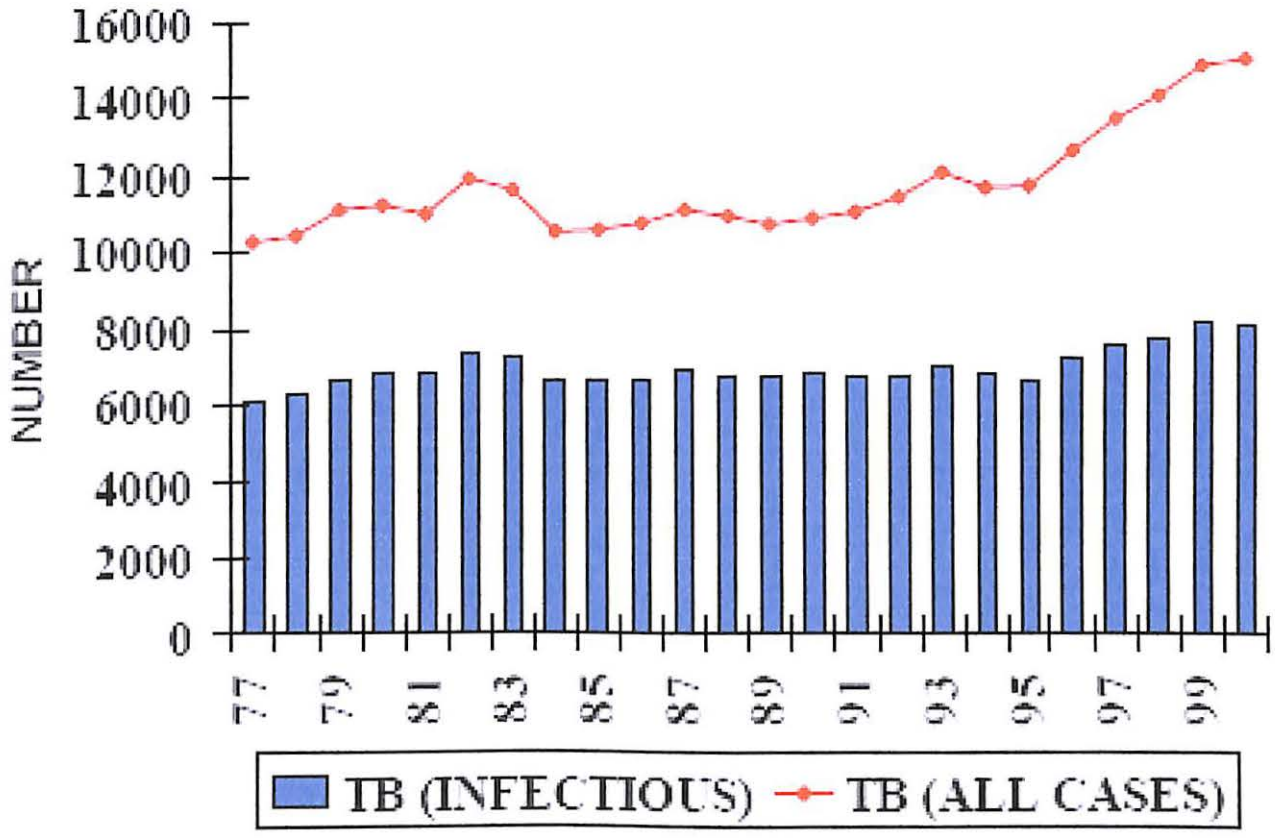

Figure 1.1: TB Notifications In Malaysia (1977-1999)

(Kuppusamy Iyawoo (2004). Tuberculosis In Malaysia: problems and prospects of treatment and control. *Tuberculosis* **84**: 4-7)

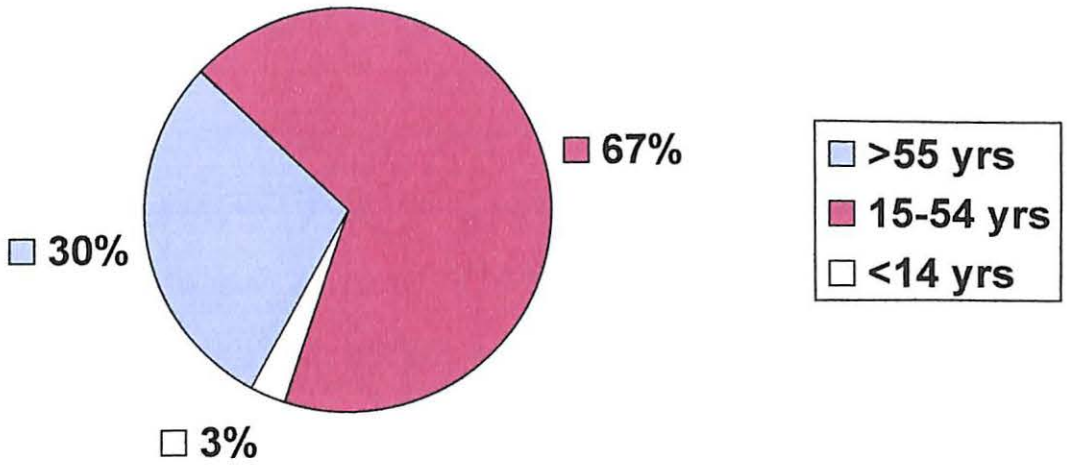

Figure 1.2:Proportion Of Tuberculosis Cases by Age Group In Malaysia by 2000

(Kuppusamy Iyawoo (2004). Tuberculosis In Malaysia: problems and prospects of treatment and control. *Tuberculosis* **84**: 4-7)

populations, especially in Kuala Lumpur and Sabah, and urban poverty and overcrowding in Kuala Lumpur and urban areas in Sabah. The rates in Kuala Lumpur tend to be inflated due to out-of-state patients being referred to or seeking treatment in the city.

Unlike malaria, the TB incidence rates increased in all states in Peninsular Malaysia over the past 10 years up to 2000, except in the north-eastern state of Kelantan (Table 1). The incidence increases in each state coincide with the rising trend in incidence observed in the latter half of the 1990s for Malaysia generally, as described above.

These figures revealed that tuberculosis remains a global and local threat. Early detection of Mycobacterium tuberculosis infection is very important, as it helps preventing further complications and spreading of the fatal disease to our community. Clinicians often encounter situations whereby the clinical findings are not classical of tuberculosis infection and the laboratory results especially sputum culture AFB are obtained only 6 weeks later. Meanwhile Tuberculin Skin Test always carry false positive results since many patients have already been vaccinated with BCG. Diagnosis may often be delayed with current diagnostic tools. Tuberculin skin tests for instance requires patient to come 48-72 hours apart from the first visit for mantoux interpretation. Unfortunately, even if it is positive it needs to be supported by other diagnostic tools and collective clinical grounds. Sputum culture AFB, which is the recognized gold-standard investigations requires 4-6 weeks to yield any mycobacterium infection.

Table 1: Incidence Rate and Mortality Rate of Communicable Disease Per 100 000 Population, Malaysia, 1999 - Tuberculosis (All Types)

State	Population ($x10^5$)	Incidence/100,000		Mortality/100,000	
		No. of Cases	Incidence Rate	No. of Deaths	Mortality Rate
Perlis	226.20	105	46.42	7	3.09
Kedah	1,579.80	746	47.22	68	4.30
Penang	1,246.80	856	68.66	52	4.17
Perak	2,118.10	1,016	47.97	68	3.21
Selangor	3,188.70	679	21.29	80	2.51
W.Persekutuan	1,407.20	1,713	121.73	46	3.27
N. Sembilan	836.50	372	44.47	22	2.63
Melaka	593.20	285	48.04	50	8.43
Johor	2,670.70	1,158	43.36	34	1.27
Pahang	1,291.50	637	49.32	40	3.10
Terengganu	1,033.50	456	44.12	85	8.22
Kelantan	1,522.20	845	55.51	109	7.16
Peninsular Malaysia	**17,714.40**	**8,868**	**50.06**	**661**	**3.73**
Sarawak	2,027.10	1,771	87.37	130	6.41
Sabah	2,970.40	4,268	143.68	142	4.78
MALAYSIA	**22,711.90**	**14,907**	**65.64**	**933**	**4.11**

(Tahunan L. Unit Tibi/Kusta, Cawangan Penyakit Berjangkit, Jabatan Kesihatan Awam, Kementerian Kesihatan Malaysia, 2000)

A rapid and reliable diagnostic tool is required to ensure those 'grey' cases that presented are not being missed from treatment of anti-TB.

1.2. Current challenges in curbing tuberculosis

The eradication of tuberculosis transmission of infection is not a reality at present. There are few challenges mentioned below that need to be considered in curbing tuberculosis.

1.2.1. Magnitude of tuberculosis infection.

The World Health Organization (WHO) estimates that approximately one third of the total population of the world harbors tubercle bacilli in a latent form. While only a small fraction of these individuals will ever develop disease, as transmission from persons with infectious tuberculosis declines, most cases will emanate from this pool. In addition to this large pool of potential cases, there is a zoonotic reservoir for both M tuberculosis and M bovis whose extent and impact are not well understood. (World Health Organization. Sub-Regional Workshop on Tuberculosis Control in the Gulf States, Muscat, Oman, December 1996)

1.2.2. Incubation Period.

Enarson D.A. *et al,*. reported that together with the appearance of the disease arising from the large pool of the community the infection may occur for many decades after the infection has occurred. While this may provide an opportunity for secondary prevention, it further complicates an elimination strategy because, even if transmission of M

tuberculosis is completely arrested, cases can be expected to appear for the entire life span of those who have been infected (for up to 70 years of age).

1.2.3 Inadequacy of the diagnostic tools and treatment strategies.

Tuberculin skin test, used to identify infection with M tuberculosis, is more than 100 years old; sputum smear microscopy is equally as old; the most recently developed drug routinely used for treatment is as old (see section 1.4 for details). Enarson D A *et al*,. 2000 had documented that tuberculin skin test even though is a tool which is capable of measuring infection in the individual or in the community, lacks specificity where environmental mycobacteria (i.e M avium and M bovis) are common and lacks predictive value in identifying those likely to develop disease in the future. Consequently, treating latent tuberculous infection remains a blunt instrument, with a large number of persons having to be treated to prevent a case of disease. Sputum smear microscopy is very useful in identifying the most potent sources of infection, however it lacks sensitivity in identifying those sources of infection that are substantially less potent.

The latent cases may play an important role as sources of infection where the proportion of such cases may be relatively higher (as is the case when tuberculosis case rates are very low), and their detection is delayed (they are less sick and therefore less likely to seek care). Bacteriological culture facilities and newer molecular techniques are not widely used where most tuberculosis patients live. Comstock G W. I 1994 claimed that treatment of tuberculosis, while effective in most patients, is still cumbersome due to the need for administration of multiple drugs over a prolonged period. The very shortest

possible treatment of a case of tuberculosis is 4 months (where the bacterial population is so low as to be undetectable by current culture techniques) and up to 24 months where resistance to both isoniazid and rifampicin is present. Although the currently recommended strategy for management of tuberculosis is feasible, sustainable and effective, it is, nevertheless, cumbersome and becomes increasingly so as the disease retreats to population groups that are marginalized and substantially less likely to adhere to a complex treatment regimen. The current strategy for effective control of tuberculosis is centered on case management. It is prolonged for the individual due to the long period of time required for treatment, and it is prolonged for the community. Suarez P G *et al.*2001 described that the point of entry relies on a symptomatic person who has already infected others by the time of clinical presentation. Those infected may harbor their bacilli for many decades prior to developing disease, necessitating a very prolonged vigilance with diminishing returns. Sustaining political commitment over such a prolonged period is very difficult, and increasingly so as the disease begins to disappear from the community.

1.2.4 Poverty

Raviglione *et al* in 1995 described poverty as always related to tuberculosis. In areas where there are high density of population with poor water supply and unhygienic environment, tuberculosis may easily be spread within the community. Many have argued that it is impossible to control tuberculosis (much less eliminate it) without addressing poverty; indeed the most significant factor associated with the decline in

tuberculosis in many industrialized countries has been economic development, an argument that is difficult to refute.

1.2.5 Poor health services

Another factor that should be address in curbing tuberculosis infection is the inadequacy or poor health services. Tuberculosis campaigns failed to achieve its objectives due to lack of resources. The diminishing quality and difficulties to access to health services had also complicate the situation. Current trends of globalization often include privatization as a component, with the view that private services are more efficient than public services. Consequently in relation to tuberculosis control program, non-profitable campaigns against tuberculosis suffers most . As reported by Brudney *et al,*.1991 that the decline in public health services has been systematically followed by rises in tuberculosis, for example, in the US and the former Soviet Union there has been accompanied by the emergence of drug-resistant strains of tuberculosis which are very difficult and costly to cure. Frieden T R *et al,*. 1993 reported that health sector reform may also result in seriously weakening tuberculosis control activities, when the reform process does not give due consideration to public health services such as tuberculosis control.

1.3. Pathophysiology of tuberculosis infection

Tuberculosis is defined as a pulmonary and systemic infectious disease caused by M tuberculosis and characterized by formation of granulomas and by cell mediated hypersensitivity, in which M tuberculosis multiply and attack different parts of the body (Daniel *et al.,* 1994). There are about more than 20 species of *Mycobacterium* aerobic, non-spore-forming rod-shaped bacteria that stain a red pigment after carbolfushsin. (AFB) stain. M tuberculosis, M bovis and M africanum are pathogenic in normal immunocompetent hosts. They grow slowly and the generation time is roughly (15-20 hrs) compared to *S. pneumoniae*~ 20 min. (in vitro).

"Atypical mycobacterium" includes M kansasii, M fortuitum and M avium *intracellular* are pathogenic in immunocompromised host (HIV and AIDS). The progression of the disease resulted from the severity of the causative agent and the immunity of the host.

1.3.1. Primary Infection

Tuberculosis is spread by airborne droplet nuclei, which are 1-5 μm particles containing 1 to 400 bacilli each. They are expelled in the air with coughing, sneezing, singing, laughing, talking etc and remain suspended in the air for many hours. They can be inhaled and subsequently entrapped in the distal airways and alveoli. There, bacilli are ingested by local macrophages, multiply within the cells, and within 2 weeks are transported through the lymphatics to establish secondary sites (lymphohematogenous spread). The development of an immune response, begins by a delayed-type

hypersensitivity reaction over the next 4 weeks leading to granuloma formation, followed by the decrease in the number of bacilli (Behr MA *et al,*. 1999).

The actual infection is initiated by alveolar implantation of organisms in droplet nuclei that are small enough (1-5 μm) escaping the ciliary epithelial cells of upper respiratory tract. Subsequent progression depends on the inoculum size and the host cellular immunity. The organisms implanted and ingested by pulmonary macrophages where they continue to grow and multiply and hence they spread to regional lymph nodes in the mediastinal and retroperitoneal areas. Approximately 5-15 days into infection, CD4 cells with antigen are activated and secrete Interferon-γ which in turn stimulate macrophages to become bactericidal. After lymph nodes are involved the organisms spread via bloodstream to other organs such as kidney, bone, liver, CNS and apical regions of the lungs. Later about 15-25 days into the infection, macrophages form granulomas that contain organisms. Since then patient will develop delayed hypersensitivity via activation of CD4 lymphocytes within 1-3 months and develop positivity towards Purified Protein Derivatives.

Some of them remain viable or 'dormant' for many years. This stage is called latent TB infection (LTBI), which is generally an asymptomatic, radiologically undetected process in humans.

Sometimes a primary complex (Ghon complex) can be seen radiographically, mostly in the lower and middle lobes, and comprises the primary lesion, hilar lymphadenopathy

plus/minus a lymphangitic track. Later, the primary lesion tends to become calcified, and can be identified on the chest radiographs for decades. Most commonly, a positive tuberculin test remains the only proof of LTBI, and therefore does not signify active disease. (Iseman MD, 2000)

Under certain conditions of immature or dysregulated immunity, alveolar macrophages and the subsequent biologic cascade could fail in limiting the mycobacterial proliferation, leading to primary progressive tuberculosis (mostly in children less than 5 years old, HIV positive or profoundly immunosuppressed individuals). Factors known to influence this unfavorable course are: patients' age, nutritional status, host immunity, and bacterial infective load.

1.3.2. Secondary Infection

Once infected with M tuberculosis, 3-5% of immunocompetent individuals will develop active disease eg, secondary progressive tuberculosis within 2 years, and an additional 3-5% later on during their lifetime. So, overall, there is a lifetime risk of reactivation of 10%, with half of it occurring during the first 2 years after infection (Sutherland *et al.*, 1976). A recent analysis (Horsburgh *et al.*,2004) showed that the lifetime reactivation rate is around 20% for most persons with PPD (Purified Protein Derivative) induration more than 10 mm and either HIV infection or evidence of old, healed tuberculosis, and is between 10 and 20% for recent PPD skin test converters, adults younger than 35 years of age with an induration more than 15 mm, or on therapy with Infliximab (a TNFα receptor blocker), and for children younger than 5 years of age and skin induration more than 10

mm. About 90% of patients experience primary disease and have no further clinical manifestations other than a positive PPD. While other 3-5% of patients experience progressive primary disease and 7-10% experience reactivation disease - that arises subsequent to hematogenous spread of the organism.

Miliary TB is a dissemination of tuberculosis infection and includes granuloma formation. In such conditions the CD4 cells are primarily involved in preventing further spread of TB. Therefore low CD4 cells as in AIDS patients have higher risks of getting infected with Tuberculosis.

1.4. Current Diagnostic Technology In Malaysia

1.4.1. Conventional Methods

1.4.1.1. Sputum Acid Fast Bacilli Smear

In countries with a high prevalence of tuberculosis (TB), direct sputum smear microscopy remains the most cost effective tool for diagnosing patients with infectious tuberculosis and for monitoring their progress on treatment. The World Health Organization strategy for tuberculosis control (DOTS) relies on a network of laboratories that provide acid fast bacilli (AFB) sputum smear microscopy.

Figure 1.3, illustrates the importance of collecting 3 serial early morning sputum samples. The first sample has 81% chance of being positive while the third sample collected represent almost 100% possibility of yielding acid-fast bacilli (Dr. John Ridderhof, CDC Atlanta. 2000)

Rouillon A. in 1976 discovered that smear-positive patients are 4-20 times more infectious and if left untreated, a smear-positive patient may infect 10-15 persons/year. They are much more likely to die if untreated. The advantages of AFB smear includes rapidity, highly specific of 99.0 % and uses simple and available equipment. However it has a very low sensitivity of 72.1%. The sensitivity and specificity of this method also depends on the centers and techniques being applied.

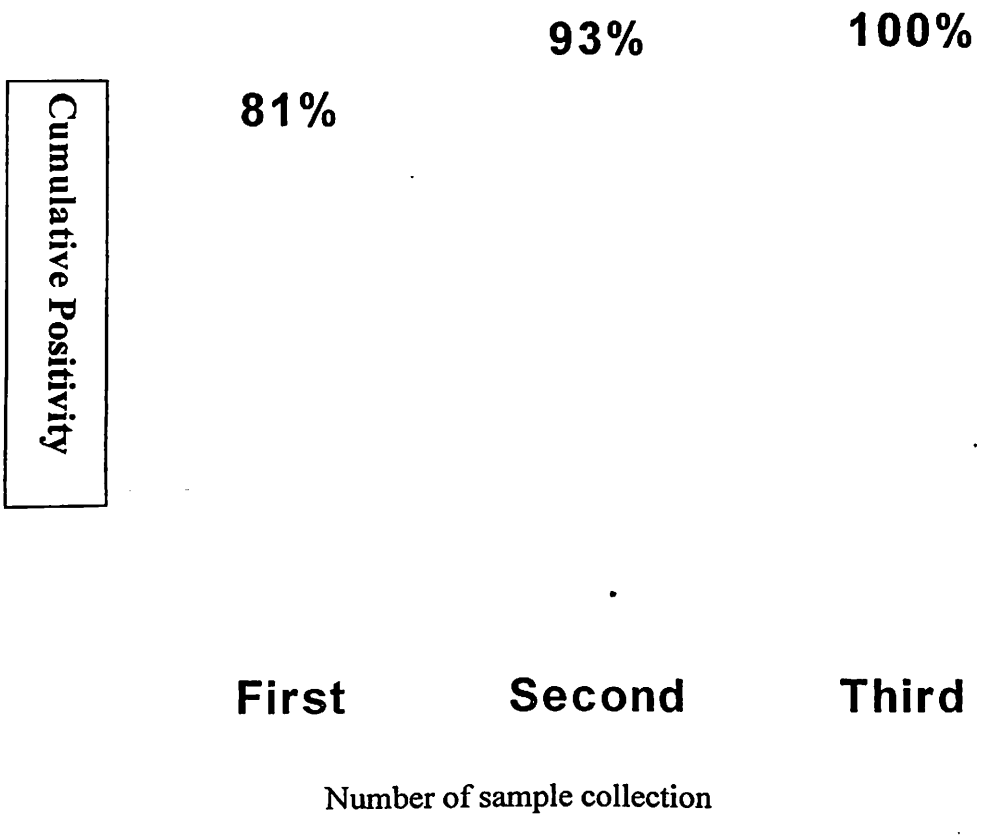

Figure 1.3: Optimum Sputum Acid Fast Bacilli smear sampling for diagnostic purposes of tuberculosis infection. (Dr. John Ridderhof, CDC Atlanta. 2000)

1.4.1.2. Chest X-Rays

Toman K. in 1979 reported that, no chest X-ray pattern is typical of TB and 10-15% of culture-positive TB patients are not diagnosed by X-ray. About 40% of patients diagnosed as having TB on the basis of x-ray alone do not have active TB. As illustrated in figure 1.4, Chest X-ray is unreliable for diagnosing and monitoring treatment of tuberculosis. About 80% of cases had been overdiagnosed as tuberculosis based from the chest x-rays.

National Tuberculosis Institute findings published in Indian Journal of Tuberculosis (1974) described that most of the suspected or initially diagnosed as tuberculosis infection from chest x-rays, only 30% of them actually have tuberculosis.

1.4.1.3. Polymerase Chain Reactions

The advantages of diagnostic molecular techniques have been so widely popularized. Therefore, there is increasing trend for clinical microbiology laboratories to either include diagnostic molecular techniques in their "research" activities or risk being left behind in the quality of service that they provide to clinicians and patients. The Polymerase Chain Reaction (PCR) is one such technique (Ashok Rattan, 2000).

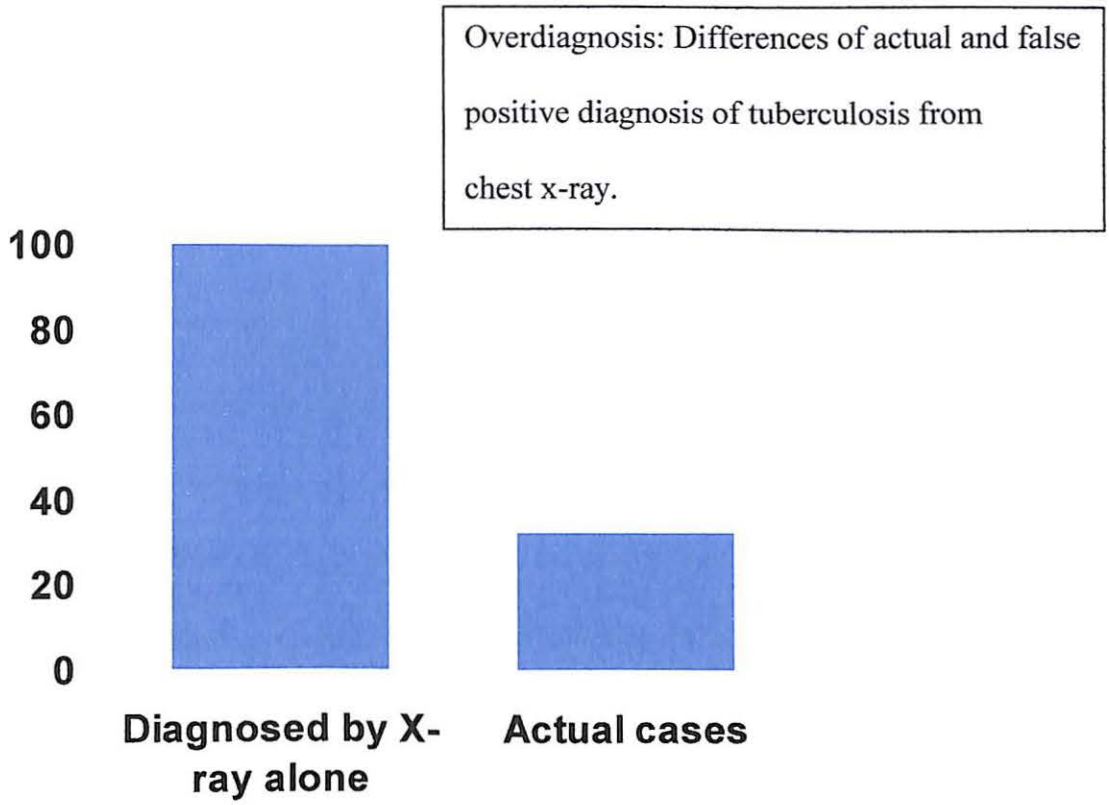

Figure 1.4: Overdiagnosis: Differences of actual and false positive diagnosis of tuberculosis from chest x-ray. (National Tuberculosis Institute, Ind J Tuberc, 1974)

In 2003, Olga L. Sarmiento *et al*,. reported in a review of meta-analysis in assessing the performance of PCR for the diagnosis of smear-negative pulmonary tuberculosis discovered the sensitivity and specificity ranged from 9 to 100% and from 25 to 100%, respectively.

According to the Centers for Disease Control, Georgia (1991), approximately 20 to 50% of patients with pulmonary tuberculosis are smear negative for Acid Fast Bacilli. 10% of these patients are culture negative. PCR reduces the time required for the identification of the *Mycobacterium* and may enhance the detection of smear-negative pulmonary tuberculosis cases. Few factors have been pointed out for the low sensitivity of PCR for the diagnosis of smear-negative pulmonary tuberculosis and the significant variability in sensitivity and specificity in different studies. Noordhoek, et al.,(1996) had proposed explanations for these negative findings that includes differences in decontamination procedures, cross contamination, sampling error, quality of the reference standard and mixture of respiratory and other specimens. S. Levidiotou, *et al*,. (2003) described the overall sensitivity of PCR in smear positive is 82.5%, specificity of 99.8%, and positive predictive values of 94.3% and negative predictive values of 99.4%.

These results are obtained in comparison to the results of M tuberculosis culture. In conclusion, the use of the Cobas Amplicor MTB-PCR assay might enable clinical microbiology laboratories with experience in molecular biology testing to perform PCR and diagnose tuberculosis infection immediately, leading to improved patient