A STUDY ON THE INFLUENCE OF HIV INFECTION IN PULMONARY TUBERCULOSIS PATIENTS ON THE SECONDARY TRANSMISSION OF PULMONARY TUBERCULOSIS TO HOUSEHOLD CONTACTS

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LIST OF ABBREVIATIONS

AFB	-	Acid-Fast Bacilli
AIDS	-	Acquired Immunodeficiency Syndrome
CDC	-	Center for Disease Control, Atlanta, USA
CXR	-	Chest X-Ray
ELISA	-	Enzyme-Linked Immunosorbent Assay
HIV	-	Human Immunodeficiency Virus
MOH	-	Ministry of Health, Malaysia
PPD	-	Purified Protein Derivatives
PTB	-	Pulmonary Tuberculosis
TB	-	Tuberculosis
TU	-	Tuberculin Unit
WHO	-	World Health Organization

ABSTRAK (BAHASA MALAYSIA)

KAJIAN PENGARUH JANGKITAN HIV DALAM PESAKIT TUBERKULOSIS PULMONARI KE ATAS TRANSMISI SEKUNDER TUBERKULOSIS PULMONARI KEPADA KONTAK ISIRUMAH

Pengenalan: Epidemik virus HIV telah membawa kesan yang besar ke atas transmisis tuberkulosis (TB). Keupayaan kes-kes jangkitan bersama HIV dan TB dalam menyebar kuman *M. tuberculosis* dan menghasilkan pertambahan morbiditi TB adalah tidak diketahui. **Objektif:** Untuk membandingkan prevalen jangkitan *M.tuberculosis* di kalangan kontak isirumah kepada pesakit HIV serta mengkaji faktor-faktor yang boleh mempengaruhi transmisi jangkitan PTB. **Metodologi dan Bahan:** Satu kajian keratan lintang telah dijalankan dengan mengkaji rekod ujian tuberkulin (Mantoux) yang telah diberi semasa penyiasatan rutin kontak isirumah pesakit TB di Klinik Dada, Hospital Kota Bharu, Kelantan dari tahun 1999 ke 2000. Status HIV pesakit ditentukan oleh keputusan ujian ELISA manakala maklumat kontak isirumah diperolehi semasa lawatan ke rumah. **Keputusan:** Seratus enam puluh lapan kontek kepada 67 pesakit HIV-negatif dan 62 kontek kepada 22 pesakit HIV-positif telah diselidiki. Tiga puluh dua peratus (20/62) daripada kontek kepada HIV-positif PTB mempunyai keputusan ujian tuberkulin positif berbanding dengan 49.4 % (83/168) kontek kepada HIV-negatif (nisbah *odds* (OR) = 0.48 95% Sela keyakinan (CI) 0.26 – 0.90, p = 0. 020). Pesakit-pesakit TB jangkitan HIV dalam kajian ini mempunyai lesi pulmonari yang kurang teruk tanpa kavitasi. Perbezaan tersebut masih lagi signifikan selepas dijalankan analisis lanjutan logistik regresi terhadap keterukan PTB serta faktor-faktor lain yang mempengaruhi transmisi TB (OR terlaras = 0.42 95% CI 0.18 – 0.95 p = 0.04). Kontak

dewasa, kepadatan isirumah yang tinggi, tinggal lebih lama dengan pesakit serta faktor sputum positif dan kavitasi pada X-ray dada pesakit mempunyai nisbah *odds* yang lebih tinggi bagi mendapat ujian tuberkulin yang positif di kalangan kontak isirumah, tetapi perbezaan yang wujud adalah tidak signifikan. **Kesimpulan:** Kajian ini menunjukkan bahawa pesakit TB HIV-positif kurang menjangkitkan tuberkulosis kepada kontak berbanding dengan pesakit HIV-negatif. Kajian ini mencadangkan bahawa apabila immunosupresi akibat jangkitan HIV menjadi semakin teruk, keupayaan untuk menjangkitkan TB pulmonari menjadi semakin rendah. Keupayaan kes-kes PTB untuk menjangkitkan mungkin bergantung kepada tahap jangkitan HIV di mana pesakit-pesakit yang mempunyai imun yang lemah akan mempunyai lesi pulmonari yang kurang teruk serta kurang kemungkinannya untuk mempunyai kavitasi, mengakibatkan keupayaan untuk menjangkitkan menjadi lebih rendah berbanding dengan pesakit-pesakit pada tahap awal jangkitan HIV. Kehadiran HIV dalam komuniti mungkin tidak memerlukan kepada perubahan pengurusan kes-kes kontak TB dalam masyarakat secara umum.

ABSTRACT (ENGLISH)

A STUDY ON THE INFLUENCE OF HIV INFECTION IN PULMONARY TUBERCULOSIS PATIENTS ON THE SECONDARY TRANSMISSION OF PULMONARY TUBERCULOSIS TO HOUSEHOLD CONTACTS

Introduction: The Human Immunodefiency Virus (HIV) epidemic has had a profound influence on the transmission of tuberculosis (TB). The potential for HIV-associated TB cases to transmit *M.tuberculosis* and to produce a secondary increase in TB morbidity is unknown. **Objectives**: To compare the prevalence of *M.tuberculosis* infection among household contacts of HIV-positive and HIV-negative pulmonary TB (PTB) patients, and to identify potential variables that associated with the transmission. **Materials and Methods:** A cross-sectional study was carried out to review records of tuberculin (Mantoux) tests administered during routine contact investigations at the Chest Clinic, Kota Bharu Hospital, Kelantan from 1999 through 2000. HIV status of patients was based on the result of ELISA tests while information on household contacts were recorded during house visit. **Results:** One hundred and sixty eight contacts to 67 HIV-negative patients and 62 contacts to 22 cases of HIV-positive PTB patients were included. Thirty-two percents (20/62) of contacts of HIV-positive PTB had a positive tuberculin compared with 49.4 % (83/168) of contacts to HIV-negative patients (crude Odds Ratio (OR) = 0.48 95% Confidence Interval (CI) 0.26 – 0.90, p = 0. 020). HIV-infected PTB patients in this study had a less severe form of pulmonary lesion without cavitations. The difference in tuberculin response among the household contacts of each group was still significant after performing multivariate logistic regression analysis to adjust for the severity of PTB and other potential variables associated with

infectiousness of TB (adjusted OR = 0.43, 95% CI 0.18 – 0.95 p = 0.04). Adult contacts, crowding of house, longer duration of stay with index cases, presence of sputum positive and cavitations on chest X-ray in index cases showed higher odds of being tuberculin positive among household contacts but the differences were not significant. **Conclusion:** The study has shown that HIV-infected PTB were less infectious to their household contacts than HIV-negative patients. The study suggests that the more advanced HIV-associated immunosuppresion among the PTB index cases, the less infectious is the PTB. The transmissibility of TB may depend on the stage of HIV infection i.e patients who are immunosuppressed are less likely to cavitate and have milder pulmonary lesions, and therefore are less infectious than those patients who are at an early stage of HIV infection. The presence of HIV in the community may not necessitate a change in the current policy in the management of contacts in the general population.

INTRODUCTION

1 INTRODUCTION

Tuberculosis (TB) is still a leading contender for the dubious distinction of being the most important plague of mankind. By now it is clear that TB is once again a major public health problem. It remains the leading cause of death by an infectious disease. Recent statistics have increased the awareness of the magnitude of the problem caused by the disease. According to the World Health Organization (WHO), one third of the world's population is already infected with *M. tuberculosis* which causing about 3 million deaths each year (Kochi et al., 1995). These statistics are surely alarming as TB is a curable disease if properly managed.

TB is an important cause of mortality amongst the infectious diseases in Malaysia. Statistical data gathered by the Disease Control Division of the Ministry of Health, Malaysia (MOH) showed a reduction in incidence of TB from 83.4 per 100, 000 population in 1980 to 60.2 per 100, 000 population in 1991(MOH, 1999). However, in 1999 the number of persons afflicted with TB increased to 65.6 per 100, 000 population. In 1995, the number of reported deaths due to TB was 571 out of 11,778 cases, giving a case fatality rate of 4.8 %. In 1999, however, there was an increase in the number of reported deaths due to TB, giving a case fatality rate of 5.2 %. This is due to a combination of factors such as HIV co-infection, delayed diagnosis and poor compliance to treatment (TB in the New Millennium, 2000). The majority of victims (about 67% in 1999) belongs to the economically productive age group of 15 to 54 years and will have long-term effects on the national economy.

At the same time, the Human Immunodeficiency Virus (HIV) pandemic is increasing rapidly in many communities worldwide; more than 30 million people are currently infected and 1.5 million deaths are estimated to have occurred in 1996. Nearly 14 million people are expected to be infected with HIV and TB by the year 2000 (WHO, 1998). In Malaysia, all TB patients have been routinely screened for HIV status since 1990. The trend of tuberculous patients with HIV infection has continuously increased since then. The cumulative number of cases of HIV-infected TB in Malaysia was 603 (about 4 % of total TB cases) of which 202 have since died (MOH, 1999). In Kelantan, there were 184 cumulated cases of TB/HIV from 1993 to 1999 (Kelantan State Health Department, 2000).

The effects of HIV on TB will be greatest where the two diseases overlap, as in the developing world, affecting major African countries (Elliot et al., 1990; Colebunders et al., 1989) but increasingly in South East Asia (Lim, 1991; Yanai *et al.*, 1996). Quite suddenly, because of the epidemic of infection with HIV, the relative effect of immunosuppression on the incidence of TBs has changed. As a consequence, large proportion of some populations, both in developed and developing countries, are immunocompromised. Moreover, HIV infection is most common among populations in whom there is a high prevalence of tuberculous infection. As a result of the superimposition of HIV infection and TB infection, rates of TB have increased dramatically in many places.

The potential for HIV-associated TB cases to transmit *M. tuberculosis* and to produce a secondary increase in TB morbidity is unknown. If alterations in the TB mediated by HIV infection result in more efficient transmission, or behavioral factors result in the

exposure of more contacts, or if HIV infection is prevalent among the contacts themselves, there is potential for an accelerated epidemic. Large outbreaks of multidrug-resistant TB among HIV-infected hospital and hospice patients and among HIV-infected prison inmates have demonstrated that those persons co-infected with HIV and TB can be highly infectious (Di Perri *et al.*, 1989; Edlin *et al.*, 1992). Few reports have specifically investigated the rate of TB in the contacts of HIV infected tuberculous index cases. It is not clear whether this rate was due to increased infectiousness of the index cases or enhanced susceptibility of the contacts, or both. Therefore, it is of considerable importance of public health concern to establish whether patients with HIV-associated PTB are more or less infectious than those with PTB alone. The purpose of this study is to identify the impact of HIV on the epidemiological transmission of TB and to determine whether changes are required in the management of contacts of PTB cases in developing countries in light of the HIV epidemic.

LITERATURE REVIEWS

2 LITERATURE REVIEWS

2.1 OVERVIEW ON TB

Tuberculosis (TB) has been recognized since the dawn of recorded history. Starting in the latter half of the 19th century, the incidence declined significantly in the industrialized nations and by the early 1980s there was a widespread opinion that the disease had virtually been conquered. It is now apparent that, far from being conquered, tuberculosis is one of the most prevalent infectious causes of human suffering and death worldwide. Indeed, there are more cases of TB in the world today than at any previous time in human history. Because of the relentless spread of tuberculosis throughout the world, the World Health Organization (WHO), in 1993, took the unprecedented step of declaring tuberculosis a global emergency (WHO, 1994).

2.1.1 Global Burden of TB

Each year, around seven to eight million people develop the disease. Estimates for 1990 were that 8 million new TB cases developed globally, with 95% in the developing world. The largest number of cases occur in the WHO's Southeast Asian, Western Pacific and African regions (Murray *et al.*, 1990). TB causes the death of around three million people annually. The disease is responsible for 7% of all adult deaths and 25% of preventable adult deaths. Mortality from TB in 1990 was estimated to be 2.9 million, making it the largest single cause of death from a pathogen in the world. Although 98% of deaths are in

the developing world, accounting for 25% of avoidable adult deaths, more than 40 000 deaths occurred in industrialized nations (Kochi, 1991).

Despite the achievement made in effective prophylaxis and therapy, TB continues to rank among the most serious of health problems throughout the world. Globally, 1.7 billion individuals, approximating to one third of the world's population, are or have been infected with the tubercle bacilli. The age distribution of the infection is dramatically different between the industrialized and developing worlds. In industrialized countries, 80% of people infected with TB are over the age of 50 whereas 75% of those in the developing world are less than 50 years old; the greatest attack rate is in the economically most productive age group (15 – 59 year olds) (Kochi, 1991).

2.1.2 Aetiology of TB

Most cases of human TB are caused by the human tubercle bacillus, *Mycobacterium tuberculosis*, but in countries where cattle TB still occurs human tuberculosis are also caused by *M. bovis* (Des Prez and Heim, 1991). In addition, some cases, principally in equatorial Africa, are caused by a rather heterogeneous group of strains termed *M. africanum* (Braunstein, 1990). Though bearing separate species names, these are really members of a single species often termed the *M. tuberculosis* complex (Wayne, 1982).

2.1.3 Risk of Becoming Infected with Tubercle Bacilli

The probability of having been infected with one of the three tubercle bacilli above (*M. tuberculosis* is by far the most common) is assessed by the size of induration caused by the tuberculin test. It is believed that the risk of becoming infected has been falling throughout most of the world, most rapidly in industrialized nations and least in sub-Saharan Africa and Indian subcontinent, where the annual rate of decline is estimated to be less than 3% per year (Kochi, 1991). Reasonably good estimates can be obtained in countries where there are enough children and young adults who have not been vaccinated with BCG to allow the risk to be estimated (Styblo, 1991).

The likelihood of having been infected among household contacts of infectious cases of TB has also declined with time, at least in the United States. In 1986, less than 30% of close contacts of all ages were positive tuberculin reactors (Rieder *et al.*, 1989a). The risk of infection is strongly associated with the probability of coming in contact with infectious tuberculosis such as crowding, the closeness and intimacy of that contact, its duration and the degree of infectiousness of the case. Grzybowski *et al.* (1975) cited a study among Indian contacts in the Canadian Provinces of Columbia and Saskatchewan, that infection risk was greater if the contact was intimate (household or sweethearts) than if it was casual (other friends or fellow employees). If sputum of the source case contained so many tubercle bacilli that they were demonstrable by microscopic examination, the risk was also greatly increased. Patients with positive sputum cultures were more infectious than those with negative cultures. Extent of pulmonary involvement was strongly associated with infectivity: 62% of contacts of cases with far-advanced disease were reactors, compared to 16% reactors among contacts to minimal

cases. The study also showed that the tuberculin reactions were also related to cough duration.

There is some evidence that the risk of acquiring infection increases with age from the infancy to adulthood (Sutherland and Fayers, 1975), probably because of increasingly numerous contacts with other persons. In term of gender distribution, in nearly all populations around the world, males are more likely to have been infected than females, again probably reflecting their opportunity for more and varied contacts in most societies (Reichman and O'Day, 1978). Riley and Moodie (1974) stated that effective chemotherapy of the source case appears to reduce infectiousness rapidly, perhaps even more rapidly than is indicated by results of sputum examination. However, when source case with drug resistant organisms had a history of prior and probably ineffective treatment, their contacts were at increased risk of being infected. This resulted from the longer duration of exposure that is associated with multiple episodes of treatment.

2.1.4 Transmission and Pathogenesis of TB

The infectious agent of TB, *Mycobacterium tuberculosis*, is carried on airborne droplet nuclei. Droplet nuclei are produced when persons with pulmonary TB cough, sneeze, speak, or sing. They also may be produced through manipulation of lesions or processing of tissue or secretions in the hospital or laboratory. Droplet nuclei are so small (1 to 5 um) that air currents normally present in any indoor space can keep them airborne for long periods of time. Once released from the host, they are dispersed throughout the room. Although patients with TB also generate larger particles containing numerous bacilli, these particles do not serve as effective vehicles for transmission of infection because

2.1.5 Signs and Symptoms of PTB

Characteristically, in pulmonary TB, there is the almost imperceptible onset of a cough. This slowly progresses over weeks or months to become more frequent and associated with the production of mucoid or mucopurulent sputum. Hemoptysis also may occur. Occasionally, there is recurring dull, aching pain or tightness in the chest. Dyspnea is uncommon and usually indicates extensive parenchymal involvement, massive pleural effusion, or other underlying cardiopulmonary disease. Some patients present with the acute onset of productive cough, fever, chills, myalgia, and sweating similar to the signs and symptoms of influenza, acute bronchitis, or pneumonia. Physical findings may include rales or signs of lung consolidation (Bailey, 1980).

2.1.6 Radiographic Examination of the Chest

In patients who have signs and/or symptoms suggesting pulmonary or pleural TB, standard posterior-anterior and lateral radiographs of the chest should be obtained. Apical-lordotic or oblique views may aid in visualizing lesions obscured by bony structures of the heart. Special imaging techniques such as computerized tomography and magnetic resonance imaging may be of particular value in defining nodules, cavities, cysts, calcifications, contours of large bronchi, and vascular details in lung parenchyma. Bronchography may be useful in the definition of bronchial stenosis or bronchiectasis. Fluoroscopy should be reserved for the demonstration of the mobility of thoracic structures and for the visualization of localized lesions to guide diagnostic procedures.

The initial radiographic manifestation of initial infection in the lung, whether in a child or an adult, is usually parenchymal infiltration accompanied by ipsilateral lymph node enlargement. The parenchymal lesion may be detected at any stage of development and in any portion of the lung, or it may be too small to be seen on the radiograph. Miller and Miller (1993) noted that in primary TB, the unusual radiographic patterns including parenchymal infiltrates, are located in the lower lobes in more than 50% of patients. Tuberculous involvement of the hilar or mediastinal nodes is usually unilateral. Lymph node changes tend to persist longer than the parenchymal shadows. Calcification of the lung lesions and the lymph nodes may occur several years after infection. TB in HIV-infected patients may have the radiographic characteristics of primary disease.

More commonly, in adults, lesions are seen in the apical and posterior segments of the upper lobes, or in the superior segments of the lower lobes. However, lesions may appear in any segment; nodular infiltrates of varying sizes are perhaps most common. Lesions may be dense and homogeneous, with lobar, segmental, or subsegmental distribution. Cavitation is common, except in immunocompromised patients. There may be thin or thick walled, solitary or numerous and some may show air-fluid level (Cohen *et al.*, 1978). One may also find lower lobe nodular infiltrates without cavitation upon radiograph observation of TB in the elderly. Other findings include atelectasis and fibrotic scarring with retraction of the hilus, and deviation of the trachea. Diffuse, finely nodular, uniformly distributed lesions on the chest radiograph characterize hematogenous tuberculosis; fever and systemic symptoms and signs may antedate this finding. The term "miliary" is applied to this appearance because the nodules are about the size of millet seeds, approximately 2 mm in diameter (Sahn and Neff, 1974). Unilateral or, rarely, bilateral pleural effusion is usually the only radiographic abnormality evident with pleural TB. Although these are the more common radiographic patterns, TB may produce almost

any form of pulmonary radiographic abnormality. Rarely, patients may present with normal radiographs, particularly patients with HIV infection and endobronchial TB.

A single chest radiograph should not be used as a guide to stability or the nature of the underlying disease. The use of words such as "old" or "fibrotic" should be avoided when interpreting a single radiograph because this often will be misleading. However, chest radiographs that show no change during a 3 or 4-month interval generally indicate "old" TB or another disease (Bass *et al.*, 1990).

2.1.7 Staining and Microscopic Examination

The detection of acid-fast bacilli (AFB) in stained smears examined microscopically is the first bacteriologic evidence of the presence of mycobacteria in a clinical specimen. It is the easiest and quickest procedure that can be performed, and provides the physician with a preliminary confirmation of the diagnosis. Also, because it gives a quantitative estimation of the number of bacilli being excreted, the smear is of vital clinical and epidemiological importance in assessing the patient's infectiousness. It is estimated that the lowest concentration of organisms that can be detected by microscopic examination is 10^4 / ml of sputum. Various studies have indicated that 50 to 80% of patients with pulmonary TB will have positive sputum smears (Bass *et al.*, 1990).

2.1.8 AFB Smears from Pulmonary Specimens

Lipsky *et al.* (1984) reviewed over 3000 specimens submitted for AFB smear and culture and found the smear sensitivity to be 33%. If only patients with positive TB cultures (removing all patients with cultures for mycobacteria other than *M. tuberculosis*) are considered, however, 65% were AFB smear positive and multiple numbers of specimens increased the smear positivity rate to 96%. Overall, the sensitivity of an AFB smear in patients with confirmed cases of pulmonary TB has been 50–80% (Bass *et al.*, 1990).

Cavitary TB is associated with a markedly increased number of AFB within the lesion, probably because of the aerobic requirements of *M. tuberculosis*. Patients with active TB cavities have an AFB smear positivity rate of 98% (Barnes *et al.*, 1988). False positive smear are usually caused by specimens from patients being treated for TB (specimens contain non-viable organisms), smear reader inexperience or overall quality control of staining techniques. When patients being treated are removed from the analysis, the positive and negative predictive values for AFB smears are greater than 95%. (Murray *et al.*, 1980).

Young children (particularly those under 3 years of age), the elderly and HIV-infected persons may not produce cavitary lesions, granulomas or sputum. This can significantly lower AFB smear positivity. In a recent study on the case of the use of AFB smear in the elderly, only one of 19 patients had cavitary disease and two of 19 (10%) were smear positive (Morris, 1991). Although only 6% of HIV-infected TB demonstrates cavitation, 85% will have chest X-ray suggestive of infection with *M. tuberculosis* and a smear positivity rate of 31-82% (Barnes *et al.*, 1991). In one case- control study, HIV-infected TB had a significantly lower rate of AFB positive smears (45%) compared with controls

(81%) (Klein *et al.*, 1989). In general, the HIV disease host response to *M. tuberculosis* is directly related to the level of immunosuppression. When immunosuppression is mild, TB disease is similar to that seen in the normal host, while increasing immunosuppression will increase the risk of atypical TB and dissemination (De Cock *et al.*, 1992).

2.2 TUBERCULIN SKIN TEST

The tuberculin skin test has been the traditional method of demonstrating infection with *M. tuberculosis*. Although currently available tuberculin skin tests are substantially less than 100% sensitive and specific for detection of infection with *M. tuberculosis*, no better diagnostic methods have yet been devised (Huebner, 1981). Intelligent interpretation of skin test results requires a knowledge of the antigen used (tuberculin), the immunologic basis for the reaction to this antigen, the technique(s) of administering and reading the test, and the results of epidemiologic and clinical experience with the test.

The tuberculin test is based on the fact that infection with *M. tuberculosis* produces sensitivity to certain antigenic components of the organism that are contained in culture extracts called "tuberculins." Two preparations of tuberculin are currently licensed for use in the United States: Old Tuberculin (OT) and Purified Protein Derivative (PPD). Tuberculin PPD is a purified protein derivatives prepared from culture filtrates of *M. tuberculosis*. WHO has adopted the use of PPD-S (Standard) prepared by Siebert and Glein since 1951. The international unit, known as the tuberculin unit (TU) is the biologic activity represented by 0.00002 mg of PPD-S (Comstock, 1981). Tuberculin PPD is

available for intracutaneous injection by the Mantoux technique and by multiple puncture devices.

2.2.1 Immunologic Basis for the Tuberculin Reaction

The reaction to intracutaneously injected tuberculin is the classic example of a delayed (cellular) hypersensitivity reaction (Bothamley and Grange, 1991). Characteristic features of the reaction include: (1) its delayed course, reaching a peak more than 24 h after testing; (2) its indurated character, largely because of cell infiltration; (3) its occasional vesiculation and necrosis. A delayed hypersensitivity reaction to tuberculin may indicate previous natural infection with *M. tuberculosis*, infection with a variety of non-tuberculosis mycobacteria, or vaccination with BCG, a live attenuated mycobacterial strain derived from *M. bovis*.

Characteristically, delayed hypersensitivity reactions to tuberculin begin at 5 to 6 hours, are maximal at 48 to 72 h, and subside over a period of days (Dvorak *et al.*, 1986). In a few persons (those who are elderly or those who are being tested for the first time), reactions may develop slowly and may reach maximum peak until after 7 days (Slutkin *et al.*, 1986). Immediate hypersensitivity reactions to tuberculin or constituents of the diluent can also occur. However, these reactions begin shortly after injection, disappear by 24 h, and are not likely to be confused with delayed hypersensitivity reactions (American Thoracic Society, 1990). Unfortunately, not all persons infected with *M. tuberculosis* or *M. bovis* will have a delayed hypersensitivity reaction to a tuberculin skin test. A large number of factors (Table 1) have been reported to cause a decreased ability to respond to tuberculin.

TABLE 1: Factors Causing Decreased Ability to Respond to Tuberculin

Factors Related to the Person Being Tested.

1. Infections
   1.1. Vital (measles, mumps, chicken pox)
   1.2. Bacterial (typhoid fever, brucellosis, typhus, leprosy, pertussis, overwhelming tuberculosis, tuberculous pleurisy)
   1.3. Fungal (South American blastomycosis)
2. Live virus vaccinations (measles, mumps, polio)
3. Metabolic derangements (chronic renal failure)
4. Nutritional factors (severe protein depletion)
5. Diseases affecting lymphold organs (Hodgkin's disease, lymphoma, chronic lymphocytic leukemia, sarcoidosis)
6. Drugs (corticosteroids and many other immunosuppressive agents)
7. Age (newborns, elderly patients with "wanes" sensitivity)
8. Recent or overwhelming infection with M. tuberculosis
9. Stress (surgery, burns, mental illness, graft versus host reactions)

Factors Related to the Tuberculin Used

1. Improper storage (exposure to light and heat)
2. Improper dilutions
3. Chemical denaturation
4. Contamination

Factors Related to the Method of Administration

1. Injection of too little antigen
2. Delayed administration after drawing into syringe
3. Injection too close

Factors Related to Reading the Test and Recording Results

1. Inexperienced reader
2. Conscious or unconscious bias
3. Error in recording

The existence of one or more of these factors does not mean that testing should not be undertaken because only a fraction of infected persons with these conditions may have falsely non-reactive tests. The presence of reactions in such persons may still identify those in whom infection is highly probable. If the lack of reaction to the test is suspected of being a false response, a repeat tuberculin skin test should be done. If generalized inability to respond is suspected, then it may also be desirable to test delayed hypersensitivity using several other antigens to which the person has very likely been exposed. Those who fail to respond to any of these antigens are more likely to be anergic, a condition suggesting that the immune system is not functioning properly and that the lack of reaction to the tuberculin test may be a false response.

2.2.2 The Intracutaneous (Mantoux) Test

The intracutaneous administration of a measured amount of tuberculin is the best means of detecting infection with *M. tuberculosis*. One-tenth milliliter of PPD is injected into either the volar or the dorsal surface of the forearm. Other areas may be used, but the forearm is preferred. The use of a skin area free of lesions and away from veins is recommended. The injection is made using a one-quarter- to one-half-inch, 27-gauge needle and a tuberculin syringe. The tuberculin should be injected just beneath the surface of the skin, with the needle bevel upward. A discrete, pale elevation of the skin (a wheal) 6 to 10 mm in diameter should be produced when the injection is done correctly. If it is recognized that the first test was improperly administered, another test dose can be given at once, selecting a site several centimeters away from the original injection. A note in the record should indicate the site chosen for the second test (Seibert and Bass, 1990).

Tests should be read between 48 and 72 hours after injection. Reading should be performed in a good light, with the forearm slightly flexed at the elbow. The basis of reading is the presence or absence of induration, which may be determined by inspection (from a side view against the light as well as by direct light) and by palpation. The diameter of induration should be measured transversely to the long axis of the forearm and recorded in millimeters. Another method to determine the induration is by using the ballpoint technique (Jordan *et al.*, 1987). However, strong conclusion regarding the variability of either method is difficult to make since a true gold standard does not exist and depend on the experienced reader (Howard and Solomon, 1989).

2.2.3 Interpretation of Skin Test Reactions

Persons with sensitivity to tuberculin are known as reactors. The definition of a tuberculin reaction size that is indicative of infection with *M. tuberculosis* is influenced by the dose, dilution, and nature of the tuberculin preparation being used, immunologic factors and the relative prevalence of tuberculin sensitivity resulting from infection with *M. tuberculosis* and that resulting from other mycobacteria in the population being studied. Reactions caused by infection with mycobacteria other than *M. tuberculosis* (cross-reactions) commonly occur in many parts of the world. The distinction between reactions that represent tuberculous infection and cross-reactions is not precise, but in general, the larger the reaction, the greater the probability that the reaction represents infection with *M. tuberculosis* (Gottlieb and Pauker, 1989). The interpretation of a tuberculin reaction should be influenced by the purpose for which the test was given and by the consequences of false classification. Errors in classification cannot be avoided, but establishing an appropriate definition of a positive reaction can minimize them.

2.2.4 Separating Tuberculous Infection from Reactions Created by Other Causes

In persons with reactive tuberculin tests, the major confounding factor is infection with and hypersensitivity to mycobacteria other than *M. tuberculosis*. These reactions (cross-reactions) tend to be smaller than reactions caused by tuberculous infection. Reactions in persons who have had recent close contact with TB and in persons with abnormal chest radiographs consistent with TB are more likely to represent infection *with M. tuberculosis* than cross-reactions. However, persons who are immunosuppressed because of disease (e.g. HIV infection) or drugs (e.g. corticosteroids) may have a limited ability to respond to tuberculin even if they have been infected with *M. tuberculosis*. Therefore, using a lower cutting point for separating positive from negative reactions is appropriate in these groups. This will ensure that very few persons infected with *M. tuberculosis* will be classified as having negative reactions, and few persons not infected with tubercle bacilli will be classified as having positive reactions.

Among persons who have not had recent close contact with TB or do not have abnormal chest radiographs consistent with TB, or are not immunosuppressed but have other risk factors for TB, a higher cutting point is appropriate. In the remainder of the population having very low probability of exposure to TB and in which cross-reactions are likely to be more common than reactions caused by tuberculous infections, a still higher cutting point should be selected.

2.2.5 Factors That Cause Persons to Be Falsely Classified as Uninfected

When the tuberculin test is used in the evaluation of persons with a process that might have a TB source, the major concern is with reactions that cause the person to be falsely classified as uninfected. It is important to recognize that a small or no reaction to a tuberculin skin test alone does not exclude the diagnosis of TB from further consideration. The generation and maintenance of a tuberculin reaction in persons infected with the tubercle bacillus requires a complex interaction of immunologic responses. Failure of single components or of their interactions may result in a nonreactive test in a person infected with *M. tuberculosis*. This failure to respond may be mild and short-lived or profound and permanent. There are many conditions that have been reported to impair delayed hypersensitivity and thus cause such false negative reactions. Some of these have been well studied, whereas others are reported only in anecdotal fashion.

2.2.6 General Classification of Reactions (American Thoracic Society, 1990)

A reaction of greater than or equal to 5 mm is classified as positive in the following groups: (1) persons with HIV infection or persons with risk factors for HIV infection who have an unknown HIV status; (2) persons who have had close recent contact with infectious TB cases; and (3) persons who have chest radiographs consistent with old healed TB.

A reaction of greater than or equal to 10 mm is classified as positive in persons who do not meet the above criteria but who have other risk factors for TB. These would include: (1) foreign-born persons from high prevalence countries in Asia, Africa, and Latin

America; (2) intravenous drug users; (3) medically under-served low income populations, including high-risk racial or ethnic minority populations, especially blacks, Hispanics, and Native Americans; (4) residents of long-term care facilities (correctional institutions, nursing homes, mental institutions etc); (5) persons with medical conditions that have been reported to increase the risk of TB. These medical risk factors include silicosis, gastrectomy, jejunoileal bypass, being 10% or more below ideal body weight, chronic renal failure, diabetes mellitus, high dose corticosteroid and other immunosuppressive therapy, some hematologic disorders (e.g. leukemias and lymphomas), and other malignancies; and (6) In addition to the groups listed above, public health officials should be alert for other high-risk populations in their communities. For example, it may be possible to identify certain geographically or sociodemographically defined groups that have a higher prevalence of TB. Also, depending on local circumstances, employees in facilities where a person with disease would pose a hazard to large numbers of susceptible persons (health care facilities, schools, child care facilities, etc.) may be considered positive at greater than or equal to 10 mm induration.

A reaction of greater than or equal to 15 mm is classified as positive in all other persons. For each of the above categories, reactions below the cutting point are considered negative.

2.2.7 Previous Vaccination with BCG

There is no reliable method of distinguishing tuberculin reactions caused by vaccination with BCG from those caused by natural mycobacterial infections. Therefore, it is usually prudent to consider large reactions to PPD tuberculin in BCG-vaccinated persons as indicating infection with *M. tuberculosis*, especially among persons from countries with a

high prevalence of TB (American Thoracic Society, 1990). There are several reasons for not assuming that a large reaction to tuberculin is due to BCG vaccination: (1) tuberculin test conversion rates after vaccination may be much less than 100%, (2) the mean reaction size among vaccinees is often < 10 mm, and (3) tuberculin sensitivity tends to wane after vaccination. The interpretation of the tuberculin skin test may be difficult in a person who has received BCG. Comstock *et al.*, (1981) showed that 8 – 15 years after BCG vaccination, 16% of person who had skin test result greater than 10 mm compared to 2% of controls. Menzies and Vissandje (1992) evaluated tuberculin reactivity in 1511 schoolchildren and young adults in Montreal in whom BCG was administered 10 – 25 years earlier. Among 1041 persons vaccinated once in infancy, only 7.9% had significant tuberculin reaction more than 10 mm, and this was not different from controls when adjusted for socioeconomic status. Because many BCG-vaccinated persons tend to come from areas of the world where transmission frequently occurs, it is important that previously vaccinated persons with significant reactions to a tuberculin skin test be evaluated for presence of disease and managed accordingly.

2.2.8 Use of the Tuberculin Test

The Mantoux test may be used as a diagnostic aid to detect tuberculous infection and to determine the prevalence of infection in groups of people. Furthermore, patterns of reactions to the test are useful in establishing priorities for follow-up and preventive therapy with isoniazid. Studies from many parts of the world have shown that there is a tendency for persons who have larger tuberculin reactions to be at greater risk of developing TB, probably because large reactions almost always represent infection with *M. tuberculosis*, whereas small reactions represent a mixture of tuberculous infections and other mycobacterial infections (Gottlieb and Pauker, 1989).

2.2.9 Diagnostic Aid of Tuberculin Test

A positive reaction to a Mantoux test demonstrates that hypersensitivity to mycobacteria has developed. A positive reaction to the skin test does not necessarily signify the presence of disease. Sensitivity develops in 2 to 10 week after initial infection with *M. tuberculosis*. Once acquired, sensitivity to tuberculin tends to persist, although it may wane with time. Tuberculin testing is useful in the evaluation of patients clinically suspected of having TB, a positive reaction supporting the diagnosis. A negative reaction makes TB somewhat less likely, although caution must be exercised in the presence of possible anergy, especially in the presence of severe clinical illness or disseminated TB. In elderly patients, it may be useful to repeat the test after 1 week, thus capitalizing on the booster phenomenon.

2.2.10 Detection of Previously Infected Persons

The Mantoux test is used to identify infected persons who might benefit from preventive therapy. It may also be used as the initial step in screening apparently well persons for tuberculous disease. Its value for this purpose increases as the prevalence of tuberculin sensitivity in the screened population decreases. When only a few are infected (as is the situation in most of the United States), initial tuberculin testing used in place of radiographic screening can lead to important reductions in expense and radiation exposure.

2.2.11 Detection of Newly Infected Persons

The tuberculin test can be especially valuable when repeated periodically in surveillance of tuberculin-negative persons likely to be exposed to TB. However, there are special problems in identifying newly infected persons. First, there are unavoidable errors in even the most carefully performed tests. For this reason, small increases in reaction size may not be meaningful. For all persons younger than 35 year of age whose previous reaction was negative, an increase in reaction size of 10 mm or more in diameter within a period of 2 years would be considered a skin test conversion. For those older than 35 years of age, an increase of 15 mm or more is considered a positive conversion, and they should be considered newly infected with *M. tuberculosis* and strongly considered for preventive therapy.

A second problem in identifying newly infected persons is the so-called booster phenomenon. Repeated testing of uninfected persons does not sensitize them to tuberculin. However, delayed hypersensitivity to tuberculin, once it has been established by infection with any species of mycobacteria or by BCG vaccination, may gradually wane over the years, resulting in reactions that are negative. The stimulus of this test may recall the hypersensitivity, which results in an increase in the size of the reaction to a subsequent test; sometimes causing an apparent conversion that is interpreted as indicating new infection. Although the booster phenomenon may occur at any age, boosting increases with age and is most frequently encountered among persons older than 55 years of age. The booster effect can be seen on a second test done as soon as a week after the initial stimulating test and can persist for a year and perhaps longer (Bass and Serio, 1981).