CLINICAL CHARACTERISTICS OF SPUTUM SMEAR NEGATIVE PULMONARY TUBERCULOSIS:

The association with HIV infection

By

Dr Hedwiga Francis Swai

A dissertation submitted in partial fulfilment of the requirements for the degree of Master of Medicine (Internal Medicine) of the University of the Dar-es-salaam.

University of Dar-es-salaam
July 2002

COLLEGE LIBRARY
MUHIMBILI UNIVERSITY
COLLEGE OF HEALTH SCIENCES

DECLARATION
AND
COPYRIGHT

I Dr Hedwiga Swai, declare that to the best of my knowledge this dissertation is my own original work and has not been submitted at any university for award of any degree.

Signature..... H Swai
26/3/02

This dissertation is copyright material protected under the Berne Convention, the Copyright Act 1996 and other international and national enactments, in that behalf, on intellectual property. It may not be reproduced by any means, in full or in part, except for short extracts in fair dealing, for research or private study, critical scholarly review or discourse with an acknowledgement,without written permission of the Directorate of Postgraduate Studies, on behalf of both the author and the University of Dar-es-salaam.

Acknowledgements

I wish to express my gratitude to my supervisor Dr Ferdinand Mugusi for supervising me throughout the project with his unlimited enthusiasm. His creativity, encouragement and patience have been invaluable for this work.

I also wish to thank my supervisor Prof Odd Morkve for his unlimited effort to make this work real despite geographical distance.

I owe many thanks to Prof Matuja (Head of Internal Medicine Department) for his motivation, and his ultimate guidance to make sure that we are ahead of time in the writing of this work.

I am very grateful to Dr Mgonda (My Academic supervisor) for his meticulous advice through out this course.

I would like to thank very much the entire teaching staffs for all their efforts to facilitate my learning.

I wish to extend my thanks to all my patients, nursing staff and doctors in the medical wards and the outpatients' clinics who provide me with necessary co-operation to enable me complete this work.

I am also thankfully to Dr Kazema and Dr Kimaro who volunteered to read all radiographs.

I owe my gratitude to Dr Makwaya, Dr Merkioyory Masatu and Mr Kazaura for their guidance in statistical analysis.

I owe my gratitude to Dr Adeline Moshi, Mrs Candida Masanja and Mr Stanley Morgan for their skillful advice.

Many thanks should also go to Dr Egwaga (ministry of healthy) who assisted in getting the mantoux reagent.

I am thankful to Dr Kundaeli John (Muhimbili NIMRI station), Mr Chonde, Mr Ngowi and Mr Edward of tubrculosis reference laboratory who facilitated sputum culture to be done.

Last but not list I wish to thank the laboratory technicians for their co-operation. Mrs V. Nalyanga and Mr shemagoda of Mwaisella annex, Mrs J Mlalasi of clinical research laboratory and Mr Sufi of of the Department of immunology and microbiology.

Finally I would specially thank my sisters Idda and Juliana for every thing they have done to me. They have helped me emotionally to cope with the stress of school.

DEDICATION

Dedication to my family for its sacrifice, my husband Mr Gasper Paradiso Urassa for his kindness, tolerance and everlasting support, despite my little participation in the family. His naturally positivity and courageous way of thinking has helped me to see the good side of my tiresome days. My children Rachael, Felista, Goodluck and Francis for missing me most of the time during this very tiring period.

Dr Hedwiga Swai.

Abstract

Background: There have been a massive increase of Pulmonary Tuberculosis(PTB) that has threatened to undermine achievements that had been gained by National Tuberculosis Control programs(NTLP) in most Sub-Saharan Africa. This increase is due to widespread of infection with Human immunodeficiency virus (HIV). The diagnosis of PTB in HIV infection is made difficult in the increased presence of sputum smear negative tuberculosis.

Objectives: To determine clinical and laboratory features of smear negative but culture positive PTB and the relationship of these features to HIV-1 sero-status.

Methodology:

Study site: The study was carried out at Muhimbili National Hospital and outpatient Tuberculosis clinics of IDC, Ilala and Mwananyamala, in Dar-es-salaam Tanzania.

Study subjects: Male and female patients aged 18 to 75 years who presented with more than two weeks history of cough, but had three negative sputum smears for AFB were recruited in the study.

Procedures: For patients with sputum smear negative, sputum was sent for culture. Counseling and HIV testing was done to all consenting recruited patients. A chest X-ray, mantoux test, and FBP were done to each patient. Those found to have features highly suggestive of PTB received treatment according to the NTLP guidelines. The remaining received a broad-spectrum antibiotic.

Results: 467 fulfilled the inclusion criteria, 127(27.2%) had sputum culture positive for Mycobacterium tuberculosis. Of the 127sputum culture positive subjects, 66(51.9%) were correctly treated with anti-TB and 61(48.1%) were missed and did not get anti TB. Of the 286 subjects' who had sputum culture negative, 107(37.4%) were wrongly treated with anti-TB and 179(62.6%) received antibiotics. HIV perse is not a predictor of culture positive.
The presence of a dry cough, a high respiratory rate, a low eosinophil count, a mixed type of anaemia and presence of a cavity were found to be predictive of smear negative but culture positive PTB

Conclusion: A third of patients who are investigated for PTB and found to have sputum smear negative results have active disease when sputum culture is done. The current practiced procedures of establishing PTB diagnosis are not sensitive enough to establish the correct diagnosis of TB. They under diagnose PTB and over treat people with no PTB.

List of abbreviations

PTB	Pulmonary Tuberculosis
HIV	Human Immunodeficiency Virus
AIDS	Acquired Immunodeficiency Syndrome
TB	Tuberculosis
NTLP	National tuberculosis Control Programme
FBP	Full Blood Picture
PA	Posterior anterior
MTB	Mycobacteria tuberculosis
NTM	Non tuberculous mycobacteria.
WHO	World health Organization
BMI	Body mass Index
PPD	Purified Protein Derivative
BT	Blood transfusion
Prev pulm dis	Previous pulmonary diseases

LIST OF TABLES

No	Title	page
1	Distribution of the study population by Age, sex and HIV sero-status	46
2a	Demographic characteristics of the study population by sputum culture results	47
2b	Demographic characteristics of the study population by sputum culture results and HIV status	48
3a	Symptoms of the study subjects at the time of interview by their sputum culture results	50
3b	Symptoms of the study subjects at the time of interview by their sputum culture results and HIV sero-status	51
4	Past medical history and health seeking behavior by their sputum culture results and HIV status	52
5a	Physical signs of the study subjects by their sputum culture results	54
5b	Physical signs of the study subjects by their sputum culture results and HIV sero-status	55
6a	Laboratory features of the study population by patients' sputum culture results	57
6b	Laboratory features of the study population by patients' sputum culture results and HIV sero-status	58
7a	Chest radiological findings of patients by their sputun culture results	60
7b	Chest radiological findings of patients by their sputun culture results and HIV serostatus	61
8	Diagnosis made using culture results and Treatment given	63
9	Sensitivity and specificity of the diagnostic procedure of patients with smear negative	63
10	Diagnosis made and Treatment given by subjects' sputum culture results and HIV serostatus	64
11	Un adjusted bivariate logistic regression results of predictors of sputum culture positive	65
12	Multivariate analysis of clinical characteristics of smear negative culture positive PTB	66

LIST OF FIGURES

No	Title	page
1	Recruited study subjects	45
2	Distribution of HIV sero-status by sex among study subjects	46
3	HIV- sero-status by culture results	56
4	Diagnosis and treatment of study subjects in relation to their sputum culture results	62

Table of contents

Title page..... i
Certification..... ii
Declaration and Copyright..... iii
Aknowledgement..... iv
Dedication..... V
Abstract..... Vi
List of abbreviations..... Vii
List of tables..... Viii
List of figure..... ix
1. Introduction..... 1
1.1 history of TB..... 1
1.2 Aetiology 2
1.3 Microbiology of mycobacterium..... 2
1.4 Mode of infection-Immunology of TB..... 3
1.5 Transmission 8
1.6 Pathogenesis..... 10
1.7 Host response to MTB infection..... 12
1.8 Clinical presentation of TB..... 15
1.9 Diagnosis of tuberculosis 16
1.10 Treatment..... 27
1.11Burden of TB..... 30
1.12Burden of HIV 31
1.13HIV infection and Tuberculosis 32
2. Study questions..... 34
3. Problem statement..... 34
4. Rationale of the study..... 36
5. Objectives..... 37
6. Methodology..... 37
7. Hypothesis..... 42
8. Results..... 43
9. Discussion..... 67
10. Conclusion..... 74
11. Recommendations..... 75
12. References..... 76
13. Appendix 1..... 88
14. Appendix 2..... 93
15. Appendix 3..... 94
16. Appendix 4..... 95

1. INTRODUCTION AND LITERATURE REVIEW

1.1 HISTORY OF TB.

Although tuberculosis (TB) was decimating tribes of American Indians in the period between 1880 and 1910, it was practically unknown in most of Sub-Saharan Africa and was uncommon in areas of North Africa during this time.[1] Hirsch reported in 1860 that the native population of an Algerian city of 25,000 had been free of tuberculosis for a period of at least 8 years.[1] Many medical observers reported the complete absence of tuberculosis in the interior of Sub-Saharan Africa. Cummins, reporting his own and other army medical officers experience noted that tuberculosis was almost unknown in those parts of Africa in which an influx of Europeans had not yet occurred.[2] Liechtenstein and Livingston found no tuberculosis in parts of South Africa as late as the 1st half of the nineteenth century[3, 4] and Graybowski estimates that tuberculosis could not have reached South Africa until about 1910.[5]

Tuberculosis was rare among Africans who lived in small remote villages in which conditions for air borne transmission of the tubercle bacillus were unfavorable.[6] As Europeans moved in, in great numbers to many areas of South and Sub-Saharan Africa, tuberculosis began to appear with increasing frequency among the Africans. Millar reported on the beginning of the spread of tuberculosis in South Africa in 1908, By the next decade, tuberculosis was recognized to be taking a heavy toll among black men working in the mines.[7] The epidemic curve of tuberculosis morbidity and mortality probably had not reached the peak in Africa before it was accelerated sharply by the advent of HIV infection in the mid 1980's.

1.2 AETIOLOGY

Tuberculosis is caused by bacteria Mycobacterium tuberculosis (MTB). This is a droplet infection, transmitted from an infectious source to susceptible persons primarily through the air (e.g. through coughing). [8]

Several types of Mycobacteria are responsible for pulmonary tuberculosis

(a) Mycobacterium tuberculosis complex that includes

- Mycobacterium tuberculosis – that causes most of infection
- Mycobacterium Bovis – that is endemic in cattle and is spread to humans by milk.[9]

(b) Mycobacterium avium, Mycobacterium intracellulare, and Mycobacterium scrofulaceum (MAIS complex) can be isolated from tap water and animal tissues. MAIS used to be a rare cause of disease but the incidence is increasing in AIDS and other immuno-suppressed patients.[9] In Cleveland, USA, in 1985 isolates of the MAIS complex outnumbered those of Mycobacterium tuberculosis.[9]

1.3 Microbiology of mycobacterium tuberculosis

Characteristics of mycobacteria tuberculosis.

Taxonomy: The families Mycobacteriaceae, along with Actinomycetales, Nocardaceae, Streptomycetaceae, and other bacterial families, belong to the order of Actinmycetales.[10] There are 8 different types of cell wall structures in this taxon. The mycobacteria cell wall consists mainly of mesodiaminopimelic acid, arabinol, and galactose. Mycolic acid, an alpha-branched beta-hydroxyl fatty acid, is an important taxonomic compound of mycobacteria.

Morphology: They are rods, occasionally filaments, and have no aerial mycelium.

Rate of growth: Time for visible colonies: 2-40 days. It is not easily lysed by lysosomes. Fifty-four different species of mycobacteria are described.[10] 26 slowly growing and 28 relatively rapid growing species. For practical purposes, slow growers are those that require more than 7 days of incubation to produce easily seen colonies from a standardized inoculum. Rapid growers are seen in less than 7 days.[10]

(b) Other mycobacterium, that can cause tuberculosis, include Mycobacterium bovis, which is endemic in cattle, and spread to human by milk.[11]

(c) Non-tuberculous mycobacteria: NTM unlike tuberculosis, these organisms are normal inhabitant, of the environment. Also unlike tuberculosis, pulmonary diseases and other infections due to the NTM are not contagious so patients with disease are not generally isolated. The species are: M. avium intracellulare, M. kansasii., M. marinum, M. fortuitum, M. chelonae , M. abscessus, M. malmoense, M. asiaticum, M. xenopi, M. simiae, M. scrofulaceum, M. nonchromogenicum, M. smegmatis, M. peregrinum, M. chelonae-like organism (MCLO), M. fortuitum third biovariant complex, M.szulgai, M.haemophilum, and M. ulcerans.[11]

1.4 Mode of infection - immunology of tuberculosis.

Immune responses in tuberculosis may be either protective, leading to resolution of disease, or tissue destroying, leading to pathological characteristics of active disease. Both types of response are cell mediated and there has been considerable controversy about whether protective and immunopathological responses are a manifestation of the same mechanisms. There is evidence that protective immune responses are

induced by antigens that are common to all mycobacterial species, while antigens restricted to tubercle bacilli are responsible for immunopathological reactions.[12]

M. tuberculosis is the prototype of an infectious agent requiring a cellular immune response for containment as well as inducing a delayed - type hypersensitivity response. Persons with defective cell mediated immunity (e.g. patient with human immunodeficiency virus (HIV) infection or chronic renal failures) are at higher risk for tuberculosis.[13] Tuberculosis is a major opportunistic infection of HIV infected persons. Tuberculosis occurs in as many as half of HIV patients. (There is high prevalence of HIV among TB patients and the reverse is true i.e. there is high prevalence of TB in HIV.[14] Infection with the human immunodeficiency virus (HIV) has transformed tuberculosis (TB) from an endemic disease into a worldwide epidemic. Early in the course of AIDS epidemic, the link between HIV and TB was not clearly recognized. However in 1994 the global program on AIDS of the World Health Organization (WHO) estimated that the prevalence of HIV infection among adults worldwide was between 13 and 14 million.[15]At the same time about 1.75 billion people (i.e. one third of the total human population were infected with Mycobacterium tuberculosis.[16, 17] Approximately 4 to 5.6 million people are co-infected world wide.[15, 18,19]

Most infected persons do not experience clinical illness, but are usually asymptomatic and non-infectious. The only evidence of infection may be a reaction to a tuberculin skin test. However infection can persist for years, and an infected person can remain at risk of developing clinical tuberculosis especially if the immune system becomes impaired.[20]

Rates of HIV seroprevalence among patients with newly diagnosed TB range from 9 to 75%.[21] In comparison with non HIV infected persons, it appears that those with HIV infection have increased susceptibility to tuberculosis infection after exposure to tubercle bacilli,[22] which means that their innate defenses are impaired, and that is clear that tuberculosis infection, whether old or recent, in HIV infected patients is highly likely to progress to clinically active disease as cell medicated and other immunologic deficiencies worsen.[16] HIV infection is the greatest risk factor ever identified that favors the progression of tuberculosis infection to tuberculosis disease. [17] M. tuberculosis grows aggressively in the absence of a cellular immune response and patients often die before laboratory confirmation by culture of organisms is available.[18,23]

Lymphocytes and Monocytes, the primary defence cell mustered versus tuberculosis infection are destroyed by HIV, [24] on the other hand TB facilitate multiplication of HIV virus because TB is a strong immune stimulator, as the immune cells in the body multiply the viral load also increase.

Data of 1988 by WHO shows that, in developing countries from 1% to 4% of the population are newly infected with MTb (or re-infected) each year, and both prevalence and risk of infection are still high, case finding is inadequate, case holding is often very poor and coverage of the population is very incomplete.[25]

CD4+ T lymphocytes and macrophages play a central role in the immune response to mycobacterial infection. HIV infection results in progressive depletion and

dysfunction of CD4+cells with defects in macrophage and monocyte function.[26] As a result, HIV infected patients have an increased risk of reactivation of latent TB as well as disease from new infection.[27] When CD4 cells falls below 500 cell/ml, TB will occur and there may be cavities, fibrosis and positive sputum smear. When CD4 cells is low (bellow 200 cells/ml) tuberculosis present as primary tuberculosis i.e. with enlarged lymphnodes, consolidation of the whole lung and sputum smear negative. In severe immunosuppression there are no cavities and sputum smear for AFB may be negative.[28]

Macrophage - based resistance against TB is due to its ability to control intracellular mycobacterial growth. CD4+ lymphocytes prime macrophage to control intracellular growth by releasing cytokines such as interferon, interleuken (IL)-1 and interluken-2, and tumour necrosis factor-alpha (TNF-), which activates blood borne macrophages.[29] An additional protective mechanism may be the generation of cytolitic T cells in response to mycobacteria.[30] Risk of developing TB is increased if one is co-infected with HIV and TB. In HIV positive patients infected with TB the chance of developing TB disease increase yearly by 9%. The interaction between TB and HIV may increase viral burden, accelerating progression to advanced stages of HIV disease. Tuberculosis has many clinical features similar to those of AIDS and is strongly associated with HIV infection, being one of the most important opportunistic diseases in AIDS patients. (Rechman 1988,WHO 1989). Two of the three major criteria for AIDS, severe weight loss and severe weakness are also major symptoms for tuberculosis. Among the frequent minor criteria of AIDS, fever and

chronic cough are found in TB patients as well. It is clear that both diseases should be considered whenever the physician thinks of one of them.[31]

In a study of 114 patients with PTB confirmed by culture in Zambia 76% of HIV negative patients had a positive sputum smear, compared with 57% of HIV positive patients.[32] With the wide spread of the HIV epidemic, extra-pulmonary tuberculosis is reported to be increasing. However, at present pulmonary tuberculosis remains the most frequent type of tuberculosis in patients infected with HIV occurring in 42-80% of cases in Africa. [33,34] A study done by Moshi in 1994, Dar es Salaam Tanzania [35] pulmonary tuberculosis was still the commonest presentation of tuberculosis in HIV sero-positive patients occurring in 74% of cases. Symptoms of tuberculosis in HIV sero-positive patients are similar to those in HIV seronegative patients, but symptoms such as night sweats and fever can be due to HIV disease itself.[35, 36]

About 85% of HIV seropositive TB patients from Africa have already experienced a significant weight loss (often more than10 kg or 20%of body weight) when they are diagnosed with tuberculosis.[21,37,38] A study done in Zaire by Mukadi et al demonstrated that weight loss and fever were more marked in HIV seropositive tuberculosis patients than HIV sero-negative.[39] Half of the cases had fever for more than one month.[38] Half of sero-positive patients have chronic diarrhea chest pain and dyspnoea.[34,38,40] Other common features accompaning the presentation of HIV associated tuberculosis in Africa include oral candidiasis,[38,40] generalized lymphadenopathy [38,40] and a recent history of herpes zoster.[34,38] All three are uncommon in the seronegative patients and therefore are valuable indicators of

concurrent HIV infection. Cough is a symptom reported less frequently in seropositive patients than in seronegative patients,[37, 38,40] probably owing to decreased local inflammation with less cavitation and end bronchial irritation. Haemoptysis which results from caseous necrosis of the bronchial arteries inside cavities is reported to be less common in sero-positive patients having been described in no more than 15% of cases as opposed to at least 20% of seronegative patients.[37]

1.5 TRANSMISSION

Tubercle bacilli may be transmitted from one person to another as an infectious aerosol, in contaminated milk, in milk products or by direct inoculation. Of these by far the most important transmission is by the air bone route. Sharing the same breathing space with an infectious patient is clearly the most important risk factor for acquiring infection.[41]

Virtually all spread of M. tuberculosis in a community is caused by patients with pulmonary tuberculosis as opposed to those with extra-pulmonary involvement; those whose sputum contains acid-fast bacilli on direct microscopic examination (i.e. are smear positive) and particularly those who are undiagnosed and hence untreated. These patients who usually present about half of all newly discovered cases of tuberculosis, are chief targets of tuberculosis control programs. Identifying and treating patients with smear positive tuberculosis is the quickest and most efficient way of breaking the chain of transmission of the disease.

Tubercle bacilli in respiratory secretions forms nuclei for liquid droplets expelled during coughing, sneezing and vocalizing. Droplets evaporate within a short distance from the mouth and thereafter desiccated bacilli remains air bone for long periods. Infection of a susceptible host occurs when a few of these bacilli are inhaled.[24] Factors that enhance transmission of the mycobacteria causing infection relate to the concentration in the droplets, viability and virulence of the organism and how effectively droplet nuclei are produced.[42] Environmental factors affect the concentration in the air and the viability of the organisms in the dropped nuclei. M. tuberculosis does not survive in dry environment; sunlight and ultraviolet light destroy organisms as well.[42] Household contact of many months is required for transmission. However patients with laryngeal tuberculosis endo-bronchial and extensive cavitary pulmonary disease are highly contagious. Infectiousness also correlates to the extent of pulmonary disease and frequency of cough.[24]

Adequate ventilation is the most important measure to reduce the infectiousness of the environment. Most patients become non-infectious within 2 weeks after the institution of appropriate chemotherapy because of the decrease in cough.

Myobacteria bovis is transmitted if one drinks unpasteurized milk. A rare form of transmission of tuberculosis by Bacillus Calmette–Guérin (BCG) has been documented. BCG is a modified strain of M.bovis, which has been used as a vaccine to prevent tuberculosis for approximately 75 years.[43] Another potential but rare form of BCG transmission has also been documented in immuno-compromised HIV–

infected individuals who received BCG immunization as part of a TB prevention program.[44, 45]

M. Africanum causes a form of infection that is similar to that caused by M.tuberculosis or M.bovis.[46, 47] Although there is no scientific documentation of the method of transmission of this bacillus, most evidence indicates that M. Africanum is a highly transmissible, pathogenic organism.[48]

1.6. PATHOGENESIS

Initial primary tuberculosis infection usually occurs in the lung but occasionally this may occur in the tonsil or alimentary tract, especially the ileo-caecal region. Inhaled droplets are so small that they can avoid the mucocilliary defenses of the bronchi and lodge in the terminal alveoli of the lungs. Infection begins with multiplication of tubercle bacilli in the lungs. This is the Ghon focus. Lymphatics drain the bacilli to the hilar lymph nodes. The ghon focus and the related hilar lymph nodes form a primary complex. In Most people, the primary infection and the associated lymphnode lesion heal and calcify. In a few, healing particularly in lymphnodes is incomplete and viable tubercle bacilli may enter the blood stream.[9] This can result to a hematogenous spread in the bones, joints and kidneys. Occasionally the node may ulcerate through the bronchial wall and discharge caseous material into the lumen, with production of acute tuberculous lesion in related lobe or segment. Infection may also be carried by lymphatics from tuberculous mediastinal lymphnode to pleural or pericardium with production of tuberculous pleurisy or pericarditis. Rarely caseous tuberculosis focus ruptures into a vein and produces acute

dissemination throughout the body, a condition known as acute miliary tuberculosis. Meningitis often complicates this condition.

The immune response (delayed hypersensitivity and cellular immunity) develops about 4-6 weeks after primary infection.[49] The size of the infecting dose of bacilli and strength of the immune response determine what happens next. In most cases the immune response stops the multiplication of bacilli, however few, dormant bacilli may persist.[49]A positive tuberculin skin test remains the only evidence of infection. The immune response in a few cases is not strong enough to prevent multiplication of bacilli and disease occurs within a few months.[49]

Post primary tuberculosis occurs after a latent period of months or years after primary infections. It may occur either by reactivation or by re-infection. Characteristics of post-primary pulmonary tuberculosis seen in the lungs include: -

1. Extensive lung destruction with cavitation
2. Positive sputum smear
3. Upper lobe involvement
4. Usually no intrathoracic lymphadenopathy. [49]

1.7. HOST RESPONSE TO MYCOBACTERIA TUBERCULOSIS INFECTION

a- Phagocytosis of M. tuberculosis: Mycobacteria tuberculosis can infect a host and then be immediately eliminated (no infection), or become dormant indefinitely inside the host (latent infection), or cause disease soon (primary tuberculosis) or many years later (reactivation tuberculosis) after infection. In only 5-10% of infected persons who have no underlying immunosuppressive disease, will the organism proliferate and cause active disease sometime in the host's life time.[50, 51]

Phagocytosis of M. tuberculosis by mononuclear cells is the first event in the host-pathogen relationship that leads to either latent infection or active disease. Most pathogens enter mammalian cells through receptor -ligand-based interaction and there may be more than one pathway of entry into the cells.

Phagocytosis of M. tuberculosis by human monocytes derived macrophages is mediated by compliment receptor CRI and CR3 after opsonizatation by compliment C3 component.[52] CR4 is also involved.[53] Virulent and avirulent strains of M.tuberculosis may have different pathways of entry, which may influence their survival inside macrophages. In addition to macrophage-directed phagocytosi, organisms themselves can initiate their uptake into mammalian cells. The pathways of entry are: airway route, drinking unpasteurized milk, transcutaneously, congenital and through organ transplant. [54,55,56]

b- Post-phagocytosis: (Phagosome-lysosome fusion) -Most microorganisms, on encountering a macrophage, become ingested and enclosed inside membrane - bound vacuoles or phagosomes. They are then digested by lysosomal enzymes, which are released into the phagosomes after their fusion with lysomes. The survival and growth of M.tuberculosis inside macrophage vary according to strain.[57]

c- Escape from phagosomes is another mechanisms by which pathogens avoid the anti-microbial effects of phagolysosomes are for them to escape the phagosomes.[58]M. tuberculosis H37RV strain has been observed to bud out of fused vacuoles in vesicle and sometimes remains free in the cytoplasm of macrophage.[59]

d- Resistance to lysosomal enzymes: Microbes that remain viable inside fused phagosomes have developed mechanisms either to prevent acidification, to elaborate products that inhibits or inactivate the lysosomal enzymes, or to develop enzyme-resistant surface structure.[58] M. tuberculosis appears to be able to remain viable inside fused phagosomes, suggesting that it is able to resist the contents of lysosomes. Even dead tubercle bacilli can be resistant to degradation by lysosomes.[60]

e- Resistance to oxidants (killing mechanism). Human macrophages can generate superoxide, hydrogen peroxide and hydroxyl radicals, which have microbicidal effect.[61, 62] Although there is evidence that those 0_2 intermediates contribute to mycobacteria killing, their role in killing the phagocytosed tubercle bacilli remain un-established.

f- Cytokines productions in tuberculosis When macrophages ingest mycobacterium tuberculosis, they produce cytokines: IL - 1, IL - 6, IL - 10, tumour necrosis factor-alpha (TFN- α) and probably IL-12.[63, 64] These cytokines have the potential to exert potent immunoregulatory effects and to mediate many of the clinical manifestation of tuberculosis.

g- Immune defenses against tuberculosis: Host defenses against M. tuberculosis depend on the cell-mediated immune system rather than humoral immunity. Persons with defective cell mediated immunity such as those with human immunodeficiency virus (HIV) infection and chronic renal failure are at markedly increased risk for tuberculosis.[65] Persons with defective humoral immunity, such as those with sickle cell disease and multiple myeloma, show no increased predisposition to tuberculosis infection. Primarily, T-lymphocytes and macrophages mediate anti-mycobacterial immune defenses, and adoptive transfer of resistance against tuberculosis in animal model is mediated by T-cells.[66]

h- CD4+ T lymphocytes: Sensitized CD4+ T-cells are readily detectable in humans exposed to mycobacterium, as evidenced by in vitro blastogenesis in response to mycobacterial antigens and by tuberculin hypersensitivity in vivo. Both are mediated by CD4+ T cell and are vigorous in individuals who have resolved infection and in patients with mild pathology. CD4+ T-cells plays a central role in the resistance to infection with mycobacterium tuberculosis. T-cell responsiveness correlates inversely with disease progress in terms of both low blastogenic responses to mycobacterial antigen in vitro [67] and reduced or absent skin tuberculin hypersensitivity[68] among patients with advanced or uncontrolled disease, also extreme susceptibility of HIV-infected individuals to tuberculosis.[69]

The fact that HIV infected individuals contract the disease before any other opportunistic infection and before their CD4+ T-cells count has appreciably diminished, suggest that T-cell populations is so central that even slight disturbance can upset the finely balanced immunity to mycobacterium tuberculosis.

1.8. CLINICAL PRESENTATION OF TUBERCULOSIS

Primary tuberculosis is asymptomatic and there is non-specific pneumonitis and hilar lymphnode enlargement. The initial lesions of tuberculosis develop before specific cell mediated immune reactions develop to contain the infection.[12]Dissemination to the regional lymph nodes causes the primary complex. Necrosis is inconspicuous or absent and, except in rare case of progressive primary disease, pulmonary cavitation is not seen. Asymptomatic cases may be found on routine examination. In other cases the symptoms include cough, sometime with haemoptysis, fever, decreased appetite and in children, failure to thrive. Signs include wheezes due to bronchial compression by enlarged lymphnodes and those of pneumonia secondary to lobar collapse. Immediate complications may include bronchopneumonia, pleural effusion and disseminated (milliary) disease.[12] Late complications are bronchioectasis and post primary tuberculosis.

Post primary This is mainly due to reactivation of old TB infection. The onset of the disease is insidious, patients may present with non-specific symptoms of fever, chills and night sweats, as well as easy fatigability, loss of appetite and weight loss.[70] Specific respiratory symptoms include; prolonged productive cough, chest pain and

hemoptysis (coughing up blood). Tuberculosis due to re-activation has a predilection for apical segments of upper lobes and superior segments of lower lobes of the lungs. Pulmonary cavities are a major source of bleeding. Another source of bleeding is aspergilloma in a chronic tuberculous cavity and bleeding in this instance may occur without persisting tuberculous disease. Rupture of tuberculous cavity into the pleural space may lead to tuberculous empyema and bronchopleural fistula.[70]

Clinical presentation in HIV positive patients

The clinical presentation of tuberculosis in HIV patients positive patients are similar to those of HIV negative patients but symptoms like night sweat and fever can be due to HIV itself.[35] Clinical presentation depends on the extent of the degree of immunosupression. If early immunosuppression they will present as post primary TB, and in late stages of HIV the presentations are atypical with smear negative and negative tuberculin skin test.[36] Weight loss and fever are more marked in HIV positive patients than HIV negative patients. HIV patients also present with diarrhea, chest pain, and dyspnoea.[34, 38,40] The accompanying presentations of HIV associated tuberculosis are oral candidiasis and generalized lymphadenopathy.[34, 38]

1.9 DIAGNOSIS OF TUBERCULOSIS

1.9.1 PPD SKIN TEST

In the Mantoux test, 0.1ml of solution containing a known number of international units (TU) of purified protein derivative (PPD) of tuberculin is injected intradermally in the forearm. Reaction is read by measuring the transverse diameter of indurations as detected by gentle palpation at 48 to 72 hours. Persons with previous positive skin

test results, documented history of tuberculosis disease or reported history of severe necrotic reaction of tuberculin should be exempted from routine tuberculin skin test screening.[70]. Neither pregnancy, lactation, nor previous vaccination with bacillus of calmete and Guerin (BCG) vaccine are contraindications for tuberculin skin testing. Tuberculin skin test is interpreted as follows: -

1- Indurations of $\geq$ 5mm is positive in HIV patients and in patients who are in close contacts with persons who have tuberculosis.
2- Reaction of $\geq$ 10mm is positive in person from population groups of elevated risk of tuberculosis
3- Reaction $\geq$ 15 mm is positive in persons from low risk general population.

Repeated skin test may boost reaction size. However repeated skin test with PPD does not lead to positive reaction in uninfected persons. Tuberculin skin tests results become negative as CD4 decline. HIV infected persons who are infected with M. tuberculosis and who do not react to tuberculin (PPD), is said to be anergic. He may be evaluated for skin test anergy by administering at least two antigens other than tuberculin (e.g. tetanus toxoid, mumps or candida), [70] however scientific basis for anergy testing is not well established. The results from supplemental anergy testing, in conjunction with PPD tuberculin skin testing, have been interpreted in two ways. A positive response to other antigens with a negative PPD test has been interpreted as evidence that the negative PPD test result is a true negative and the person tested is not infected with mycobacteria tuberculosis. Lack of response to other antigens in conjunction with a negative PPD skin test as evidence that a person is unable to mount a positive response to PPD even if infected with mycobacteria tuberculosis.[71]False positive PPD reactions may be caused by errors in administering

the test or in reading the results. However false positive results are commonly attributable to the presence in tuberculin antigens shared with other mycobacteria. False negative tuberculin skin test reaction may be caused by non-responsiveness to delayed type hypersensitivity.

1.9.2. Acid-fast smear: Though less sensitive than culture, the acid-fast smear is an essential adjunct to the diagnosis of tuberculosis. A specimen with a count of 10^4 colony-forming units (CFU)/ml is more likely to result in a positive smear while a negative smear is likely if the count is lower.[72] The acid-fast smear examination is specific, rapid and simple to perform. While a single smear of a respiratory specimen has a reported sensitivity of between only 22% and 43%, when multiple specimens are examined, the sensitivity rises as high as 96%. [73] The sensitivity of smears of other specimen sources (e.g. pleural fluid, urine, and ascitic fluid) however is not as great.[74]

While smears are an important adjunct to the detection of M. tuberculosis, they are not adequate criterion alone, and must be followed by culture. Three specimen of sputum can be stained by the Ziehl-Neelsen method and examined microscopically when bacilli are numerous, whereas auramine phenol fluorescent test is of value in detection of small number of bacill.[9]

Quality control of sputum smears procedures: A positive and negative control slide should be included with each run of stains. This will verify the correct performance of the procedure as well as the staining intensity of the acid-fast organisms. Control

slides should be reviewed before patient smears are read to confirm that mycobacteria stain acid fast. If the results of the quality control slides are acceptable, go on to the patient smears. If however the control slides are unacceptable, review the procedure and reagent preparations.[75]

1.9.3 Auramine phenol stain: This is a sensitive and preferred screening test. It is simple to perform and permits more rapid examination of a smear but requires fluorescent microscopy and not easy to perform out of hours. Less specific for mycobacteria than Ziehl- neelsen stain. It is usual to confirm results by overstaining with Ziehl-Neelsen. If the number of daily specimen to be examine exceed 30, and if electricity is continuously available, fluorescence microscopy might become more cost-effective. Fluorescence stain utilizes basically the same approach as Ziehl-Neelsen staining, but carbol fuschin is replaced by fluorescent dye, the acid for discoloration is gentler, and counterstain is not essential although useful to quench background fluorescence. Both sensitivity and specificity of fluorescent microscopy are comparable to the operating characteristics of Ziehl-Neelsen technique. The most important advantage of fluorescence staining is that slides can be examined at lower magnification, thus allowing the examination of a much larger area per unit time. In fluorescence microscopy, the same area that needs examination for 10 minutes with light microscopy might be examined in 2 minutes. Its disadvantage lies on the cost of capital investment.[75]

Quality control for Auramine phenol stain procedure: A positive and negative control slides should be included with each run of stain. This will verify the correct

performance of the procedure as well as the staining intensity of the acid-fast organisms. Control slides should be reviewed before the patient smears are read to confirm that the mycobacteria stain acid fast. If the results of the quality control are acceptable, go on to the patient's smears. If however the control slides are unacceptable, review procedure and reagent preparations. When the problem has been identified and corrected, remake and stain all the patients slides from the problem run along with a new set of control. [75]

WHO recommends that sputum should be collected 3 times. At the first visit spot specimen is collected; thereafter the patient is given a sputum container for collection of an early morning specimen (over night specimen) before his second visit. At the second visit the overnight specimen is delivered and a further spot specimen is collected.[76] In this way TB can be diagnosed within 2 days. Pulmonary tuberculosis is present when there are two positive results of sputum examinations or a positive culture report. If only one positive smear report is available and the other 2 are negative diagnosis is made basing on chest X-ray and clinical examination[76]. Broncho-alveolar lavage fluid, fasting gastric washings or laryngeal swab may be used for AFB smear and culture if patients cannot produce sputum or sputum culture when smears are negative.[9] Tubercle bacilli can also be identified in urine, body fluids or tissue of patients.[76]

1.9.4 SPUTUM CULTURE

A variety of laboratory media for the cultivation of mycobacteria have been described. The traditional components for such media include an egg and potato

base such as Middle brook 7H10 or 7H11, or albumin in an agar base such as Löwenstein-Jensen (L-J), Petragnani, or American Thoracic Society (ATS) media. These media are able to support the growth of even small numbers of organisms and allow for the determination of characteristic feature of colonial morphology, growth rate, and pigment production. Since there are advantages to each type of medium, and not all strain of mycobacteria can be recovered on a single substrate, it has been recommended that one representative of each substrate type be used for primary isolation.[73, 74,77] Without doubt the most popular medium in clinical laboratories is the Löwenstein Jensen Media. The use of liquid media such as Middle brook 7Hg is recommended for subcultures and in-vitro tests.

The usual methods of cultivation of mycobacteria take several weeks. Substantial improvement in the time of detection and total number of positive cultures can be done by the use of broth-based growth system such as BACTEC.[78] BACTEC is a machine, which uses radiometric liquid medium culture system. The BACTEC bottle contains a nutrient broth with a radioactive isotopic label C14 attached to the palmitic acid contained in the broth. If a mycobacterial cell is contained in the specimen, it will metabolize the palmitic acid releasing the isotopes as $C14O_2$, which is detected by the instrument. The rate and amount of $^{14}CO_2$ produced is directly proportion to the rate and amount of growth occurring in the medium. The radiometric technique can detect very small quantities of $^{14}CO_2$, which allows detection of mycobacterial growth at a very early stage. With the BACTEC system, the average time to the recovery of M. tuberculosis from smear positive specimen is 8 days, compared to 18 days for conventional media.[78] When culture positive, smear

negative samples were examined, the mean time to detection with the BACTEC was 14 days versus 26 days for conventional media.[79] Clearly, the BACTEC AFB system has become the standard against which others must be compared. However, since sample proceeding with the BACTEC 460 is not automated, multiple instruments may be required to maintain efficiency in laboratories with very high volumes. Another broth-based system is the recently introduced septic-check AFB (BBL), which combine broth and solid media into a single device. Septic check AFB contains 20 ml of modified Middle brook 7H9 broth in a carbon dioxide-enriched culture bottle. After inoculation of the sample the bottle is capped with a slide containing three solid media: a non-selection Middle brook 7H11 agar an egg based medium, and chocolate agar.

Limitations of BACTEC:

1.Colony morphology cannot be observed.

2.If even a single contaminating bacterium is present, the entire medium will be contaminated.

3.Mixed growth of more than one type of mycobacterium cannot be detected.

4.A subculture on a Middlebrook agar plate is needed to isolate a mixed culture.[79]

1.9.5.Serology tests: Enzyme linked immunosorbent (ELISA) technique.[24]

Serology tests are based on recognition of serum 1gG antibody to selected mycobacterial antigen.[24]

1.9.6.Polymerase chain reaction (PCR)- Gene amplification has been used with greatest specificity and sensitivity to identify mycobacteria DNA. It offers great promise for rapid diagnosis. This technique is capable of amplifying minute amounts

of DNA into millions of identical copies.[80] PCR is a way to make millions of identical copies of a specific DNA sequence, which may be a gene, or part of a gene, or simply a stretch of nucleotides with a known DNA sequence, the function which may be unknown. PCR is accomplished in the following way: A specimen that contains the DNA sequence of interest is heated to denature double stranded DNA. Then specific synthetic oligonucleotides, 'primers' (short single stranded pieces of DNA), bind to the unique DNA sequence of interest, and a heat-stable DNA polymerase extends the primer to create a complete and complimentary strand of DNA. This process is typically repeated sequentially 25 to 40 times, thereby creating millions of copies of the target DNA sequence. The amplified sequence can then be detected easily by gel electrophoresis. If the target DNA sequence is not present in the sample examined the primers have nothing to bind to, and no amplification occurs. This PCR offers potentially un matched sensitivity and specificity as a way to copy a DNA sequence many times over. The insertion sequence IS6110 is of uncertain functional significance (it is not a gene, for example) and belong to a class of molecules known as tranposons, which are self-replicating stretches of DNA that can become permanently integrated into the host genome.[81]Since the identification of IS6110 in M. tuberculosis, this sequence has been found in the M. tuberculosis complex of organisms (M.tuberculosis M. africanum, M. microtic and M. bovis) but in no other mycobacterial species.[82] PCR may be applied to specimens in culture well before the 4 to 8weeks periods necessary for isolates to be identified with confidence. PCR is extremely reliable compared with conventional culture and speciation techniques. Using the IS6110 sequence (insertion element) as an

amplification target, there is virtually no misidentification of M. tuberculosis with any other mycobacterial species.[82]

1.9.7. Restriction fragment-length polymorphism (RFLP) -DNA genetic fingerprint

Restriction fragment-length polymorphism (RFLP) is a new tool that has recently been applied to TB. RFLP is a test that identifies specific strains of microorganisms. It permits tracking of specific strain of M. tuberculosis. It can be used to establish transmission links and to identify individual and environmental risk factors. Moreover application of DNA fingerprinting can provide important insights into the pathogenesis of tuberculosis and may assist in identifying strains of M. tuberculosis that have specific properties such as tissues tropism or virulence. When carefully done with standardized methodology, DNA fingerprinting of M. tuberculosis provides a powerful tool for examining the transmission dynamics of tuberculosis. The combination of conventional epidemiology and DNA finger printing is yielding important insight in the transmission and pathogenesis of TB.[82]

The use of RFLP analysis to identify pathways of the transmission of tuberculosis within a community is based on the premise that epidemiologically unrelated patients develop tuberculosis as a result of reactivation of latent infection. The acquisition of infection will likely have occurred at a point remote in time and, often, in location. For these reasons, isolates of M. tuberculosis isolated from epidemologically unrelated patients in a community have unique RFLP patterns. It can be assumed that one member of the cluster was the source case and other was secondary case. Such

assumption provides a conservative estimate of the number of patients in whom recent transmission has occurred.[82]

1.9.8 High performance chromatographic techniques are capable of rapidly identifying mycobacteria species by their characteristic lipid. It can also identify non-tuberculous mycobacteria species after a solid media culture is found to have growth. Fatty acid are extracted from mycobacteria and analyzed by chromatographically. Briefly, treated samples of mycobacterial cell wall components are separated chromatographically. The chromatographic pattern is used to identify the mycobacteria species. At present, the system can identify 27 species of mycobacteria with good accuracy. It takes approximately 1 hour of preparation time and 30 min of chromatography time to identify mycobacteria.

1.9.9.CHEST X-RAY PA

A posterior anterior view of the chest is the standard radiography [70]. Chest X-ray can demonstrate whether there is hilar lymphadenopathy, cavities or multinodular infiltrate. Chest radiography is an important tool for both Screening diagnosis and evaluation of tuberculosis.

In primary tuberculosis occurring as a result of recent infection there is middle or lower lung infiltrate, often associated with ipsilateral hilar adenopathy.

In post primary tuberculosis there is abnormality in the upper lobes of one or both lungs. Cavitation is common and the most frequent site of involvement is the apical

and posterior segments of the right upper lobe, and the apical posterior segment of the left lobe.

In patients with HIV infection the nature of radiographic findings depends on the degree of immucompromise produced by the infection. Tuberculosis that occurs early in the course of HIV infection tends to have the typical radiographic findings as that of post primary tuberculosis. With more advanced HIV disease, the radiographic findings become more atypical, cavitation are uncommon, and lower lung zone or diffuse infiltrates and intrathoracic adenopathy are frequent. [36]

1.9.10 HAEMATOLOGICAL TESTS: THE FULL BLOOD COUNT

In HIV the most frequent abnormalities in the blood cells are anaemia, thrombocytopenia and neutropenia either individually or combined and lymphopenia with reduced number of CD4 lymphocytes. Anaemia of chronic HIV infection is usually mild normochromic and normocytic. Platelate counts are moderately reduced but may fall dramatically (10-20x10^9/L) producing easy bleeding and bruising.

The mechanism of cytopenia include infections (opportunistic, Mycobacterium avium intracllulare, Cytomegalovirus), malignancies (Lymphoma), autoimmune reactions and impaired marrow production either due to HIV infection or progenitors or lack of growth factors perhaps due to HIV infection of accessory cells. The drugs used in the therapy especially azidothymidine (AZT) but also ganciclovir, pentamidine and trimethoprim may also cause cytopenia especially if the patient is

folate deficient. Serum B_{12} levels tend to be low in AIDS probably due to intestinal B_{12} malabsorption but megaloblastic anaemia due to B_{12} deficiency is unusual.[83, 84]

1.10. TREATMENT OF TUBERCULOSIS

Chemotherapy should be started without delay, once bacteriological and biopsy specimens have been obtained, even if the diagnosis has not yet been confirmed. Initial treatment is with three or four drugs as recommended for pulmonary tuberculosis.[85] Corticosteroid drugs should be added in severally ill patients. Prednisolone 30-60 mg daily suppresses fever and improves the condition of the patient. Thereafter the dose of prednisolone is reduced to a minimum to prevent return of pyrexia or other symptoms.

1.10.1 Recommended treatment for tuberculosis

A. For AFB +ve and complicated tuberculosis like extra-pulmonary tuberculosis e.g. TB pericarditis, TB meningitis, milliary TB, TB peritonitis.

Initial phase – Two months of treatment

Rimactazid – Rifampicin 600mg + Isoniazid 300mg daily

Pyrazinamide - 1600mg daily

Ethambutol - 1200mg daily

Continuation – six months of treatment

Isoniazid - 300mg daily

Ethambutol) - 800mg daily

B. For AFB –ve and un complicated TB

Initial phase – Two months treatment

Rimactazid – Rifampicin 600mg + Isoniazid 300mg alternate days

Pyrazinamide - 1600mg alternate days

Ethambutol - 1200mg alternate days

Continuation – six months of treatment

Isoniazid - 300mg daily

Ethambutol) - 800mg daily

1.10.2 Tuberculosis preventive Therapy:

The recommended regimen for preventive therapy in adults is a single daily dose of 300mg of isoniazid for 6-12 months. Regardless of their ages, persons in the following high-risk groups should be evaluated for preventive therapy if they have a positive skin-test result.[70]

- Persons known to be infected with HIV who have a skin-test results of (=5 mm indurations;
- Persons who are at risk of HIV infection (including injecting-drug users whose HIV status is unknown) and who have an indurations of (=5 mm
- Person who have had close contact with a person who has infectious TB and who have an indurations of (=5 mm)
- Persons who have chest-radio-graph findings suggestive of previous TB but who have received inadequate or no treatment and who have an indurations of (=5 mm

- Injecting-drug users who are known to be HIV negative and who have an indurations of (=10 mm)
- Persons who have medical conditions known to increase the risk of TB disease and who have an indurations of >10 mm.: and
- Persons whose tuberculin skin-test result converted from negative to positive within the preceding 2 years and who have a (=10 mm increase in the size of indurations if (35 years of age or a (= 15 mm increase if (35 years of age.[70].

1.11 BURDEN OF TUBERCULOSIS:

1.11.1 Global burden of tuberculosis: The global prevalence of mycobacteria tuberculosis (MTb) infection in 1997 was estimated to be about 32% (1.86 billion people) and the prevalence of MTB and HIV co-infection worldwide was reported to be 0.18%.[86] At the end of 1998 it was estimated that there were 8 million new cases of tuberculosis, and 3 million deaths occurred due to TB during the same time, worldwide. About 9% of global TB cases were attributed to HIV infection and this was projected to increase to about 14% by the year 2000. Many countries in Sub-Saharan Africa have annual TB notification rates of about 10% while rates of HIV infection in new TB patients have been reported to exceed 50%.[86] The global burden of tuberculosis remain enormous mainly because of poor TB control in South East Asia, Sub-Saharan Africa and Eastern Europe and because of high rates of mycobacteria and HIV co –infection in some African countries.[87]

1.11.2 Burden of tuberculosis in Tanzania. In 1999, in Tanzania 52,437 cases of tuberculosis were notified, in which 24,125 (46%) were new sputum smear positive, 1,737 (3%) relapses, while the remaining 26,575 (51%) were smear negative and extra-pulmonary tuberculosis combined.[88] As seen in all other high HIV prevalence areas, the smear positive rates among TB patients have declined compared to 1984 (pre HIV era). In 1984 more than 62% of the newly diagnosed TB patients were sputum smear positive against 46% in 1999. In contrast, the proportion of smear negative and extra-pulmonary TB cases has increased nearly one and half fold from 35.5% to 50.7%.[88] Age and sex distribution of cases notified in 1999 shows that the most affected age group is 15-34 year age group among females and 25-44 year age

group among males. The current distribution of PTB falls within the age distribution of HIV in the general population.

The total number of cases of TB notified annually has increased five times over the last decade because of the HIV/AIDS pandemic, leading to an upsurge of tuberculosis cases especially in urban areas. The National TB and Leprosy annual report of 1998 showed that the contribution of Dar-es-salaam, the largest urban city in Tanzania, was more than 26% of all cases notified in Tanzania.[88]

1.12 BURDEN OF HIV.

1.12.1 Global burden of HIV. The AIDS epidemic update of December 2000 showed that, the number of people living with HIV/AIDS totalled 36.1 million, of which adults are 34.7 million, women 16.4 million, and children less than 15years old 1.4million. Total number of AIDS death since the beginning of the epidemic up to Dec 2000 was 21.8 million, of which adults were 17.5 million, women 9million and children less than 15 years were 4.3 million.[89]

People newly infected with HIV in 2000 totalled 5.3 million, of which adults were 4.7 million, women were 2.2 million and children less than 15 years were 600,000. Total number of AIDS death in 2000 was 3 million of which adults were 2.5million, women 1.3 million and children less than 15 years 500,000.[89]

1.12.2 Burden of HIV in Tanzania. HIV infection in Tanzania was first reported in 1983 in the northwestern region bordering Uganda. Since then the infection has spread to affect all 20 regions of Tanzania. The prevalence of HIV in Tanzania is

estimated to be about 10% (ranging from 5-20%). [86] The prevalence of HIV infection in Mbeya region at the end of 1998 was reported to be 15.4% and that of Moshi rural was about 20%. In a study of Vitamin supplementation among pregnant women done in Dar-es-salaam between 1995 and 1997, the HIV sero-prevalence was reported to be 13.5% in 1995, 12.4% in 1996, and 14.8% in 1997.[86] HIV screening among blood donors showed an overall prevalence of HIV infection to be about 9%, being slightly higher in female donors (11.8%) compared to male blood donors. (8.5%) In the 1998 report of the National AIDS Control Programme 50% of reported AIDS cases were below the age of 32 years and 88.6% were between 15 to 49 years.[86]

1.13. HIV INFECTION AND TUBERCULOSIS

In Africa: HIV sero-prevalence in TB patients is reported to be around 40% with a range of 20-73%[18,24,40]. In Uganda and Zambia more than 60% of TB patients were found to be HIV sero-positive, while 30% of newly diagnosed TB patients in Kenya were also reported to be HIV seropositive[40, 90,91.] The prevalence of HIV among smear positive TB in Mainland Tanzania varies from 24.3% to 75%, with an average of 40%. The prevalence of over 75% and 80% was recorded in smear negative PTB and extra pulmonary PTB in Rukwa region.[86]

During the 10 years period of 1990 to 1999, it was estimated that 3.9 million (25%) of the expected 15 million people in Sub-Saharan Africa who develop TB would be HIV seropositive.[92] A large proportion of persons infected with HIV live in the developing regions of the world, especially Sub-Saharan Africa, where as previously

indicated, there are high prevalence of tuberculosis infection and high incidence of tuberculosis.[14]Tuberculosis is an extremely important complication of HIV infection in Sub-Saharan Africa.

2.STUDY QUESTIONS.

1. What are the clinical characteristics associated with smear negative culture positive pulmonary Tuberculosis?
2. Are clinical characteristics associated with smear negative pulmonary tuberculosis different between HIV positive and HIV negative patients?

3. Problem statement

There have been a sharp rise in the incidence of pulmonary tuberculosis worldwide since the mid 1980`s, but more so in the Sub-Saharan African region. This has been attributed mainly to the appearance and wide spread of HIV infection on the continent.[38,40,93] The tuberculosis epidemic has threatened to undermine whatever achievements that had been gained by National Tuberculosis Control Programs in Sub-Saharan Africa.[25] Diagnosis of tuberculosis in HIV infected patients has become more difficult because of the increased frequencies of sputum smear negative and extra-pulmonary cases[88, 94] .For the diagnosis of pulmonary tuberculosis the detection of Acid Fast Bacilli (AFB) in expectorated sputum is still crucial, especially in developing countries of Sub-Saharan Africa, where other facilities including sputum culture for MTB are unavailable or are just prohibitively expensive. When AFB is detected in sputum then the diagnosis of PTB is certain. However diagnostic problem start when patients with suspected PTB is sputum smear negative.[94] It has always been recognized that a proportion of patients are sputum smear negative using the Ziehl-Nelseen (ZN) stain, the commonly used stain in most laboratories in the region to detect AFB in sputum. This is a simple, rapid and cheap test but lacks sensitivity of a single sputum test. About 5000 bacilli per milliliter of sputum must be present

for it to be positive. However multiple sputum tests in a good laboratory can give a sensitivity of about 90%. Sputum smear using ZN stain for AFB seems to be even less sensitive in patients with HIV associated pulmonary tuberculosis. With the sharp rise of PTB in countries, which are worst affected by the HIV epidemics, the number of patients with suspected PTB who are sputum smear negative has increased. In some reports the number accounts for as high as 40% of all possible cases of PTB. For every case of smear positive pulmonary tuberculosis, there is usually one case of smear negative pulmonary TB or extra-pulmonary disease. On this basis it is estimated that in the third world there are each year 4-5 million new smear positive cases and another 4-5 million smear negative and extra-pulmonary cases.[25]

Chest radiography is not always helpful in smear negative patients. The radiographic distinction between active and inactive tuberculosis can be difficult and appearance may be atypical or due to other infections in HIV positive patients.[94] However substantial numbers of patients are treated for tuberculosis without definitive diagnostic criteria.[25] With the advent of HIV associated tuberculosis with more frequent smear negative tuberculosis, the role of culture in TB control programs may need to be reassessed.[94]

In countries where resources are limited, and where the use of chest x -rays may be limited due to the cost as well as atypical appearances seen in HIV infected patients, clinical and/or laboratory characteristics which are able to identify smear negative but culture positive Pulmonary tuberculosis are therefore required. This may help in the development of diagnostic algorithms for the purpose of diagnosing individual

patients with suspected pulmonary tuberculosis but are smear negative, but could be culture positive.

4. Rationale of the study

Little information exists regarding differences in clinical presentation between sputum smear negative but culture positive pulmonary tuberculosis and sputum smear negative culture negative in patients who present with chronic chest infection. This is also true between HIV positive and HIV negative patients. Hence there is a need to know the clinical characteristic of patients presenting with smear negative tuberculosis, to guide physicians on the selection of patients who will need Anti TB treatment.

5. OBJECTIVES

5.1 BROAD OBJECTIVE

To determine clinical and laboratory features of smear negative, culture positive pulmonary tuberculosis and the relationship to HIV sero-status.

5.2 SPECIFIC OBJECTIVES

5.2.1 To determine clinical and laboratory features of smear negative but culture positive pulmonary tuberculosis.

5.2.2 To determine clinical and laboratory features of smear negative culture positive pulmonary tuberculosis in relation to the patients HIV serostatus.

6. METHODOLOGY

Study Setting: The study took place in Dar es Salaam. The city accounts for over 26% of all new cases of Tuberculosis reported each year in Tanzania [76]. The study was conducted at Muhimbili National Hospital, a University Teaching and National referral Hospital, and out patient Tuberculosis clinics at the Infectious disease clinic (IDC) and Mwananyamala, Temeke and Ilala district hospitals' TB outpatient clinics".

Study Design: This was mainly a descriptive study with an analytical component, which described the clinical, and laboratory characteristics of smear negative but culture positive pulmonary tuberculosis. A comparison of the clinical characteristics in relation to HIV serostatus was also made.

Study subjects: The target study population was all those with respiratory symptoms who were sputum smear negative. The population that was accessible for the study

was all those patients admitted to the medical wards at Muhimbili National Hospital and those seen at the outpatient tuberculosis clinics of IDC, Ilala , Mwananyamala and Temeke district hospitals.

Inclusion criteria

a) All adults who were 18 – 75 years
b) Presenting with symptoms of chronic cough of ≥ 2weeks
c) Those who had sputum smear negative for Acid Fast Bacilli (AFB) three times
d) Individuals who had an ability to give consent for the study including a consent for HIV testing

Exclusion criteria

a) Known patient of Tuberculosis and those with a past history of PTB
b) Patients on anti TB including Preventive therapy)
c) Patients with chronic respiratory diseases like Asthma, COPD and bronchiectasis
d) Patients with heart failure

Sampling of study Subjects: Consecutive patients with new respiratory disease (chronic cough of ≥2 weeks), admitted to the medical wards of Muhimbili Medical Center and those seen at the 4 out patient Tuberculosis clinics were enrolled if they fulfilled the inclusion criteria.

Sample size

Among symptoms, which could be used to differentiate tuberculosis from a non-tuberculous respiratory disease, is the frequency of hemoptysis. This has been reported in some studies to be 15% in non-tuberculous patients with respiratory disease, and 37% in patients suspected to have tuberculosis.[95]

•**P1** is the proportion of patients with sputum smear negative but culture positive expected to have hemoptysis.

•**P2** is proportion with sputum smear and culture negative for AFB expected to have hemoptysis.

I would like the difference between P1 and P2 to be only 10%. Then P2 =27

E=6

E is the standard error.

$$\bullet N = \frac{P1\,(100-P1)+P2\,(100-P2)}{E^2}$$

•**N**=119.5 =120 subject per group.

Procedures

Those patients who fulfil the inclusion criteria were interviewed and examined by the study Physician to elicit symptoms and signs of respiratory or any other disease. The information obtained was filled in a clinical record form (annex 1). Sputum was collected on three consecutive days and sent for smear (ZN staining) and culture to the Tuberculosis Reference Laboratory at Muhimbili National Hospital. Smears were considered positive if any of the three sputum's samples demonstrated Acid Fast Bacilli (AFB). Patients found to have sputum smears positive were sent to treatment centres where they received treatment for tuberculosis according to the National TB and Leprosy program recommendations. Those found to be AFB negative on three occasions were then actively enrolled in the study. Patients with sputum AFB negative were started on anti-TB therapy if they met the criteria for a presumptive diagnosis of AFB negative PTB as recommended by NTLP which were:

- History of fever and cough for over 2 weeks
- History of weight loss of >10% body weight.
- Highly suggestive Chest X- ray
- Raised ESR >50mm/hr
- Positive mantoux test

Prior to recruitment into the study patients were informed about the study. Consent to participate in the study and for HIV testing was obtained from every individual patient.

A purified protein derivative (PPD) skin test was done on each of the enrolled patients by injecting 0.1 ml of PPD on the medial aspect of the arm, the result of the test was read 48 hrs later and was graded as

1- Negative if the size of indurations was less than 5 mm in diameter,

2- Weak positive if it was 5-10 mm in diameter,

3- Positive if it was 10-15 mm and

4- Strongly positive if it was above 15 mm.

Venous blood was drawn from each study subject and the following laboratory tests were done at the clinical research laboratory:

- A FBP; which included Hemoglobin, red blood cell morphology, and white blood cell count both total and differential. The blood was analyzed using a coulter counter.
- Erythrocyte sedimentation rate was set using the Westergren method within 2 hrs of drawing blood.

- HIV test was done according to the Tanzania National AIDS Task Force guidelines. Pre and post-test counseling was done to every patient. HIV testing was done using a dual ELISA algorithm. Sera which were non-reactive on first ELISA were considered HIV antibody negative, and those reactive on first ELISA were retested by a second ELISA based on a different test principle. Sera reactive on both ELISA tests were considered seropositive. Samples with discordant test results were confirmed by Western blot (WB). Western blot interpretation was done according to the World Health Organization Criteria for Western blot.[96]
- Posterior-anterior chest radiography was taken for each enrolled patient. Films were reviewed and reported on using a structured format (Appendix 2) by two independent radiologists. In case of disagreement in their reporting, they were asked to review the radiographs again together and come to a consensus.

The following abnormalities were looked for in the chest radiographs:

1- Predominant Parenchymal lesions

2- Presence of cavitations

3- Predominant Location

4- Presence of pleural fluid

5- Lymphadenopathy (Hilar and mediastinal)

- Culture of sputum for MTB was done on all sputum using the Löwenstein-Jensen culture media.

- For those presenting with lymphadenopathy, ascites or pleural effusion, further relevant investigations was done.

7. Hypothesis

Null hypothesis: There is no difference in the frequency of hemoptysis between patients with sputum smear negative but culture positive for Mycobacterium tuberculosis, and those with sputum smear and culture negative.

Ethical consideration

Informed oral/written consent was requested from all subjects. The Ethical committee of Muhimbili National Hospital approved the study. Throughout the study period both TB and non-TB patients were treated and followed up in exactly the same way as corresponding patients outside the study.

Data analysis and statistical procedure

Data that was collected was analyzed using statistical package for social sciences (SPSS) and EPI info. X^2 test was used for comparison of categorical data and a student t-test was used for continuous data. Logistic regression analysis was applied and the direct effects of the predictors were assessed by their 95% confidence intervals.A p-value of < 0.05 was considered to be statistically significant.

8. RESULTS

The study population.

During the study period of 3 months, a total of 3246 patients with respiratory symptoms that could produce sputum were seen at the 5 treatment centres, and investigated for pulmonary tuberculosis. Four hundred and three (12.4%) of those who submitted sputum for smear were found to have sputum smear positive and were started on anti TB treatment using the short course chemotherapy. Four hundred sixty seven (16.4%) patients of those who had smear negative sputum for AFB, fulfilled the inclusion criteria and consented to participate in the study. Their sputum was taken for Mycobacterium tuberculosis culture. The remaining 2376 (83.6%), were not included, 1628 (68.5%) did not consent and some 748 (21.5%) did not meet the inclusion criterial. Among those who did not meet the inclusion criteria, 550 (23.2%) subjects were being investigated for TB relapse , 102(4.3%) were found to have heart failure, 67(2.8%) were below the consenting age of 18 years and 29(1.2%) patients presented with feutures of chronic obstructive pulmonary diseases.

Sputum for culture was found to be positive for MTB in 27.2% (127/467) of the study subjects; and in 11.1% (52/467) patients, sputum culture was reported to be contaminated. The remaining 61.7% (288/467) had sputum culture negative for MTB at 8 weeks. (Fig 1). Of the 467 study subjects 68.1% (318/467) were found to be HIV positive. Two study subjects among those who were culture negative their ELISA results were missplaced. The 52 patients with contaminated cultures and the 2 patients with missing ELISA results were excluded from further analysis, and thus a total of 413 study subjects were analysed. Of the 413 analysed patients 30.8%

(127/413) were MTB culture positive and 69.2% (286/413) were culture negative and 287 were HIV positive while 126 were HIV negative.

Demographic characteristics of the study population

Age, sex and HIV distribution of the 413 subjects included in the study are shown in Table 1. The mean (SD) age of all study subjects was 34.16 (10.84) years ranging from 18 to 75 years. Women were much younger with a mean (SD) age of 31.7 (9.25) yrs compared to men whose mean (SD)age was 36.26 (11.86), $p< 0.001$. There was no age differences between patients with sputum culture positive and those who had sputum culture negative results. The prevalence of HIV positivity was higher among females 79.6% compared to that of males 60.4%. $p =0.000023$ fig 2.

Figure 1 Recruited study subjects

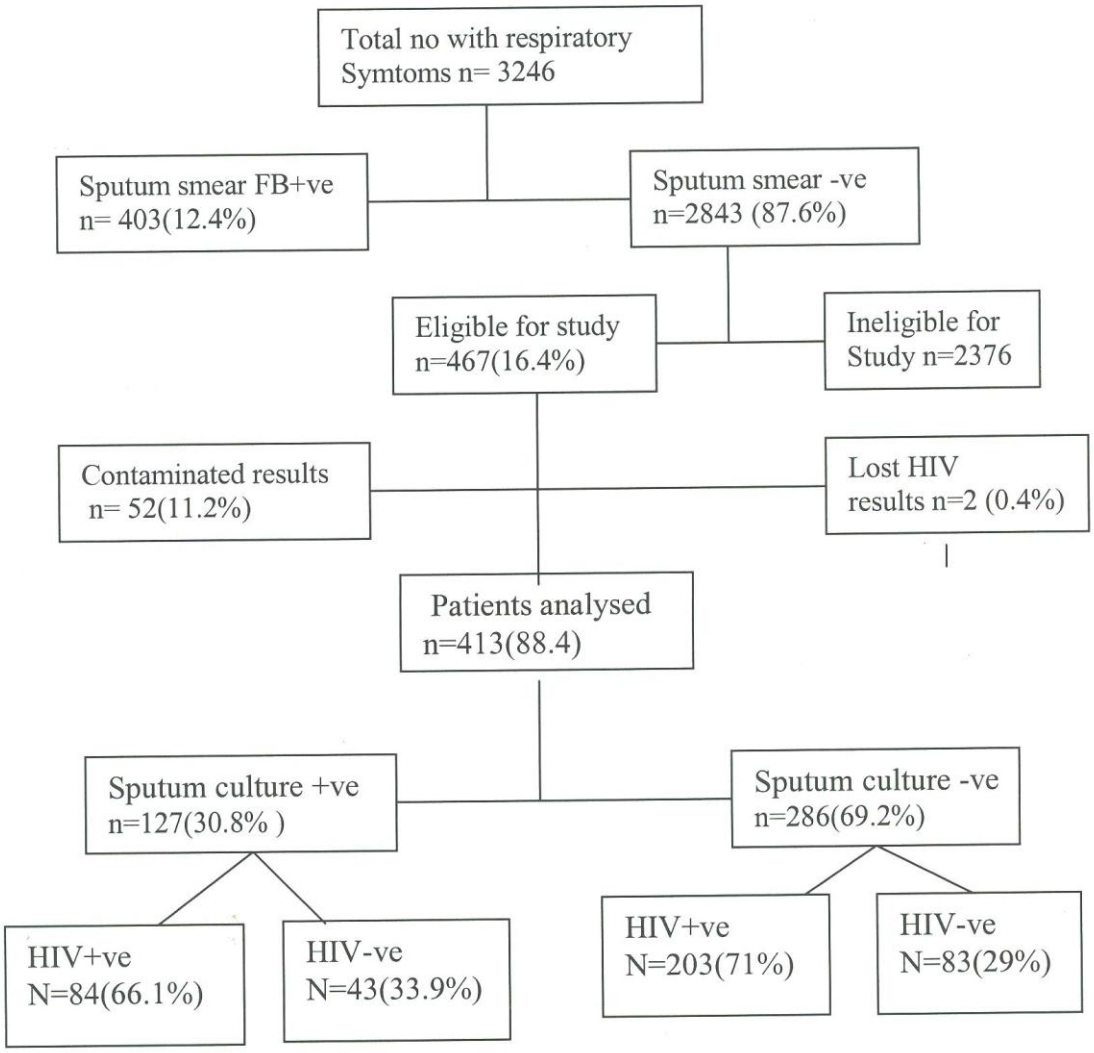


Table 1

Distribution of the study population by Age, sex and HIV sero-status (n=413)

Age group	Male		Female	
	N=217(52.5)		N=196(47.5)	
	HIV +	HIV –	HIV +	HIV-
	N=131(60.4%)	N=86(39.6%)	N=156(79.6%	N=40(20.4%)
18-29	28(21.4)	37(43.0)	64(41.0)	20(50.0)
30-39	53(40.5)	19(22.1)	66(42.3)	9(22.5)
40-49	36(27.5)	11(12.8)	19(12.2)	6(15.0)
50-59	12(9.2)	13(15.1)	7(4.5)	4(10.0)
60-69	2(1.5)	5(5.8)	0(0.0)	0(0)
≥70	0(0.0)	1(1.2)	0(0.0)	1(2.0)

Fig 2 Distribution of HIV serostatus by sex among study subjects (n=413)

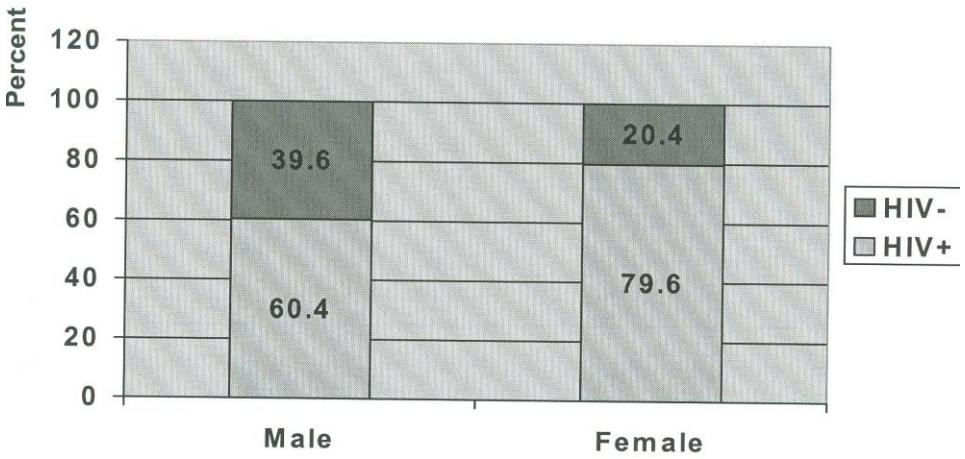


Table 2a gives a summary of the social demographic characteristics of the study population in relation to their sputum culture results. There were more males in our study population. A big proportion of the study population (74.1%) had attained at least primary school education and this was similar in both sputum culture result groups. Most study subjects in both culture groups were self-employed. However there were more married study subjects in the culture negative group compared to the culture positive (54.2% and 41.7% respectively). (P=0.023).

Table 2a

Demographic characteristics of the study population by their sputum culture results

		Culture Positive	Culture Negative	P= value
		N=127	N=286	
Sex	Males	69(54.3)	147(51.4)	0.625
	Females	58(45.7)	139(48.6)	
Educat	None	31(34.8)	66(23.1)	0.849
	Gone to school	94(75.2)	212(74.1)	
Occupat	Un employed	30(25.6)	85(31.4)	0.257
	Self-employed	75(64.1)	162(56.6)	0.462
	Employed	12(10.3)	23(8.5)	0.462
Marital	Singles	49(39.2)	90(31.5)	**0.053**
	Married	53(41.7)	155(54.2)	**0.023**
	Divorced	19(15.2)	32(11.1)	0.245
	Widowed	4(3.2)	9(3.1)	0.789
Smoking	Yes	21(16.5)	60(20.9)	0.308
	No	106(83.5)	226(79.0)	
Drinking	Yes	40(31.5)	81(28.3)	0.53
	No	87(68.5)	205(71.7)	

Table 2b shows that, in both culture positive and negative groups HIV positive patients were more than HIV negative patients, however there were more female

HIV positive patients in both culture negative and positive groups than males, this difference was statistically significant. (P<0.0002). There were no differences among the four groups in relation to their occupational status, marital status, drinking and smoking habits.

Table 2b
Demographic characteristics of the study population by sputum culture results and HIV status.

		Culture positive		p-value	Culture negative		p-value
		HIV + 84	HIV – 43		HIV+203	HIV -83	
Sex	Males	37(44.0)	32(74.4)	0.001	93(45.8)	54(65.1)	0.003
	Females	47(56.0)	11(25.6)		110(54.2)	29(34.9)	
Educat	None	16(19.3)	15(35.7)	0.044	47(23.9)	19(23.5)	0.943
	Gone to school	67(80.7)	27(64.3)		150(76.1)	62(76.5)	
Occupat	Un employed	19(24.7)	11(27.5)	0.72	65(34.4)	20(24.7)	0.004
	Self-employed	47(61.0)	28(70.0)	0.315	105(55.6)	57(70.4)	0.000
	Employed	11(14.3)	1(2.5)	0.315	19(10.1)	4(4.9)	0.000
Marital	Singles	29(35.4)	20(46.5)	0.18	57(28.1)	33(39.8)	.053
	Married	34(41.5)	19(44.2)	0.78	111(54.7)	44(53.0)	0.797
	Divorced	16(19.5)	3(7.0)	0.07	26(12.8)	6(7.2)	0.174
	Widowed	3(3.7)	1(2.3)	0.87	9(4.4)	0(0)	0.11
Smokin	Yes	13(15.5)	8(18.6)	0.653	37(18.2)	23(27.7)	074
	No	71(84.5)	35(81.4)		166(81.8)	60(72.3)	
Drinkin	Yes	30(35.7)	10(23.3)	0.153	58(28.6)	23(27.7)	0.883
	No	54(64.3)	33(76.7)		145(71.4)	60(72.3)	

Presenting symptoms of study subjects

The frequency of symptoms of study subjects is shown in Table 3a in relation to their sputum culture results. Nausea, vomiting, dysphagia and anorexia were observed significantly more common in the culture positive than negative group. Dry cough was reported more commonly in the culture positive group (p=0.0005) while

productive cough was more common in the culture negative group (p=0.0005). Other symptoms found to be more frequent in culture positive than negative were; dyspnoea at rest, numbness, limb weakness and arthralgia. The frequencies of fever, night sweats, weight loss, odynophagia, diarrhoea, abdominal pain and constipation were similar in both groups. This similarity was also observed with other symptoms such as; itching, skin disease, haemoptysis, pleuritic chest pain, non-pleuritic chest pain, and dyspnoea on exertion.

Table 3b shows that, weight loss was common in patients with sputum culture positive irrespective of whether they were HIV positive 73(86.9%) or negative 35(81.4%), while in culture negative patients weight loss was common in those who were HIV positive 168(82.8%) than those who were HIV negative 57(69.5) (p=0.025). Nausea, vomiting, odynophagia, diarrhoea, itching, and skin disease were significantly more common in those subjects who were HIV positive in both groups.

Table 3a

Symptoms of study subjects at the time of interview by their sputum culture results

Feature	Culture Positive N=127(%)	Culture Negative N=286(%)	P value
Fever	113(89)	256(89.5)	0.85
Weight loss	108(85.0)	225(78.7)	0.155
Nausea	42(33.1)	51(17.8)	**0.0005**
Vomiting	31(24.4)	42(14.6)	**0.015**
Anorexia	46(36.2)	75(26.2)	**0.042**
Dysphagia	7(5.5)	4(1.4)	**0.037**
Odynophag.	16(12.6)	26(9.0)	0.276
Diarrhoea	22(17.3)	31(10.8)	0.067
Itching	21(16.5)	36(12.5)	0.271
Skin dis.	22(17.3)	47(16.4)	0.271
Dry cough	29(22.8)	29(10.1)	**0.000**
Prod.Cough	98(77.2)	257(89.9)	**0.0005**
Haemopt.	13(10.3)	37(12.9)	0.467
Dysp.at rest	39(30.7)	51(18.0)	**0.004**
Dysp.on Ex	43(33.9)	102(36.0)	0.707
Numbness	43(33.9)	69(24.1)	**0.036**
Paraesthesia	21(16.5)	55(19.1)	0.534
Limb weak	62(48.8)	93(32.5)	**0.002**
Arthralgia	41(32.3)	49(17.1)	**0.0007**

Table 3b
Symptoms of study subjects at the time of interview by their sputum culture results and HIV sero-status.

Feature	Culture Positive		P value	Culture Negative		P value
	HIV +84	HIV – 43		HIV + 203	HIV - 83	
Fever	75(89.3)	38(88.4)	0.876	183(90.1)	73(88.0)	0.582
Weight loss	73(86.9)	35(81.4)	0.408	168(82.8)	57(69.5)	**0.013**
Diarrhoea	18(21.4)	4(9.3)	0.087	25(12.3)	6(7.3)	0.220
Itching	21(25.0)	0(0)	**0.000**	29(14.3)	7(8.4)	0.176
Skin dis.	20(23.8)	2(4.7)	**0.007**	42(20.7)	5(6.0)	**0.002**
Dry cough	15(17.9)	14(32.6)	0.062	20(9.9)	9(10.8)	0.801
Prod.Cough	69(82.1)	29(67.4)	0.062	183(90.1)	74(89.2)	0.801
Haemopt.	8(9.5)	5(11.9)	0.679	26(12.8)	11(13.3)	0.919
Pleur pain	16(19.0)	7(16.3)	0.701	30(14.8)	10(12.0)	0.546
Non pl.Pain	41(48.8)	21(48.8)	0.998	82(40.4)	31(37.3)	0.633
Dysp.at rest	28(33.3)	11(25.6)	0.370	39(19.2)	12(14.5)	0.340
Dysp.on Ex	28(33.3)	15(34.9)	0.861	75(36.9)	27(32.5)	0.479
Numbness	31(36.9)	12(27.9)	0.311	54(26.6)	15(18.1)	0.126
Limb weak	42(50.0)	20(46.5)	0.710	79(38.9)	14(16.9)	**0.000**

Past medical History and health seeking behaviour

A previous history of pulmonary disease (other than PTB) was observed more common in patients who had sputum culture positive 40.2% (51/127) than those who had sputum culture negative 19.4% (56/286) ($p<0.0001$).(Table 4) Most patients had similar health seeking behaviour, few admitted to have visited traditional healers and none had attended spiritual healing, over 94% of patients had received treatment for respiratory conditions in other clinics or hospitals before coming to the TB clinics.

Table 4
Past medical history and health seeking behaviour by their sputum culture results and HIV status.

Past medical History	Culture+ N=127	Culture- N=286	Sputum culture+ n=127		Sputum culture- N=286	
			HIV+ N=84	HIV- N=43	HIV+ N=203	HIV- N=83
BT	5(3.9)	16(5.6)	4 (4.8)	1(2.3)	11 (5.4)	5(6.0)
Prev. pulm. Dis.	**51(40.2)**	**56(19.4)****	35(41.7)	16(37.2)	43(21.2)	12(14.5)
Health seeking behaviour						
Traditional healers	3(2.4)	3(1.0)	2(2.4)	1(2.3)	**0(0)**	**3(3.6)***
Modern medicine	122(96.1)	274(95.8)	79(94.0)	43(100)	195(96.1)	79(95.)

* P<0.05
** p <0.001

Physical signs in study subjects

Table 5a shows physical signs observed in the study population at the time of interview in relation to subjects' sputum culture results. Fever (defined as axillary temperature ≥ 37.5^0C), tachypnoea (RR >20/min) tachycardia (>99/min), pallor, the presence of lymph nodes especially matted lymph nodes and features of consolidation were observed more frequently in those who had sputum culture positive. Features that were found to be more frequent in patients with sputum culture negative included; the presence of sebhoreic dermatitis and angular stomatitis. Other Physical findings were similar in the two groups including the presence of pleural effusion.

Table 5b shows that, the proportion of those who had a temperature of ≥ 37.5^0C was higher in patients with sputum culture positive compared to those with culture negative, this was even higher in those who were HIV positive, being very high in

culture positive HIV positive group (p-0.031). It was also noted that 19.3% of culture positive HIV positive group and 16.7% of those with culture positive but HIV negative had high pulse rate compared to the other three groups (p=0.0085). The presence of matted lymphadenopathy was observed more in subjects with sputum culture positive compared to those who had sputum culture negative. This was more pronounced in those who had sputum culture positive and HIV positive. (P=0.033). Although the presence of hair changes was not significantly different between those who had sputum culture positive compared to those who had negative cultures. These changes were seen significantly more frequent among HIV positive subjects in both groups (p<0.0001). Pallor was common in culture positive HIV positive (42.9%), followed by culture positive HIV negative subjects (27.9%) and culture negative HIV positive (24.1%) and lastly culture negative HIV negative subjects (12.0%) (P<0.0001).

Other physical findings that were more frequently found in HIV positive patients in both groups included; pitting oedema (p=0.056) kaposi's sarcoma (p=0.0024) seborrheic dermatitis (p<0.0001), Papular pruritic eruption (p<0.0001), angular stomatitis (p<0.0001), oral candidiasis (p<0.0001) and the presence of herpes zoster (p-0.022). Consolidation was common in patients with sputum culture positive regardless of their HIV sero-status. (P=0.007).

Table 5a
Physical signs of study subjects by their sputum culture results

	Culture+ N=127	Culture- N=286	P-value
BMI <18.5 kg/cm^2	53(46.5)	106(39.3)	0.188
BMI >18.5 kg/cm^2	61(53.5)	164(60.7)	0.188
Temp >37.8/min	62(50.8)	105(37.2)	**0.01**
High pulse >99/min	23(18.4)	24(8.4)	**0.003**
Respiratory Rate	50(39.7)	44(15.3)	**0.000**
Lymphadenopathy Matted	15(11.8)	15(5.2)	**0.016**
Discrete	18(14.2)	36(12.5)	0.64
Cervical	30(24.0)	47(16.4)	0.06
Axillary	11(0.8)	27(9.4)	0.8
Hair changes	24(18.9)	48(16.7)	0.7
Pallor	48(37.8)	59(20.6)	**0.000**
Pitting oedema	7(5.5)	8(2.7)	0.37
Kaposis sarcoma	9(7.1)	12(4.2)	0.28
Seborrh.Dermatitis	6(4.7)	36(12.5)	**0.012**
Herpes zoster	7(5.5)	10(3.5)	0.334
Herpes simplex	5(4.0)	12(4.2)	0.925
Tinea	10(7.9)	35(12.2)	0.196
PPE	26(20.5)	62(21.6)	0.69
Angular Stomatitis	10(7.9)	49(17.0)	**0.014**
Oral candidiasis	16(12.6)	22(7.6)	0.137
Crepitations	77(60)	156(54.4)	0.235
Consolidation	51(40.2)	67(23.4)	**0.000**
Plural effusion	24(18.9)	36(12.5)	0.087

Table 5b
Physical signs of study subjects by their sputum culture results and HIV sero-status

	Sputum culture+ N=127		P-value	Sputum culture- N=286		P-value
	HIV+ N=84	HIV- N=43		HIV+ N=203	HIV- N=83	
BMI <18.5 kg/cm^2	38(50.7)	15(38.5)	0.21	76(40.4)	29(36.3)	0.5
BMI >18.5 kg/cm^2	37(49.3)	24(61.5)	0.21	112(59.6)	51(63.8)	0.5
Temp >37.8/min	43(53.1)	19(46.3)	0.48	78(39.0)	25(31.3)	0.224
High pulse >99/min	16(19.3)	7(16.7)	0.722	20(9.9)	3(3.7)	0.081
Respiratory Rate	34(41.0)	16(37.2)	0.683	34(17.0)	10(12.3)	0.331
Lymph nodes Matted	11(13.1)	4(9.3)	0.53	15(7.4)	0(0.0)	**0.02**
Discrete	11(13.1)	7(16.3)	0.626	26(12.8)	10(12.0)	0.86
Cervical	20(24.1)	10(23.8)	0.972	37(18.3)	10(12.0)	0.195
Axillary	6(7.2)	5(11.9)	0.383	22(10.9)	5(6.0)	0.198
Hair changes	23(27.4)	1(2.3)	**0.001**	43(21.2)	5(6.0)	**0.002**
Pallor	36(42.9)	12(27.9)	0.1	49(24.1)	10(12.0)	**0.02**
Pitting oedema	7(8.3)	0(0)	0.094	6(3.0)	2(2.4)	1.0
Kaposis sarcoma	9(10.7)	0(0)	**0.028**	12(5.9)	0(0)	**0.052**
Seborrh.Dermatitis	6(7.1)	0(0)	0.073	33(16.3)	3(3.6)	**0.003**
Herpes zoster	7(8.3)	0(0)	**0.051**	10(4.9)	0(0)	**0.040**
Herpes simplex	5(6.0)	0(0)	0.165	8(3.9)	4(4.8)	0.750
Tinea	8(9.5)	2(4.7)	0.493	24(11.8)	11(13.3)	0.738
PPE	23(27.4)	3(7.0)	**0.007**	55(27.1)	7(8.4)	**0.001**
Angular Stomatitis	10(11.9)	0(0)	**0.016**	46(22.7)	3(3.6)	**0.000**
Oral candidiasis	16(19.0)	0(0)	**0.002**	22(10.8)	0(0)	**0.002**
CrepitationS	53(63.1)	24(55.8)	0.427	116(57.1)	38(46.3)	0.098
Consolidation	4(40.5)	17(39.5)	0.918	49(24.1)	18(22.0)	0.694
Plural effusion	14(16.7	10(23.3)	0.369	24(11.8)	12(14.5)	0.542

Laboratory features of study population

Laboratory features by patients' sputum culture positivity are shown in Table 6a. There were no differences in a number of haematological parameters between sputum culture positive and negative patients, including Hb, WBC and ESR. However there were differences in WBC differentials; eosinophilia and lymphocytosis were more common among sputum culture negative compared to sputum culture positive. However table 6b shows that, the highest proportion of subjects with haemoglobin <12g/dl were observed in patients who were HIV positive

in both sputum culture groups (p<0.0001). Most patients with sputum culture negative had a normal neutrophil count more so in those who were HIV negative (p=0.005), while neutrophilia was noted to be more common in patients with sputum culture positive and HIV positive (28.8%) compared to the other three groups (p=0.003) (table 6b). Lymphopenia was common in HIV positive subjects in both culture positive and culture negative groups (p=0.004) while lymphocytosis was noted in culture negative HIV negative subjects (p=0.019). High ESR was noted in HIV positive subjects in both groups (p=0.004). Anaemia was reported in 73.6% of all study subjects. Normocytic anaemia was reported more commonly in HIV negative subjects in both culture positive and culture negative groups (p<0.0001), while a mixed type of anaemia was noted to be more prevalent in HIV positive subjects in both culture positive and culture negative groups (p=0.0001). As expected HIV negative subjects in both culture positive and culture negative groups (p<0.0001) had a positive mantoux test. There were no difference in HIV seroprevalence in the two culture groups (p= 0.32) (fig 3).

Figure3 HIV sero- status by culture results

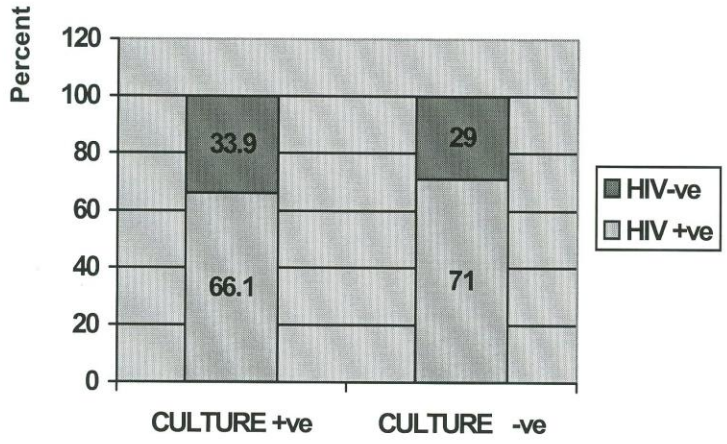


Table 6a

Laboratory features of the study population by patients' sputum culture results

Feature		Culture +ve N=127	Culture -ve N=286	P-value
Haemoglobin	<12g/dl	97(82.2)	207(72.3)	0.207
WBC	<4x10^9/l	35(29.4)	102(35.7)	0.112
	4-11x10^9/l	78(65.5)	152(55.7)	0.068
	>11x10^9/l	6(5.0)	18(6.6)	0.555
Neutrophil	<2x10^9/l	2(1.7)	4(1.5)	0.776
	2-7.5x10^9/l	87(73.1)	229(80.1)	**0.012**
	>7.5x10^9/l	30(25.2)	40(14.6)	**0.011**
Lyphocytes	<1.5x10^9/l	19(14.9)	22(7.8)	0.075
	1.5-4x10^9/l	73(57.4)	151(52.7)	0.353
	>4x10^9/l	27(22.7)	100(34.9)	**0.030**
Basophils	>1x10^9/l	15(13.4)	53(18.5)	0.08
Eosinophils	<0.4x10^9/l	40(31.5)	55(19.2)	**0.003**
	>0.4x10^9/l	72(64.3)	205(78.8)	**0.003**
Monocytes	>0.8x10^9/l	85(77.3)	202(70.6)	0.52
ESR (mm/hr)	<50	16(12.5)	43(15.0)	0.57
	>50	103(86.6)	231(80.8)	0.57
RBC Morphology				
Normocytic		50(42.4)	136(47.5)	0.155
Microcytic		22(18.6)	53(19.4)	0.859
Macrocytic		0(0)	5(1.8)	NS
Mixed		46(39.0)	78(28.6)	**0.042**
Mantoux (PPD)				
Negative		85(66.9)	197(68.8)	0.71
positive		43(33.1)	89(31.3)	0.71

Table 6b

Laboratory features of the study population by patients' sputum culture results and HIV sero-status.

Feature	Sputum culture+ n=127		P-value	Sputum culture- N=286		P-value
	HIV+ N=84	HIV- N=43		HIV+ N=203	HIV- N=83	
Haemoglobin <12g/dl	72(91.1)	25(64.1)	**0.000**	164(84.5)	43(55.8)	**0.000**
WBC <4x10^9/l	24(30.0)	11(28.2)	0.8	75(38.7)	27(34.6)	0.5
4-11x10^9/l	53(66.3)	25(64.1)	0.85	109(56.2)	43(55.1)	0.806
>11x10^9/l	3(3.8)	3(7.7)	0.64	10(5.2)	8(10.3)	0.11
Neutrophils <2x10^9/l	1(1.3)	1(2.6)		3(1.5)	1(1.3)	
2-7.5x10^9/l	56(70.0)	31(79.5)	0.25	157(80.5)	72(92.3)	**0.017**
>7.5x10^9/l	23(28.8)	7(17.9)	0.19	35(17.9)	5(6.4)	**0.015**
Lymphoc <1.5x10^9/l	16(20.0)	3(7.7)	0.08	20(10.3)	2(2.6)	**0.036**
1.5-4x10^9/l	45(56.3)	28(71.8)	0.09	109(55.9)	42(53.8)	0.71
>4x10^9/l	19(23.8)	8(20.5)	0.66	66(33.8)	34(43.6)	0.11
Basophils >1X10^9/l	11(14.5)	4(11.1)	0.626	37(20.3)	16(21.9)	0.778
Eosinophils<0.4x10^9/l	29(38.2)	11(30.6)	0.433	44(23.7)	11(15.1)	0.128
>0.4x10^9/l	47(61.8)	25(69.4)	0.433	142(76.3)	62(84.9)	0.128
Monocytes 0.8x10^9/l	56(73.7)	29(85.3)	0.179	146(80.2)	56(80.0)	0.969
ESR (mm/hr) <50	8(10.0)	8(20.5)	0.115	22(11.3)	21(26.6)	**0.002**
>50	72(90.0)	31(79.5)	0.115	173(88.7)	58(73.4)	**0.002**
RBC Morphology						
Normocytic	25(31.6)	25(64.1)	**0.000**	83(42.8)	53(67.9)	**0.000**
Microcytic	14(17.7)	8(20.5)	0.71	41(21.1)	12(15.4)	0.298
Macrocytic	0(0)	0(0)		3(1.5)	2(2.6)	0.93
Mixed	40(50.6)	6(15.4)	**0.000**	67(34.5)	11(14.1)	**0.000**
Mantoux (PPD)						
Negative	82(97.6)	3(7.0)	**0.000**	192(94.6)	5(6.0)	**0.000**
positive	2(2.4)	40(93.0)	**0.000**	11(5.4)	78(94.0)	**0.000**

Radiographic appearances

Table 7a shows radiological findings of the study population from PA chest radiographs. Upper zone involvement was reported more commonly among patients with sputum culture positive 96(75.6%) compared to those who had culture negative 147(51.9%). The commonest appearance were patchy opacification (45.7%), reticular nodular shadowing (24.4%) and homogeneous opacity (5.5%). Cavitations were noted more among culture positive 25(19.7%) subjects than negative 5(1.8%) ($p<0.000$). Cavities were seen more in the upper and middle lobes in both left and right side ($p<0.05$). Among patients who had sputum culture positive, cavities were more frequent in HIV negative compared to HIV positive (Table 7b).

Table 7a

Chest radiological findings of patients by their sputum culture results

		Culture+ N=127	Culture- N=286	P-value
RT upper				
	Small nodule	16(12.6)	22(7.8)	0.119
	Patchy	30(23.6)	52(18.4)	0.219
	Homogenous	6(4.7)	7(2.5)	0.369
	Cavity	14(11.0)	1(0.3)	**0.000**
RT Middle				
	Small nodule	23(18.1)	26(9.2)	**0.01**
	Patchy	48(37.8)	128(44.7)	0.140
	Homogenous	7(5.5)	9(3.2)	0.394
	Cavity	7(5.5)	4(1.3)	**0.018**
RT. Lower				
	Small nodule	17(13.4)	28(10.3)	0.35
	Patchy	39(30.7)	84(29.8)	0.85
	Homogenous	7(5.5)	18(6.4)	0.73
	Cavity	4(3.1)	3(1.0)	0.27
LT upper				
	Small nodule	15(11.8)	17(6.0)	**0.04**
	Patchy	28(22.0)	45(15.9)	0.13
	Homogenous	1(0.8)	4(1.4)	0.96
	Cavity	15(11.8)	1(0..35)	**0.000**
LT middle				
	Small nodule	15(11.8)	26(9.2)	0.41
	Patchy	58(45.7)	112(39.2)	0.27
	Homogenous	2(1.60	7(2.5)	0.83
	Cavity	9(7.1)	2(0.6)	**0.0007**
LT lower				
	Small nodule	13(10.2)	27(9.5)	0.82
	Patchy	38(29.9)	78(27.3)	0.67
	Homogenous	6(4.7)	16(5.7)	0.69
	Cavity	4(3.1)	2(0.6)	

Table 7b

Chest radiological findings of patients by their sputum culture results and HIV serostatus

		Sputum culture+ n=127		P-value	Sputum culture- N=286		P-value
		HIV+ N=84	HIV- N=43		HIV+ N=203	HIV- N=83	
RT upper							
	Small nodule	10(11.9)	6(14.0)	0.7	17(8.5)	5(6.2)	0.53
	Patchy	19(22.6)	11(25.6)	0.7	35(17.5)	17(21.0)	0.47
	Homogenous	4(4.8)	2(4.7)	0.67	4(2.0)	3(3.7)	0.67
	Cavity	8(9.5)	6(14.4)	0.451	0(0)	1(1.2)	0.287
RT Middle							
	Small nodule	18(21.4)	5(11.6)	0.17	20(10.0)	6(7.4)	0.5
	Patchy	31(36.9)	17(39.5)	0.77	92(46.0)	36(44.4)	0.8
	Homogenous	4(4.8)	3(7.0)	0.91	7(3.5)	2(2.5)	0.93
	Cavity	3(3.6)	4(9.3)	0.18	3(1.5)	1(1.2)	1.00
RT. Lower							
	Small nodule	13(15.5)	4(9.3)	0.33	22(11.1)	6(7.4)	0.37
	Patchy	28(33.3)	11(25.6)	0.37	59(29.6)	25(30.9)	0.84
	Homogenous	4(4.8)	3(7.0)	0.91	13(6.5)	5(6.2)	0.93
	Cavity	1(1.2)	3(7.0)	0.077	2(1.0)	1(1.2)	1.00
LT upper							
	Small nodule	11(13.1)	4(9.3)	0.53	11(5.5)	6(7.4)	0.725
	Patchy	15(17.9)	13(30.2)	0.11	28(14.0)	17(21.0)	0.138
	Homogenous	0(0)	1(2.3)		3(1.5)	1(1.2)	0.692
	Cavity	6(7.1)	9(20.9)	**0.023**	0(0)	1(1.2)	0.287
LT middle							
	Small nodule	10(11.9)	5(11.6)	0.96	20(10.0)	6(7.4)	0.51
	Patchy	34(40.5)	24(55.8)	0.100	74(37.0)	38(46.9)	0.12
	Homogenous	1(1.2)	1(2.3)		5(2.5)	2(2.5)	0.67
	Cavity	4(4.8)	5(11.6)	0.154	0(0)	2(2.5)	**0.025**
LT lower							
	Small nodule	8(9.5)	5(11.6)	0.95	21(10.5)	6(7.4)	0.43
	Patchy	26(31.0)	12(27.9)	0.72	52(26.0)	26(32.1)	0.32
	Homogenous	4(4.8)	2(4.7)	0.67	11(5.5)	5(6.2)	0.96
	Cavity	1(1.2)	3(7.0)	0.077	1(0.5)	1(1.2)	0.493
	Hilar LN	3(3.6)	2(4.8)	1.000	14(6.9)	6(7.4)	0.879

The sensitivity and specificity of diagnostic criteria for respiratory conditions at the clinics.

Table 8 shows presumptive working diagnosis and treatment offered to study subjects in relation to later diagnosis based on sputum culture results. 173 (41.8%) patients had a presumptive working diagnosis of Pulmonary Tuberculosis and were started on anti TB drugs and 240(58.2%) were treated with other antibiotics for their respiratory problems. 66(51.9%) of those who had sputum culture positive were correctly started on anti TB. However 107 (37.4%) patients of those who had sputum culture negative were also started on anti TB, Fig 4. Only 38.1 % (66) of those 173 treated for pulmonary tuberculosis had TB confirmed by culture. 61(48.1%) of patients who had sputum culture positive were missed and were incorrectly treated. 179(62.6%) were correctly identified to have no PTB. As observed the diagnostic procedure at the clinics had a sensitivity of 52% and a specificity of 62.6%. The positive predictive and the negative predictive diagnostic value were 38% and 74.5% respectively.

Fig 4. Diagnosis and treatment of study subjects in relation to their sputum culture Results

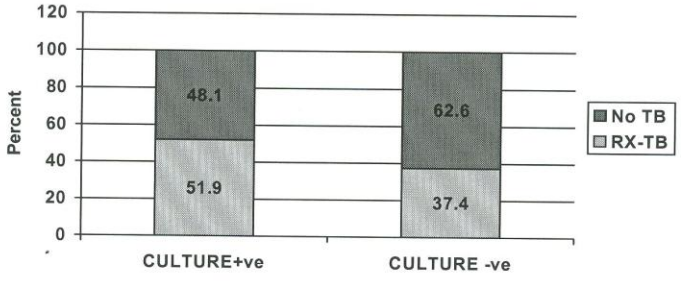


Table 8

Diagnosis made using culture results and Treatment given

Treatment given	Culture +ve	Culture –ve	Total
	n= (127)	n=(286)	N=413
Ant-TB	66(51.9)$	107(37.4)$	173(41.8)
	(38.1)#	(61.9)#	
Antibiotics	61(48.1)$	179(62.6)$	240(58.2)
	(25.4)#	(74.6)#	

$ - Column percentages

- Row percentages

Table 9

Sensitivity and specificity of the diagnostic procedure of patients with smear negative

TB treatment	Disease status		Total
	Culture +ve	Culture -ve	
Yes	66	107	173
No	61	179	240
Total	127	286	413

Sensitivity = $\frac{TP}{TP+FN} = \frac{\text{True positive}}{\text{Total positive}} = \frac{66}{127} = 52\%$

Specificity = $\frac{TN}{TN+FP} = \frac{\text{True negative}}{\text{Total negative}} = \frac{179}{286} = 62.6\%$

Positive predictive value = $\frac{\text{Diseased}}{\text{Positive test}} = \frac{66}{173} = 38.1\%$

Negative predictive value = $\frac{\text{Health individual}}{\text{Test negative}} = \frac{179}{240} = 74.5\%$

Table 10
Diagnosis made and Treatment given by subjects' sputum culture results and HIV sero-status.

Treatment given	Culture +ve		Culture –ve	
	HIV +ve N=84	HIV –ve n=43	HIV +ve n=203	HIV-ve n=83
Anti-TB	41(48.8)	25(58.1)	70(34.5)	37(46.3)
Antibiotics	43(51.2)	18(39.5)	133(65.5)	46(53.7)

Of those who were presumptively diagnosed to have TB, the diagnosis of TB was correctly established in HIV negative (58.1%) more than positive subjects (48,8%).

Clinical and laboratory characteristics predictive of sputum culture positive PTB

As shown in table 11, using an unadjusted logistic regression to determine characteristics that can predict sputum culture positivity six characteristics were found to be associated with positive sputum culture results. Patients with matted lymph node had three times more risk of being culture positive compared to those who had no matted lymphadenopathy (p-0.03). Those who had tachypnoea (RR >20) had 2.5 more risk of being culture positive than those who had normal respiratory rate (p=0.005), likewise those who reported to have a dry cough had an OR of two for having sputum culture positive compared to those who had no dry cough (p-0.028). Eosinophilia was also found to be associated with a 50% less chance of being sputum culture positive. (p=0.01). The presence of a cavity had an OR of 74 for sputum culture positive results compared to absence of cavity (p-0.0001). Presence of mixed type of anaemia had two times more risk of being culture positive

than those who had normocytic anaemia (p-0.03), while presence of microcytic anaemia was associated with a 20% less risk of being culture positive.

Table 11
Unadjusted bivariate logistic regression results of predictors of sputum culture positive.

Characteristics		OR	Confidence interval	P-value
Dry cough	Yes	2	1.1-4.6	**0.0287**
	NO	1		
Resp rate	>20/min	2.5	1.3-4.8	**0.005**
	<20/min	1		
Lymphnode	None	1		
	Matted	2.8	1.1-7	**0.03**
	Discrete	1.2	0.5-2.7	0.6
Eosinophilia	<0.4	1		
	>0.4	0.5	0.2-0.8	**0.01**
Cavity	Abscent	1		
	Present	74	9.2- 598	**0.0001**
	Cavity right mid	11	0.8-139.7	**0.06**
	Cavity other sites			NS
RBC orphology	Normocytic	1		
	Microcytic	0.8	0.4-1.7	0.6
	Macrocytic	0.01	0.000-407516	0.7
	Mixed	2	1.1-3.5	**0.003**

In an attempt to develop supplemental method for diagnosing smear negative pulmonary tuberculosis multiple logistic regression analysis of the data was done to try establish clinical and laboratory characteristics that will predict the presence of sputum culture positive. Using this analysis, dry cough, high respiratory rates, low eosinophil counts, mixed type of anaemia and the presence of cavities on X-rays were associated with culture positivity, as shown in table 12.

Table12
Multivariate analysis of clinical characteristics predictive of smear negative culture positive PTB

Characteristics		OR	Confidence interval	P-value
Dry cough	Yes	2.5	1.2-5	**0.0097**
	NO	1		
Respiratory rate	>20/min	2.4	1.2-4.4	**0.0054**
	<20/min	1		
Eosinophilia	<0.4	1		
	>0.4	0.5	0.2-0.8	**0.0094**
Cavity	Abscent	1		
	Present	74.3	9.4- 587	**0.0000**
RBC morphology	Normocytic	1		
	Microcytic	0.8	0.4-1.7	0.6
	Macrocytic	0.01	0.000-39171193	0.69
	Mixed	2	1.2-3.7	**0.01**

9. Discussion

Study population

The study population was drawn from a population of people attending TB clinics on suspicision of having pulmonary tuberculosis because of the respiratory symptoms that patients had, that were suggestive of pulmonary tuberculosis. Out of the 3246 subjects who sent sputum for AFB smear, only 403(12.4%) had sputum smear positive and were started on anti-TB. This low level of sputum smear positivity for AFB, may be contributed by the high proportion of HIV positive among individuals suspected to have PTB. The co-existence of HIV and Tuberculosis has been cited to be associated with a high proportion of sputum smear negative when examined under the light microscope using Ziehl-Neelsen stain for AFB.[88,94], In this study the proportion of HIV positive among our study population was high 68.1%.

The study population consisted of young and middle aged patients with a mean age of 34.16. The study consisted of males (52.5%) more than females (47.5%) indicating a high prevalence of tuberculosis among males, similar findings have been reported.[88] Patients seen at these clinics reported rather late for their respiratory problems, they had respiratory symptoms for at least three months before seeking medical advice. This observation has also been reported by other researchers.[97] The delay may have been contributed by a number of factors including the ability of study subjects to pay for their medical services and poor patient education

HIV sero-prevalence in the study population

This study has shown a very high HIV prevalence of sero-positivity (68.1%) among smear negative patients who attended the TB clinics on suspicion of having pulmonary tuberculosis. These results are similar to reports from other studies, which indicated that HIV sero-prevalence ranged from 20-73% among patients with pulmonary tuberculosis in sub-Saharan Africa.[18,24,40] However there were no significant differences in HIV sero-prevalence between those patients who had sputum culture positive and negative results. The existing association of HIV with respiratory conditions other than tuberculosis may have blunted the differences.[49] HIV positive sero-prevalence among our study patients like in many other studies was higher in the young and middle age groups and more so in females than males. For example more than 40% of all HIV positive patients were in the age group 30-39 years. One study by Man Po Lee et al found that the majority of patients co-infected with TB and HIV were actually males.[98] Like in many other studies HIV infected males were significantly older than female patients. These findings are also similar to those documented by the Tanzania National AIDS control programme which reported that HIV prevalence was higher in young age group among women compared to men.[86]

Sputum examination

The gold standard for the diagnosis of Tuberculosis involves the isolation and identification of Mycobacterium Tuberculosis (MTB) using cultures.[99] However because of the cost involved in the procedure and the absence of Laboratories capable of doing cultures in most developing countries, sputum smear results are

used for initiation of treatment and monitoring of patients' treatment and cure.[49, 99] In some cases when sputum smears are negative but the patient has features highly suggestive of pulmonary tuberculosis, broad-spectrum antibiotics are recommended for at lest 10-14 days and sputum smears repeated. If the patient's condition does not improve on broad spectrum antibiotics and sputum smears are still negative, a chest radiograph is done and if found to be abnormal, a presumptive diagnosis of pulmonary tuberculosis is then established and the patient is started on anti-Tuberculosis treatment, and he/she is classified to have AFB negative pulmonary tuberculosis.[99] From this study it is apparent that about one third (27.2%) of all those who had three negative sputum smear had active TB as evidenced by positive culture results of their sputum.

Presenting symptoms and signs of patients

Study patients presented with symptoms that were highly suggestive of Tuberculosis, such as, cough both dry and productive. Other symptoms included constitutional symptoms such as fever, weight loss, nausea, vomiting, anorexia, and dysphagia. There were however differences in presenting symptoms among patients whose sputum cultures were positive and negative. While dry cough was reported more common among sputum culture positive, productive cough was more in culture negative patients. Even in cases where patients reported to have dry cough, these patients were able to produce sputum when properly instructed. Dyspnoea at rest, numbness, limb weakness and arthralgia were also found to be significantly higher in culture positive patients than culture negative patients. No explanations for these findings could be found.

Tuberculosis is a wasting disease regardless of ones sero-status, however it is more so in the presence of HIV infection, which is also a very significant wasting disease. Weight loss was observed in culture positive patients regardless of their sero status because of the strong influence of Pulmonary Tuberculosis. Weight loss that was found in sputum culture negative patients were mainly in those who were HIV positive (p-0.025). In a book by Mwaluko et al. it was shown that weight loss although a feature of Tuberculosis is also a manifestation among Tanzanian patients infected with the HIV virus [31]. Symptoms, which were noted to be more common in HIV positive patients in both culture positive and culture negative patients, were odynophagia, itching, and the presence of skin disease.[9]

Patients with previous pulmonary diseases other than tuberculosis were found to have a higher frequency of sputum culture positivity. These previous respiratory infections could have been pulmonary tuberculosis that was misdiagnosed as pneumonia or any other respiratory diseases. The majority of patients who had a previous pulmonary disease and sputum culture positive were HIV positive probably indicating that co-infection of HIV and TB may lead to negative sputum smears.

Fever, high pulse rate, high respiratory rate, matted lymphadenopathy, pallor and consolidation were more common in patients whose sputum culture was positive than negative. The presence of fever, matted lymph nodes, pallor and consolidation are features reported in patients with TB[100]. These features were common in sputum culture positive regardless of their HIV sero status. However hair changes, kaposi's sarcoma, seborrheic dermatitis, pruritic eruption, and angular stomatitis were

common in HIV positive patients in both culture positive and culture negative patient groups. ($p<0.05$). Some features such as the presence of Kaposi's Sarcoma are AIDS defining, other signs are also common in patients with HIV infections in Africa.[34,38,40]

Other Laboratory features

Laboratory observations were essentially similar between sputum culture positive and negative patients, except for higher proportions of patients with neutrophilia in culture positive (25.2%) compared to negative patients (14.6%) (p-0.011) and Lymphocytosis in patients with culture negative (33.6%) compared to the culture positive group 22.7% (p-0.03). These findings are similar to those found by Aris EA [100] and Moris,[101] in which they reported neutrophilia and lymphopaenia to be more common in patients with active pulmonary tuberculosis. Red blood cell morphology showed that those who had sputum culture positive had a higher proportion of a mixed type of anaemia. Tuberculosis as a chronic disease is associated with normochromic, normocytic anaemia[101], however because most patients with pulmonary tuberculosis may also have other causes of anaemia in our set up including nutritional deficiencies, most patients may present with a mixed type of anaemia.

Anaemia, Lymphopenia and an elevated ESR were observed more commonly in HIV positive patients in both sputum culture positive and negative groups than HIV negative patients. These haematological derangements have been reported in a number of studies among HIV positive patients.[83,84,102] These derangements may be

partly due to HIV infection itself which does suppress bone marrow function leading to some degre of pancytopenia.[83,84] As a feature of immunosuppresion negative PPD tests were also observed commonly in HIV positive than negative patients indicating a profound immunosuppresion in some of the HIV positive that leads to anergy.[9, 103,104]

Chest radiographs

Although chest radiography alone does not establish the diagnosis of Tuberculosis, it is however a very important screening tool that may indicate the need for further investigation for MTB or help to establish a presumptive diagnosis of pulmonary tuberculosis. Common features that help in establishing the diagnosis of pulmonary tuberculosis in HIV negative patients include; patchy opacification mainly affecting the apical segments, hilar and mediastinal adenopathy and the presence of cavities.[105,106,107,108,109,110,] However as noted by other studies, chest radiographic findings in HIV infected patients with pulmonary tuberculosis could be atypical, with lower and middle lobe involvement, lack of cavities, a high proportion of hilar and mediastinal adenopathy.[9,111,112] In this study, most radiographs were reported to be normal even in patients who were found to have active tuberculosis. This further confirms the need to examine the patients' sputum in those suspected to have active tuberculosis by their symptoms even when they have normal chest radiographs. For those radiographs reported to be abnormal, reticular nodular shadowing mainly in the middle lobe was reported to be more common in HIV positive patients with sputum culture positive (p-0.0057) Cavities mainly in the upper lobes as expected were observed more in patients with sputum culture negative patients who were HIV

negative. (p-0.00023). Radiological findings associated with severe immunosuppression in HIV infection are similar to those seen in primary tuberculosis.[36]

Diagnosis and treatment of study subjects

Patients whose sputum smears were AFB negative, were evaluated by the treating doctors at the clinics or hospital, a presumptive diagnosis of AFB negative pulmonary tuberculosis was made in 41.8%(173) of all study subjects, and patients were started on anti-TB treatment as recommended by the Tanzania National TB and Leprosy program. The remaining 58.2% (240) patients were assumed to have other forms of respiratory diseases and were treated accordingly. However less than a half (only 38.1%) of those who were presumed to have active TB and started on Anti TB actually had TB by sputum culture results. More than 60% received an unnecessary 8-month treatment of a probably non-existing TB disease. Likewise about 48% (61) of patients who had active tuberculosis by the results of sputum culture were missed and they received inappropriate treatment leaving them to develop severe disease and to be a source of TB infection in the community. The current diagnostic procedure leading to the establishment of a presumed diagnosis of AFB smear negative pulmonary tuberculosis is inefficient, it over diagnoses pulmonary tuberculosis and misses a lot of people with active disease as indicated by the low sensitivity and specificity of the whole procedure. In an attempt to improve on the diagnostic procedure, the following clinical features were found to be highly predictive of sputum culture positive and hence to identify individuals with sputum smear negative who could be having Active TB, these include: a reported dry cough, high respiratory

rates, low eosinophil counts, a mixed type of anaemia and the presence of cavities on chest radiographs.

Clinical characteristics of PTB in HIV infection

The prevalence of HIV positive among patients with sputum culture positive was similar to those who had sputum culture negative, this similarity as indicated above may be due to the fact that HIV positivity may also be associated with other respiratory diseases other than Tuberculosis that are common in HIV infected individuals. A univariate and multivariate analysis aimed at identifying features predictive of sputum culture positive did not find ones serostatus to be a good predictor of sputum culture positivity.

10.Conclusion

- All patients who are investigated for pulmonary tuberculosis and found to have sputum smear negative results at least a third have active disease when sputum culture is done
- The current procedures of establishing AFB negative pulmonary tuberculosis are not sensitive enough to establish the correct diagnosis of active tuberculosis. They under diagnose pulmonary tuberculosis and over treats people with no pulmonary tuberculosis.
- The presence of a dry cough, a high respiratory rate, a low eosinophil count, a mixed type of anaemia and presence of a cavity were found to be predictive of smear negative but culture positive pulmonary tuberculosis.

- The prevalence of HIV among patients with sputum culture positive was similar to those who had sputum culture negative

11. Recommendations

1. Adherence to the WHO recommended algorithm [99] should be encouraged to minimize errors in the diagnosis and treatment of smear negative pulmonary tuberculosis
2. A much more sensitive diagnostic algorithm for smear negative pulmonary tuberculosis should be developed to be able to identify those individuals who are actually sputum culture positive for AFB
3. Features such as dry cough, high respiratory rate, low esinophil count, mixed type of anemia and other symptoms and signs should be further evaluated to determine their role in predicting smear negative but culture positive pulmonary tuberculosis
4. Follow up of individuals who are smear negative, treated with antibiotics should be done to identify individuals who have pulmonary tuberculosis•

Limitations of the study:

The study was not able to do CD4 cell counts to determine the level of immuno suppression that would have helped to find out whether the level of immuno-suppression influences the sputum smear and culture results.

12. References

1. Hirsch A, Handbook of Geographical and Historic al Pathology, Vol. III. London: The new Syndeham Society 1886.
2. Cummins SL. Tuberculosis in primitive tribes and its bearing on the tuberculosis of civilized communities. Int J. Pub Health 1920; 1:137
3. Lichtenstein H; Plumptre A (trans). Travel in Africa 1803, 1804, 1805, 1806 (reprint). Cape Town: The van Rierberck Society, 1928
4. Livingstone D. Missionary Travels and Research in South Africa. London: Ward Lock, 1857.
5. Grzybowski S. Tuberculosis in the third world. Thorax 1991; 46:689-703
6. Donald A., Earson John F. Murray. Global epidemiology of Tuberculosis. William N. Rom, Stuart M. Garay (Eds). Tuberculosis first edition. Brown and company 1996.
7. Millar JG. On the spread and prevention of tuberculosis disease in Pond land, South Africa. BMJ; 1908; 1:380-383.
8. American Thoracic Society/CDC. Diagnostic standards and Classification of Tuberculosis. Am Rev. Respir Dis. 1990; 142:725-35
9. Davidsons. Tuberculosis. Haslett C, Chilvers ER, Hunter JAA, Boom NA (Eds). Principles and Practice of Medicine 18th Edition. Pg347. Churchill Livingstone 1999.
10. Wayner LG, Kubica GP. The Mycobacteria. In PHA sneath et al (Eds), Bergey's Manual of systematic Bacteriology, Bultimore: Williams and Wilkeens, 1986. Pg. 1435 – 1457.

11. Timpe A, Runyon E.H.: The relationship of "atypical" acid fast bacteria to human disease: A preliminary report. J. Lab Clin Med 1954; 44: 202.

12. Davies P.D.O, Girling D.J and Grange J.M. Tuberculosis and its problem in developing countries. Weatherall DJ, Ledingham, Warrell DA (eds). In Oxford text book of Medicine. 3rd edition, vol 1 pg 639. Oxford University Press 1996.

13. American Thoracic Society. Treatment of tuberculosis and tuberculosis infection in adults and children and children. Am Rev Respir Dis 1986; 134: 355-363.

14. Murray JF, et al. Pulmonary complications of the acquired immunodeficiency syndrome. Report of the National Heart, Lung, and Blood institute Workshop. N. Engl. J. Med 1984; 310: 1682-1688.

15. World Health Organization, Global Programme on AIDS. The current global situation of the HIV/AIDS pandemic. Wkly Epidemiol Rev. 1994; 69: 189-196

16. Barnes PF, Barrows SA. Tuberculosis in the 1990s. Ann Intern. Med. 1993; 119: 400-410

17. Murray JF, J. Burns Amberson lecture. The White plaque: Down and out, or up and coming? Am. Rev. Respir. Dis. 1989; 140: 1788-1795.

18. Raviglione MC, Narain JP, and Kochi A. HIV associated tuberculosis in developing countries: Clinical features, diagnosis and treatment. Bull WHO 1992, 70: 515-526

19. Sudre P, ten Dam G, Kichi A, et al. Tuberculosis: A global overview of the situation today. Bull WHO 1992; 70: 149-159.

20. Alan B. Bloch, MD. M.P.H. Screening for Tuberculosis and Tuberculosis infection in High-Risk populations. Recommendations of the Advisory Council for the Elimination of Tuberculosis 1995. MMWR 44(RR-11); 18-34.

21. Standaert B, Niragua F, Kadende P, Pilot P. The association of tuberculosis and HIV infection in Burundi. AIDS research and human retroviruses 1989; 5: 247 – 251.

22. Nunn P et al. The effect of human immunodeficiency virus type 1 on the infectiousness of tuberculosis. Tubercle Lung Dis. 1994; 75: 25-32

23. Barnes PF, Block AB, Davison PT, Sruder DE, J. Tuberculosis in patients with human immunodeficiency virus infection. N. Engl. J. Med 1991; 324: 1644-1650

24. Thomas M. Daniel. Tubeculosis. Isselbacher KJ, Brawnwald E, wilson JD, Martin JB, Fauci AS, Kasper DL (Eds) In: Harrison's principles of Internal Medicine. Vol. 1 13th edition pg 710,713 and 714. Mc Graw-Hill, Inc 1994.

25. World Health Organization Geneva 1988. TB Control as an Integral part of primary health care pg. 11.

26. Bender BS, Davidson BL, Kline R, Brown C, Quinn TC. Role of mononuclear phagocyte system in the Immunopathogenesis of human immunodeficiency syndrome Rev. Infect Dis 1988; 10: 1142-1154

27. Selwyn PA, Harfel D, Lewis VA, Schoenbaum E, Vermund S, Klein R, et al. A prospective study of the risk of tuberculosis among intravenous drug users with human immunodefiency virus infection N. Engl. J. Med 1989; 320: 545-550

28. Guelar A, Gatell ajm, Averdejo J et al. A prospective Study of the risk of tuberculosis among HIV-infected patients. AIDS 1993; 7: 1345-9.

29. Orme IM, Andersen P, Boom WH. T cell response to Mycobacterium tuberculosis. J. Infect. Dis. 1993; 167: 1481-1497

30. Orne IM, Miller ES, Roberts, et al T. lymphocytes mediating protection and cellular cytolysis during the course of mycobacterium tuberculosis infection. J. Immunol. 1992; 148: 189-196

31. Mwaluko G.M.P, Kilama W.L, Mandara M.P, Mturu M, Maipherson C.N.L. Health and disease in Tanzania pg. 73. Harper Collins Academic 1991.

32. Elliott AM, Halwandi B, Hayes RJ, Luo N, Tembo G, Machiels L, et al. The impact of human immunodeficiency virus on presentation and diagnosis of tuberculosis in a cohort study in Zambia. J. Trop. Med Hyg. 1993: 96; 1-11

33. D. Cock K.M., Gnaire E, Adjorlolo G, et al: Risk of tuberculosis in patients with HIV – 1 and HIV-2 infection in Abidjan Ivory Coast. BMJ 1991; 302: 496 – 499.

34. Kelly P, Barnhan G, Roadford C: HIV seropositivity and tuberculosis in rural Malaria hospital. Trans Royal Soc Trop Hyg 1990 ; 84 : 725 – 727.

35. Moshi A.H.: A study of outcome in HIV seropositive and HIV seronegative patients with pulmonary tuberculosis admitted to the medical ward in Muhimbili Medical Centre DSM, Tanzania. A dissertation submitted in partial fulfillment of the requirement for the degree of master (MMED) in internal medicine in University of Dar-es-salaam 1994.

36. Man Po LEE, Johnny Wai Man Chan, Ka Ku Philip NG and Patrick Chung KiLI. Clinical manifestation of Tuberculosis in HIV-infected patients. Respirology 2000; 5:423-426

37. Eric PP, Okwera A, Aisu T, Morrissey AB, Ellner JJ, Danul T.M.: The influence of HIV infection on TB in Kampala, Uganda. Am Rev Respir Dis 1991; 143: 185 – 187.

38. Colebunders RL, Ryder RW, Nzilambi N, Dikilu K, Willame JC, Kabito M, et al. HIV infection in patients with tuberculosis in Kinshasa, Zaire. Am Rev Respir. Dis 1989; 139:1082-1085.

39. Mukadi Y, Perriens J, Michael E, Brown C, Prignot Sr. Louis ME, Willame JC, et al. Spectrum of immunodeficiency in HIV infected patients with PTB in Zaire. Lancet 1993; 342: 143 – 46.

40. Elliot AM, Luo N. Impact of HIV infection in Zambia A cross sectional study. BMJ 1990; 301; 412-415

41. Shaw JB, Wynn-William N, Infectivity of pulmonary tuberculosis in relation to sputum status. Am Rev Tuberc. 1954; 69:724-732

42. Jack J. Adler, David N. Rose. Transmission and pathogenis of tuberculosis. William N. Rom, Stuart M. Garay (Eds). Tuberculosis fist edition pg 129. Little, Brown and company 1996.

43. Gupta RC, Lavengood R, Smith JP. Military tuberculosis due to intravesical bacillus calmette-Guerin therapy. Chest 1988; 94: 1296-8

44. Ninane J, Grymonpriz A, Burtonboyg, et al. Disseminated BCG in HIV infection. Arch Dis Child 1988; 63: 126-89

45. Disseminated Mycobacterium bovis infection from BCG vaccination of a patient with acquired immunodeficiency syndrome. MMWR 1985; 34: 227-8

46. Frottier J, et al. Les Infectious a Mycobacterium African. Bull Acad Nat Med 1990: 174:29-33

47. Alfredson S, Saxegaard F. An outbreak of tuberculosis in pigs and cattle caused by Mycobacterium African. Vet Rec 1992; 131:51-3.

48. Grange JM, Yates MD Incidence and nature of human tuberculosis due to Mycobacterium Africanum in South East England: 1977-87 Epidem. Inf. 1989; 103: 127-32

49. Harries AD, Maher D. TB/HIV A clinical manual, World Health Organization 1996 pg 20.

50. Centers for disease control. National action plan to combat multidrug resistant tuberculosis. MMWR 1992; 41(RR-11): 1-48

51. Des Prez RM, Heim CR. Mycobacterium tuberculosis. In GL, Mandell RF Douglas Jr, JE Bennett (Eds), Principles and Practice of infectious Disease (3rd ed) New York: Churchill Livingstone 1990. Pp 1877-1906

52. Schlesinger LS, Bellinger-Kahawara CG, Payne NR, Horwitz MA, Phagocytosis of Mycobacterium tuberculosis is mediated by human monocyte complement receptors and complement component C3. J. Immunol 1990; 144: 2871-2780

53. Hirsch CS, Ellner JJ, Russel DG, Rich EA. Complement receptor- mediated uptake and tumor necrosis factor alpha mediated growth inhibitor of Mycobacterium tuberculosis by human alveolar macrophages. J. Immunol. 1994; 152: 743-753.

54. Luck, Chan YF, Har CMY. Congenital tuberculosis Aut Paediatr. J. 1989; 25: 366-7

55. Cartwell MF, et al. Congenital tuberculosis. N. Engl J. Med 1994; 14: 1051-5

56. Peters TB, Reiter CG, Bosell RL. Transmission of tuberculosis by kidney transplantation. Transplanta 1984; 38: 514-16.

57. Amstrong JA, D Arcy Hart P. Response of cultured macrophages to Mycobacterium tuberculosis, with observation on fusion of lysosomes with phagosomes. J. Exp. Med. 1971; 134:713-740

58. Moulder JW. Comparative biology of intracellular parasitism Microbial Rev. 1985; 49: 292-337

59. Mc Donough KA, Kress Y, Bloom BR. Pathogenesis of tuberculosis interaction of Mycobacterium tuberculosis with macrophages. Infect Immn. 1993; 61: 2763-2773

60. Stahelin H, Karnovsky ML, Inter E. Studies on the interaction between phagocytes and tubercle bacilli. The action of phyocytes upon 14C – labeled tubercle bacilli. J. Exp. Med 1956; 104: 137-150

61. Murray H.W, Juangbhanich CW, Nathan CF, Cohn ZA. Macrophaye 02-dependent antimicrobial activity. J. Exp Med 1979; 150: 950-964

62. Nathan CF, Murray HE, Wiebe ME, Rubin by Identification of interferon and as the lymphokine that activates human macrophage oxidative metabolism and antmicrobial activity. J. Exp Med. 1983; 158: 670-689

63. Barnes PF, Chatterfee D, Abrams JS, et al. Cytokine production induced by M. tuberculosis lipoarabinomannan. Relationship to chemical structure. J. Immunol 1992; 149:541-547

64. D. Andren A, Rengaraja M, Valiante NM, et al. Production of natural killer cell stimulating factor (interleukin 12) by peripheral blood mononuclear cells. J. Exp Med 1992; 176: 1387-1398.

65. Roach TI, Barton CH, Chatterjee D, et al. Mainphage activation: Lipoarakinomannan from avinilent and virulent strains of Mycobacterium

tuberculosis differentially induces the early genes c-fos, KC, JE and tumor necrosis factor & J. Immunol 1993; 150:1886-1896

66. Orme IM, Collins FM. Protection against Mycobacterium tuberculosis infection by adaptive immunothrapy. J. Exp Med. 1983; 158:74-83
67. Toossi Z, Kleinherz ME, Ellner JJ. Defective IL-2 production and responsiveness in human tuberculosis. J. Exp. Med. 1986; 163: 1162-1172
68. Krause AK. A few observations on immunity to tuberculosis. Am Rev Respir Dis 1922: 19: 306-308
69. Slutkin G, Leowki J, Mann J. The effect of the AIDS epidemic on the tuberculosis programme. Bull Int Union Tuberc Lung Dis 1988; 63: 21-24
70. Susan M. Graham, M.P.H., Phyllis E. Cruize. Prevention and Control of Tuberculosis in Correctional Facilities 1996. MMWR 45 (No. RR-8).
71. Anergy skin testing and preventive therapy for HIV infected persons: Revised Recommendations. MMWR 1997/vol46/No.RR-15 US.
72. Allen BW, Mitchson DA. Counts of viable tubercle bacilli in sputum related to smear and culture gradings. Med Lab Sci 1992; 49: 94-98
73. Roberts GD, Koneman EW, Kim YK. Mycobacterium. In a Balows (Ed). Manual of clinical Microbiology (5^{th} ed). Washington, D.C.: American Society for Microbiology 1992. Pp 304-309
74. Kent PT, Kubica GP. Public Health Mycobacteriology: A guide for the level III laboratory. Atlanta: US department of Health and Human Services Centres for Disease Control 1985.
75. Rieder HL, Chonde TM, Myking H .The Public Health Service National Tuberculosis Reference Laboratory and the National Laboratory network.

Minimum requirements, role and operation in a low-income country. International Union Against Tuberculosis and Lung Disease (Eds).1998.

76. Manual of the national Tuberculosis/Leprosy programme in Tanzania pg. 12 – 19 .1995.

77. Middlebook G, Cohn ML, Shaifer WB. Studies on isoniazid and tubercle bacilli III. The isolation, drug susceptibility and catalase testing of tubercle bacilli form isoniazid treated patients 1954; 70:852-872

78. Robert GD, Goodman NL, Heofets L, et al. Evaluation of BACTEC radiometry method for recovery of mycobacterium and drug susceptibility testing of mycobacterium tuberculosis from acid-fast smeer positive specimens. J. Clin Microbiol 1983; 18: 689-696

79. Morgan MA, Hirstmeer CD, De young DR, et al. Comparison of a radiometric method (BACTEC) and conventional culture media for recovery of mycobacteria from smear-ve specimen. J. Clin Microbiol; 1983 ; 18:384-388

80. Eisenstein BI. The polymerase chain reaction. A new diagnostic method of using molecular genetics for medical diagnosis. N. Engl J. Med 1990; 322: 178-183

81. Cave M, Eisenach K, McDermitt PF, et al. IS6110, an IS-like element of M. tuberculosis complex Nucl Acid Res. 1990; 18: 188-192

82. Mazurek GH, Cave M, Eisenach K, et al Chromosomal DNA fingerprint patterns produced with IS6110 as strain-specific markers for epidemiologic study of tuberculosis. J. Clin. Microbiol 1991; 29: 2030-2033

83. Hoffbrand A.V, Pettit J.E. Essential Haematology .Third edition . Blackwell Scientific 1993.

84. Kumar and Clark. Clinical features of HIV infection. Clinical Medicine 4th edition. Edited by Parveen Kumar and Michael Clark. Chapter 1, pg 111-112.W.B Sunders 1998.

85. K.M Citron and D.J Girling. Tuberculosis. Weatherall DJ, Ledingham JGG, Warrel DA (Eds). In Oxford Textbook of Medicine. Vol 1 pg.5.288.2nd editon. Oxford University Press 1988.

86. United Republic of Tanzania. National AIDS Control Programme HIV/AID/STD Surveillance. Report No.13, December 1998.

87. Dye C, Phil D. Global burden of Tuberculosis remains enormous. JAMA 1999;282:677-686.

88. National Tuberculosis and Leprosy program. Annual report 1999. dar-es-salaam, Tanzania.

89. AIDS epidemic update December 2000-UNAIDS.

90. Eric PP, Okwera A, Aisu T, Morrissey AB, Ellner JJ, Daniel T.M.: The influence of HIV infection on TB in Kampala, Uganda. Am Rev Respir Dis 1991; 143:185-187.

91. Nunn P, Gicheha C, Hayes R, Gathua S, Brindle R. Kibuga D. et al. Cross sectional survey of HIV infection among patients with tuberculosis in Nairobi, Kenya. Tubercle Lung Dis 1992; 73: 45-51.

92. Dolin PJ, Kaviglione MC, Kochi A. Global tuberculosis incidence and mortality during 1990-2000. Bull WHO 1994; 72: 213-220.

93. Timpe A, Runyon E.H.: The relationship of "atypical" acid-fast bacteria to human disease: A preliminary report. J. Lab Clin Med 1954; 44: 202.

94. Barker A. Sputum amear negative PTB. Tropical Doctor 1993;23:145-146

95. Field f. Willingham, Tracyl.Schmitz, Maibeth Contreras, Sheela E. Kalangi, Aldo M Vivar, Luz Carviedes, Eduardo Schiantar Ellii, Paola Maurtua Neumann, Caryn Bern, Robert H. Gilman and working group on Tuberculosis in Peru. Hospital control and multidrug resiststant Pulmonary tuberculosisin female patients, Lima, Peru. Emergency infectious 20001;7:747-754.

96. WHO Global programme on AIDS. Proposed criteria for interpretation of results from Western blot assay for HIV-1, HIV-2 and HTLV11, Wkly Epidemiol Rec 1995,37:281-283.

97. E.R Wandwalo, O. Morkve. Delay in tuberculosis case finding and treatment in. Mwanza, Tanzania. Int J Tuberc Lung Dis 2000:4(2) 133-138

98. DE Snider JR, R.M and Kochi A. 1994 Global burden of Tuberculosis. In tuberculosis: pathogenesis, protection and control. B. Br, ed ASM Press, Washington DC, p3.

99. Treatment of Tuberculosis guidelines for National Programmes. World Health Organization 1997.

100. Aris EA. Diagnostic criteria in sputum smear negative tuberculosis patients admitted to the medical wards in Muhimbili Medical Center Dar-es –salaam, Tanzania. A dissertation submitted in partial fulfilment of the requirement for the degree of master of medicine (MMED) in internal medicine in university of Dar-es-salaam 1995.

101. Morris CDW, Bird AR. The hematological changes in severe pulmonary. tuberculosis. Q J Med 1989; 272: 1151-1159

102.Bedell SE and Bush BT. ESR from folklore to facts. AM J Med 1985; 78:1001-1009

103.Huebner, R.E M.E Villarino, and D.Esnider, Jr 1992. Tuberculosis skin testing and HIV epidemic. JAMA 267: 409.

104.Zumla A, Malon P, Handerson J, Grange JM. Impact of HIV infection on Tuberculosis. Postgrad Med J 2000; 76; 259-268.

105.Greebbaun M, Beyt B.E Jr, Murray PR. The accuracy of diagnosing pulmonary Tuberculosis at a teaching hospital. Am Rev Respir Dis 1980; 121: 477-481.

106.Miller WT, Millar WT Jr. Tuberculosis in the normal host: Radiological findings. Semin Roentgenology 1993; 28(2) 109-118.

107.Mc-Adams HP, Erasmus J, Winter JA, Radiological manifestations of pulmonary tuberculosis. Radiol Clin North Am 1995; 33(4): 655-78.

108.Woording Jh, Vandivierere HM, Fred AM, et al. Update: The radiographic features of pulmonary tuberculosis. AJR 1986; 146: 497-506.

109.Choyke PL, Sostman DH, Curtis AM, et al. Adult on set pulmonary tuberculosis. Radiology 1983; 148: 357-362.

110.Farman DP, Speir WA. Initial roentgenographic manifestations of bacteriologically proven Mycobacterium tuberculosis: typical or atypical? Chest 1886; 89: 75.

111.L. Machiels, J.Pobee, P Nunn, R, J Hayes, and K.P Mc Adam. Impact of Hiv on. Tuberculosis in Zambia a Cross sectional study. BMJ 1990; 301:412

112. Pitchinik, A.E and H.A Rubinson. The radiographic appearance of tuberculosis in patienta with the acquired immune defficiency syndrome (AIDS) and pre AIDS. Am Rev Respir Dis 1985; 131: 393.