Improving the Diagnosis of Pulmonary Tuberculosis in Resource Constrained setting and the Role of Micronutrients in the Treatment of Pulmonary Tuberculosis in Abuja, Nigeria

Thesis submitted in accordance with the

requirements of the University of Liverpool

for the degree of

Doctor in Philosophy

by

Dr Lovett Lawson

Department of Tropical Medicine

Liverpool School of Tropical Medicine

University of Liverpool

October 2005

DECLARATION

I declare that this thesis is the result of my work. None of the material contained in this thesis has been presented in the past or currently, either wholly or in part for any other degree or other qualification.

Recruitment of patients, clinical examinations and collection of sputum and blood samples of the patients were carried out at 8 district hospitals in the Federal Capital Territory Abuja, Nigeria. All laboratory work was carried out at Zankli Research Laboratory, Zankli Medical Centre in Abuja, Nigeria with the exception of C-reactive protein, which was carried out at the chemistry laboratory of Alder Hay Hospital.

Sputum samples were examined by two independent microscopists unaware of each others readings and graded according to the IUATLD scale. A third technician read the slides with discrepant results and the readings were discussed to reach a consensus. Sputum was cultured on a BACTEC 960 system.

An external assessor, Dr Andy Ramsay, from LSTM visited the laboratory for quality control and conducted an external quality assessment. Both UK and local supervisors visited the study site on several occasions and advised on the preparation of this thesis.

Dr Lovett Lawson

November 2005

DEDICATION

This book is dedicated to my late mother, Olayinka Antoinette Tychus-Lawson, my very supportive wife, Dr Olufunke Juliana Lawson, my three wonderful boys, Rotimi, Femi and Tope and all the staff of Zankli Medical Centre.

ACKNOWLEDGEMENTS

I thank God Almighty for giving me the foresight, inspiration and fortitude to conclude this project.

My sincere gratitude to my darling wife, Funke, and my children, Rotimi, Femi, and Tope, for their support during the course of my study and for the sacrifices they have had to make.

My profound gratitude to Dr Luis Cuevas, Professor Peter Davies and Dr Tom Thacher, who in addition to being my supervisors extended a hand of friendship to me. I thank them for their support and guidance throughout the course of this project.

My special thanks to Dr Mohammed Yassin for his availability and assistance, and to my project advisors, Professor Bernard Brabin and Dr Brian Coulter for their support and advice throughout this project.

My special appreciation to members of the "TB group" in Abuja, Mrs Judith Giwa-Amu, Dr Aisha Abdul and Auwal Usman whose contributions at the very beginning led to the crystallization of this project. I pray that upon conclusion of all our individual projects we can come together to form a strong medical research team.

Without the TB research staff of Zankli Medical Centre, this project would not have been possible. They deserve individual mention. Laboratory staff; Isiramen, John Negedu, Nnamdi, Nathaniel and Elias; Community health workers; Joseph (Asokoro),

Chinyere (Wuse), Bolaji (Maitama), James (Gwagwalada), Simon (Kubwa), Rebecca (St. Mary's), Lucy (Gwarinpa) and Daniel (Nyanya). Data managers; Bose and Alabi. I am indeed indebted and forever grateful to them. My very special thanks to "The Pilot" Saddam Awa, our driver, whose personal coordinating skills in the field was of immense value to the success of this project. My thanks also go to Dr Andy Ramsay who travelled to Abuja to examine the quality control of the research laboratory and to Dr (Mrs) Olantunji of the National Hospital, Abuja for her contribution towards the X-ray readings.

Special thanks to the Chairmen, doctors and staff of the eight participating hospitals for the special roles they played in the recruitment and examination of the patients drawn on for this project.

I thank all members of the Federal Ministry of Health especially those at the National Tuberculosis and Leprosy Control Programme (NTBLCP); Dr. Shofola, Dr. Gwarzo, Dr. Chukwuekezie, Dr. Mohmoh and Mrs Tubi for their moral support and for ensuring the supply of drugs to the patients.

Special thanks to my friends in Liverpool, Serge, Olagbegi, Pam and Roland for their support, inspiration and friendship.

Finally, I will like to express my appreciation to all staff of Zankli Medical Centre for their support, understanding and dedication throughout the period of my study.

CONTENT

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AFB	Acid-fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
BCG	Bacilli Calmette-Guerin
BTS	British Thoracic Society
CMI	Cell-mediated Immunity
CT	Computed Tomography
DNA	Deoxyribonucleic Acid
DOTS	Directly Observed Therapy Short-course
ELISPOT	Enzyme-linked Immunospot
ESAT-6	Early Secretary Antigenic Target 6
ESR	Erythrocyte Sedimentation Rate
FCT	Federal Capital Territory
HIV	Human Immunodeficiency Virus
INF-γ	Interferon Gamma
IUATLD	International Union Against Tuberculosis and Lung Diseases
JTC	Joint Tuberculosis Committee
LFT	Liver Function Test
LJ	Lowenstein Jensen
LSTM	Liverpool School of Tropical Medicine
MDR-TB	Multi-drug Resistant Tuberculosis
MTB	*Mycobacteria tuberculosis*
MT	Monotest
NPI	National Programme on Immunization
NTBLCP	National Tuberculosis and Leprosy Control Programme
OPD	Out-patient Department
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
PHC	Primary Health Centre
PI	Principal Investigator
PPD	Purified Protein Derivative
PTB	Pulmonary Tuberculosis

RNA	Ribonucleic Acid
TB	Tuberculosis
TNFα	Tumour Necrosis Factor Alfa
TST	Tuberculin Skin Testing
WBC	White Blood Cell
WHO	World Health Organization
ZN	Ziehl-Neelsen

Abstract

This thesis aimed to validate the use of bleach digested sputum with ZN for the diagnosis of PTB against culture, describe the sensitivity and specificity of sputum smears with scanty results, describe the prevalence of HIV among patients with smear positive and smear negative TB, develop algorithms to select individuals with high risk of having PTB with and without HIV infection and assess the efficacy of micronutrients as adjunct for treatment of patients with PTB. Patients with clinical suspicion of TB were screened with microscopy and culture at 8 district hospitals in Abuja, Nigeria from September 2003 to April 2005. 1321 patients were screened and had their blood samples taken for biochemical and HIV serology. 774 (59%) participants were male and 547 (41%) female. The mean (SD) age for males was 35 (11) and females, 33 (12) years. 399 (30%) participants were positive for TB according to the WHO definition of smear-positive TB and 731 (62%) of 1286 cultured sputa were positive for TB. Of 1045 patients screened for HIV, 566 (54%) were positive and 317 (56%) of positive patients were male.

756 patients were enrolled to evaluate the use of bleach for the preparation of ZN. One sputum sample was digested, bleached and stained by the ZN method. 2251 (99%) of 2268 direct smears, 736 (97%) bleached smears and 756 BACTEC cultures were prepared. Of 756 patients cultured, 455 (60%) were positive for TB. Standard ZN identified 51% of the cases using the WHO case definition for TB. In comparison, a single digested smear identified 229 (50%) of the cases. In effect, a single digested smear identified the same number of patients with PTB as the 3-sputum strategy with similar specificity and predictive values. In HIV positive patients, 3 direct smears identified 235 (51%) of the 458 patients PTB and a single digested smear identified 229 (50%) of these patients. Independent of the prevalence of HIV, a single digested smear would improve the efficacy of diagnosis of TB providing a one-stop diagnosis.

The clinical presentation and risk factors for patients with TB were compared with those without TB and smear-positive TB patients were compared with smear-negative patients. Only a BMI <18.5%, weight loss, anaemia, conjugated bilirubin $\geq$ 0.2 mg/dl and granulocyte count $\geq$ 65% were independently predictive. Being female, having anorexia, BMI < 18.5, anaemia, hypoalbunaemia, raised ESR and SGPT were independent predictors of TB and HIV co-infection. Contact with PTB, chest pains, rhonchi, lymhocytopaenia, hypoalbuminaemia and a raised alkaline phosphatase were positive predictive factors for smear-positive TB. In HIV positive patients, haemoptysis and anaemia were positively predictive for smear positivity, while, weight loss, anorexia, BMI < 18.5 anaemia, lymphopaenia, raised ESR and SGOT and hypoalbuminaemia were independently predictive for smear-negative patients.

A total of 350 patients were enrolled in a clinical trial to assess the efficacy of zinc or zinc plus vitamin A as adjunct for the treatment of TB. A greater number of patients receiving zinc or zinc plus vitamin A had earlier resolution of their clinical symptoms and improvement in the laboratory tests, though these were mostly not statistically significant. A higher proportion of patients receiving these supplements cleared the bacilli from their sputa than patients receiving placebo by both microscopic examination and culture. Only a mean difference of 1 week sputum time clearance was observed between patients. No significant differences were observed between the mean sputum time clearance in patients receiving zinc or zinc plus vitamin A.

Improving the Diagnosis of Pulmonary Tuberculosis in Resource Constrained setting and the Role of Micronutrients in the Treatment of Pulmonary Tuberculosis in Abuja, Nigeria

CHAPTER ONE

General Introduction

Tuberculosis (TB) is one of the most common causes of death in adults in the world. The World Health Organisation (WHO) estimates that more than two billion people, a third of the world's population, are infected with the tubercle bacilli (WHO 2002). Of these, eight million develop TB each year and each person with active TB infects 10 to 15 persons yearly. TB kills about 3 million people each year, 95% of these occurring in the developing world with the majority in sub-Saharan Africa (Raviglione et al., 1995; Grzybowski 1991; Kochi 1991; Sudre et al., 1992).

The cornerstone of global TB strategies is rapid and accurate diagnosis of symptomatic patients. The identification of the tubercle bacilli on examination of a direct smear of sputum is still the most important and cheapest method of making a diagnosis of Pulmonary TB (PTB). The standard practice requires staining at least three sputum smears with the Ziehl-Neelsen (ZN) method. The WHO case definition of smear positive TB require a minimum of two positive sputum smears, as culture of the bacilli is expensive and slow (WHO 2000a). The development of cheap, reliable and simple tests for detecting *Mycobacterium tuberculosis* will improve the case

detection rate and help the early identification of infected persons. The digestion of sputum with home bleach has been used to improve the sensitivity of ZN staining, by permitting easier identification of bacilli than ZN alone (Yassin et al., 2003; Gebre et al., 1995; Habeenzu et al., 1998). A single smear using this technique has been reported to be as effective as 3 standard smears (Yassin et al., 2003). Its increased sensitivity could prove of great benefit to resource poor areas. There is however, concern of reduced specificity for the test, which has not been validated against a gold standard of culture (Van Deun et al., 2000) and this validation is addressed in chapter 5.

Patients with TB and HIV co-infection expectorate low numbers of acid-fast-bacilli (AFB) and direct smear microscopy has a lower sensitivity in these patients compared to patients without HIV probably through modifying the TB disease process, increases the proportion of patients with culture positive, smear negative TB (Elliott et al., 1993d). Techniques that could improve the detection of scanty bacilli are therefore needed to improve the performance of smear microscopy in patients with HIV. This will be critical in HIV positive individuals who often present with lower bacilli loads. The increased sensitivity of the bleach digestion method could prove of great benefit to improving the performance of smear microscopy in patients with HIV.

The WHO and the International Union Against TB and Lung Disease (IUATLD) recommended that a single smear with scanty AFB should not be accepted as diagnostic unless confirmed by further smears (Enarson et al., 2000). However it not uncommon to report sputum smears and classify them as scanty as their presence have been observed to constitute a high proportion of smear results (Van Deun et al.,

2004). In sub-Saharan Africa, the number of patients presenting with scanty bacilli has increased due to high number of patients co-infected with TB and HIV in the region (Elliott et al., 1993d) (Elliott et al., 1993; Karstaedt et al., 1998). Very few recent studies have validated scanty smear results against culture, which is considered the gold standard. Studies that compare the value of a single smear with scanty AFB against culture in areas of high HIV prevalence would be critical for the proper management of patients.

Tuberculosis has re-emerged as an important public health problem in the world since the 1980s. This has been associated, with the advent of the HIV epidemic. Both infections create a lethal combination, speeding each other's progress. TB is a leading cause of death among people who are HIV-positive, accounting for 11% of AIDS deaths worldwide (Stanford et al., 1991; Davies et al., 1996; WHO 2002; WHO 2000b). In Africa, HIV is the most important factor responsible for the increased incidence of TB in the past 10 years (Cantwell et al., 1996; Davies et al., 1996; De Cock 1996). Despite the magnitude of the problem, there are only few documented reports on the prevalence, clinical presentation and risk factors associated with HIV infection among culture-positive TB patients probably due to the inaccessibility and high cost of culture facilities in the settings where these diseases are most prevalent. This thesis compares the prevalence, clinical presentation and risk factors of patients with culture-positive and culture-negative TB with and without HIV infection to determine the differences between the groups.

With the resurgence of TB in the mid 1980s, attention has been focused on its early identification and treatment (Fairchild et al., 1998) Early identification of patients

with TB, whether they are smear-positive or smear-negative is necessary, both to enable appropriate isolation procedures and to provide a basis for early institution of therapy. Several studies have described the clinical presentation and risk factors of persons with AFB smear-positive sputum, who are the most infectious group of TB patients. Sputum smear examination for AFB of these patients can only diagnose 30 to 60% of true cases in well-equipped laboratories (Aber et al., 1980). In countries with high prevalence of TB and HIV, the detection rate is even lower owing to the paucibacillary nature of PTB in patients with HIV (Hargreaves et al., 2001a; Elliott et al., 1990; Hargreaves et al., 2001b; Long et al., 1991). In countries with low prevalence of PTB, smear-negative TB is said to pose less of a threat to public health compared to smear-positive PTB, based on its low infectivity potential, lesser extent of disease, and good prognosis even without treatment (Long 2001). However, smear-negative patients who are diagnosed with TB by culture, radiology or other means are also capable of transmitting the infection (Dutt et al., 1994). Most publications on the clinical features of PTB have not differentiated between smear-positive and smear-negative disease as these patients may have different clinical presentation and radiological findings. The directly observed therapy short-course (DOTS) programme in most developing countries are run at the level of health centres (Enarson 1995), it is important to assist the health care workers identify potential smear-positive or smear-negative TB patients.

The association between malnutrition and infection is well established (Scrimshaw 1991; Scrimshaw et al., 1997). For centuries, TB has been a disease closely associated with poverty (Grange et al., 2001a). Epidemiological, clinical and experimental studies suggest a strong relationship between micronutrient deficiencies and infection.

Micronutrient deficiencies alter the innate and acquired immune responses to pathogens and influence pathogen mutations directly, affecting virulence and clinical outcome. Zinc supplements improve outcomes in diarrhoeal diseases (Roy et al., 1997; Bhutta et al., 1999; Roy et al., 1999) and acute respiratory infections. (Bhutta et al., 1999). They also promote growth in childhood (Thu et al., 1999). Similarly, Vitamin A supplements reduce overall childhood mortality by 20 to 30% (Fawzi et al., 1993), reduce death from measles complications and acute lower respiratory infections in hospitalised children (Hussey et al., 1990). Furthermore, micronutrient deficiencies of zinc and vitamin A have been associated with increased susceptibility to TB (Karyadi et al., 2000). More recently, zinc replenishment has been shown to improve the PPD responses of children exposed to adults with tuberculosis (Cuevas et al., 2002). A small trial in Indonesia demonstrated that adults with pulmonary tuberculosis who received a combination of daily zinc and vitamin A supplements cleared the bacilli at a faster rate than those who did not receive supplements (Karyadi et al., 2002). Evidently, by including micronutrients in the current TB treatment, it may be possible to reduce the dosage of anti-tuberculosis drugs or introduce shorter regimens. Shorter regimens will lead to better drug compliance, fewer adverse effects and reduced cost to the patient. Faster sputum conversion will also reduce the risk of tuberculosis transmission, which is a major benefit to the community. To this end therefore, we also compare the effects of the outcome of including zinc with zinc + Vitamin A into a standard TB treatment regimen.

CHAPTER TWO

Tuberculosis: Literature review

2.1 Historical Perspective

Great hopes were built by the end of the 20th century that with the advent of antibiotics and generally improved living standards, nutrition and social conditions, the threat of an early death from TB and other infectious diseases would be outdated (D'Arcy Hart et al., 1939). Other factors that should have reduced tuberculosis rates include selective genetic pressure (Davies et al., 1996), immunisation and political measures (Grange et al., 2001b). In contrast, by early 21st century, this expectation has turned out to be a big fallacy, as these diseases have not only re-established themselves but also became deadlier.

In the course of history, tuberculosis has been called various names including Phthisis, Scrofula, Tabes, Hectic fever, Lupus, and Consumption. The name "Tuberculosis" was coined during the second half of the 19th century when in 1882 a German bacteriologist named Robert Koch isolated the infectious agent of the disease, which he named *Tuberculosis bacteria* or tubercle bacilli using his own Koch's postulates (Koch 1932).

Various attempts at treatment, some bordering on folk remedies and others on diet and warm fresh air, failed. Hermann Brehmer, a German botanical student, who was himself a sufferer, opened the first sanatorium in 1854. Other sanatoriums developed

in the UK, USA and many other parts of the world, from the second half of the 19th century, becoming a major part of the management of the disease into the middle of the 20th century.

The post war discovery of antibiotics, between 1945 and 1960, revolutionised the treatment of TB and for the first time a therapeutic regime was available, offering the real prospect of a cure for TB in the vast majority of patients. Streptomycin was discovered in 1948. There was a rapid decline in the incidence of TB in the middle of the 20th century, which led to the assumption that the scourge of TB would then be over. This led to complacency and neglect by the wealthier nations with decline in research and political interest in the control of the disease. The WHO monitoring and control operation was reduced to its barest minimum and by 1986, Medical Research Council (MRC) units were disbanded.

By 1986, the decline witnessed during the previous three decades had halted in many countries and the world experienced a resurgence of TB in both developed and developing countries, reoccurring in countries where it had ceased to be a problem. Between 1987 and 1993, prevalence rates in England and Wales rose by 35% and 15% respectively (HMSO 1988-2001). This resurgence was associated with the spread of HIV and further aggravated by the emergence of multiple drug resistant TB (MDR-TB), with immigration as a major factor. By compromising the immune system, HIV was the most important single factor for the progression of dormant TB into clinical disease (Antonucci et al., 1995; Raviglione 2003; Raviglione et al., 1995). This resurgence was most severely felt in the resource poor countries of sub-Saharan Africa.

With the increasing TB burden, several organizations decided to act. For instance WHO declared TB a global emergency in 1993 (WHO 1994), the first such designation ever made by that organization. The G8 group in 2000 committed itself to achieving the WHO target of reducing deaths from this disease by 50% by 2010.

Peru has achieved a 50% reduction in incidence rate in the last decade and China has seen a fall in the prevalence rate, both attributable, in part, to the introduction of effective case detection and short course chemotherapy (WHO 2001). While most developed countries have witnessed reduction in incidence rates, TB notification rates have risen up to fourfold in many of the countries of the developing world particularly in Sub- Saharan Africa (Maher et al., 2004) and few countries are likely to succeed in achieving this target utilising existing strategy and technology.

2.2 Epidemiology

The leading disease agent in Tuberculosis infection is *M. tuberculosis* and humans are the principal host. An estimated 8-9 million new cases of TB occurred in 2000. Of these 2-4 million cases were pulmonary sputum smear positive (Corbett et al., 2003) and each of these cases is capable of infecting 10-15 other individuals each year. Sub-Saharan Africa has by far the highest incidence of TB with a rate of 290/100000, but the largest number of infected people is in Southeast Asia accounting for over half of the global burden. The total number of new TB cases probably increased by 1.7%/year from 1997 to 2000 with the incidence rate per capita at 0.4%/year. At this rate, it is expected that 9-10 million new patients will be infected by 2010. This

increase will be most evident in sub-Saharan Africa (Dye et al., 2005). Eighty percent of new cases occur in 22 high burden countries as shown in figure 2.1.

It is estimated that 3 million people die from TB each year with over 95% in developing countries, primarily in sub-Saharan Africa (Grzybowski 1991; Dye et al., 2005; Corbett et al., 2003; Raviglione 2003; Antonucci et al., 1995; Raviglione et al., 1995). Among infectious agents, TB is the world's second greatest killer of modern times behind HIV/AIDS (WHO, 2001). TB accounts for 14% of adult deaths in patients suffering from HIV and 11% of adult AIDS death (Stanford et al., 1991; Davies et al., 1996; De Cock 1996), with the vast majority in Africa (Cantwell et al., 1996; Davies et al., 1996; De Cock 1996). The prevalence rates of TB by continents are shown in table 2.1.

TB is predominantly an adult disease with a peak incidence in young adults. Based on previous studies, it is believed that more men than women suffer from TB (Liberato et al., 2004; Kimerling et al., 2002). Compared with other infectious diseases, TB infection progresses very slowly, through low transmission rates, weak immunity and long generation time (Anderson et al., 1991). The importance of TB is attributable to the high case-fatality rate among untreated or poorly treated cases. Approximately two-thirds of untreated smear positive cases will die within 5-8 years, the majority within the first 2 years (Raviglione et al., 1994; Styblo 1991). The case fatality rate for untreated smear-negative patients is lower but still between 10-15% (Rieder 1995). Even among smear-positive patients having treatment, the case-fatality rate can exceed 10%, especially in cases of poor drug compliance, HIV infection and drug resistance (WHO 2000b).

Figure: 2.1 Estimated number of new TB cases by country, 2001 (lancet, 2003)

Table: 2.1 Prevalence and annual number of deaths by continent (Davies 2003a)

Region	Prevalence	Annual Deaths
Africa	3,586,000	770,000
Americas	980,000	160,000
Eastern Mediterranean	1,035,000	173,000
Europe	710,000	118,000
South-East Asia	6,553,000	1,095,000
Western Pacific	3,429,000	591,000
Total	**16,301,000**	**2,907,000**

Nigeria has a population of over 120 million people and ranks 4th among the world's 22 countries with a high TB burden. Among African countries, Nigeria has the highest estimated number of new TB cases with nearly 368,000 new cases in 2002 (WHO 2005). Of the above, 159,000 (43%) were pulmonary sputum smear-positive cases. The HIV infection rate among TB patients as of 2002 was estimated at 27% (WHO 2005).

2.3 Pathophysiology

Tuberculosis is spread by airborne droplets, containing *M. tuberculosis*. These droplets are moist small particles between 1-5μm in diameter that remain airborne for hours after expectoration, by patients with pulmonary tuberculosis (PTB). Individuals in close proximity to patients may inhale the infectious droplet nuclei from the atmosphere. The inhaled bacilli reach the alveoli and after being ingested by macrophages establish a local focus of disease called the Ghon focus. Bacilli are

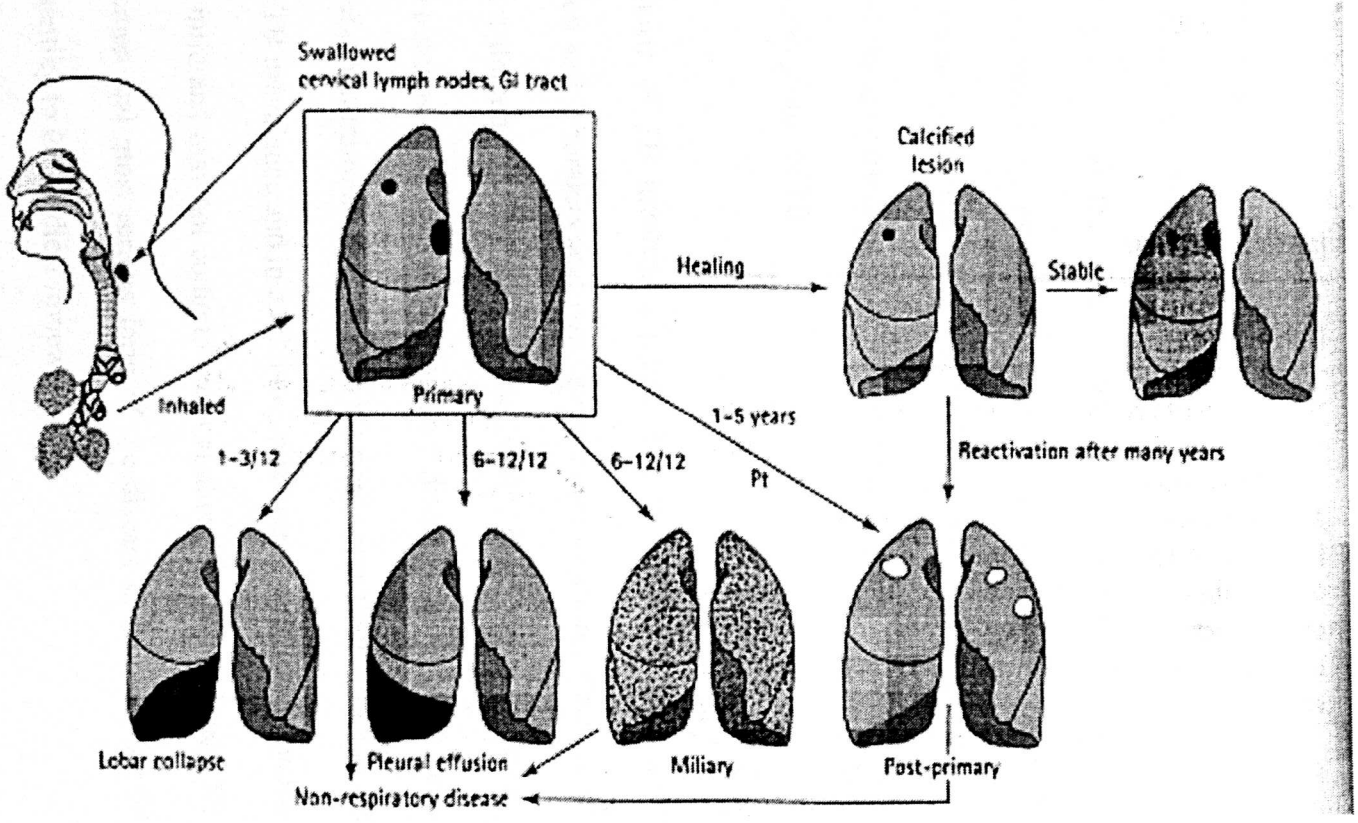
transported to the hilar lymph nodes where additional foci of infection develop. Hilar lymphadenopathy together with the Ghon focus forms the primary complex. There may be containment of the infection at this stage or progression to active disease.

In most infected individuals, cell mediated immunity (CMI) develops 6 to 8 weeks after infection. T-lymphocytes are activated and together with macrophages form granulomas enclosing the bacilli and limiting its further replication and spread. Unless a subsequent defect arises in CMI, the infection typically remains contained and active disease may never occur. This stage is usually associated with the development of a positive tuberculin skin test.

At the cellular level, alveolar macrophages infected with *M. tuberculosis* interact with T-lymphocytes through various important cytokines. The macrophages release interleukins 12 and 18 that stimulate T-lymphocytes, especially the CD4+ T-lymphocytes, to release interferon γ (INF-γ) (Sodhi et al., 1997; Ellner 1997). The cytokines then stimulate the phagocytes of the bacilli in the macrophages by stimulating the release of tumour necrosis factor α (TNFα), which is important in the formation of granulomas and limiting the extent of the infection (Flynn et al., 1993; Flynn et al., 1995; Mackaness 1968).

When the host immune response cannot contain the replication of *M. tuberculosis*, such as in HIV infection, active disease occurs. HIV infection is the most important single factor in the reactivation and progression of infection in adults. Other medical conditions can also compromise the immune system and lead to reactivation of infection. These include malnutrition, diabetes, malignant diseases, renal failure,.

Figure: 2.2 Development and spread of TB in the lungs (Davies 2003a)



steroid therapy, zinc deficiency (Pant et al., 1987) and vitamin A or D deficiency (Karyadi et al., 2002; Wilkinson et al., 2000)

2.4 Clinical Diagnosis of TB

The *M. tuberculosis* complex includes three closely related species, *M. tuberculosis, Mycobacterium bovis* and *Mycobacterium Africanum*. The term "Latent tuberculosis infection" refers to to the situation when an individual is infected but has no symptomatic, radiologic or pathologic evidence of disease. The evidence for this infection is mainly a positive skin test. "Tuberculosis disease" refers to infection with evidence of disease such as positive acid-fast smear, positive culture, and radiographic or clinical symptoms of TB.

The diagnosis of TB involves a proper history of clinical presentation, physical examination and laboratory and radiographic investigations. Because of the way the human host defends against the tubercle bacilli, there are two distinct form of the disease in humans: primary and post-primary disease. The primary form is caused by infection of a non-immune host and this is usually prevalent in childhood in countries with high TB prevalence and in adulthood in countries with low prevalence. It is often relatively mild and may not come to medical attention. Post-primary TB is by far the most common and most serious form of the disease accounting for 90% of all TB cases presenting to the medical reviewer.

2.5 Clinical Presentations

Presenting symptoms

An important diagnostic clue for diagnosis is a history of previous exposure to an individual with expectorating TB. The patient might be asymptomatic, but often there is a history of cough of more than three weeks duration, usually productive of mucoid, purulent and/or blood stained sputum. Patients may complain of fever and night sweats. Chest pain with unresolved pneumonia is a frequent symptom. As the disease progresses, patients may complain of breathlessness, localized wheeze, frequent colds, weight loss, lassitude, anorexia, dyspepsia and in some women, amenorrhoea. Any or all of these symptoms might be present in a TB patient (Brandli 1998; Davies et al., 1996)

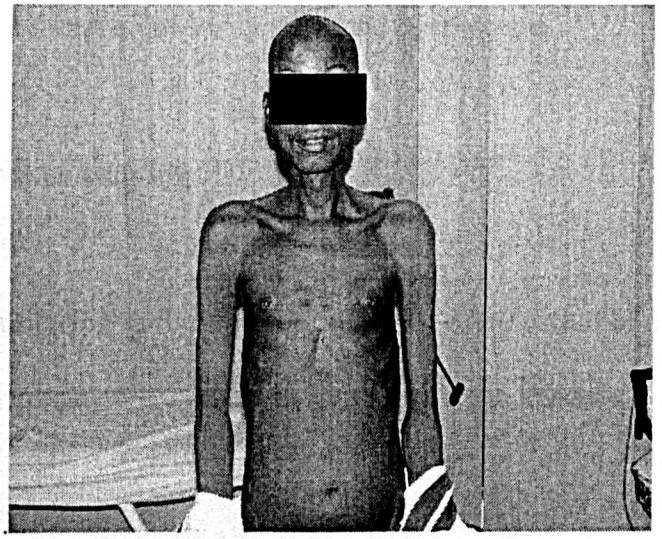
Clinical signs

Signs of TB vary according to the severity of the disease. Most patients are pyretic and anaemic. There is moderate to severe loss of weight and in very severe cases, patient's gait is affected. In some cases, there are signs of pneumonia or pleural effusion or both. Signs of extra-pulmonary TB, such as cervical lymphadenopathy, finger clubbing and phlyctenular conjunctivitis may be associated with PTB disease.

Tuberculin Skin Testing

The natural history of TB is characterized by the distinction between latent infection and active disease. The preferred and standard skin test for detecting persons with latent TB, is the tuberculin skin test (TST) of which the Mantoux test is the most frequently used. It involves injecting 5 TU (0.1ml) of purified protein derivative (PPD) intradermally and assessing the induration within 48 to 72 hrs. The importance of the TST is its ability to predict active disease in latently infected individuals. Studies have shown that treatment of latent TB infection based on positive TST reduced the risk of active disease by approximately 60% (Pai et al., 2004). The test is more useful in areas of low TB prevalence and low cross-reactions (Huebner et al., 1993). Almost 20% of patients with TB have negative skin test and immunosupressed patients such as those co-infected with HIV, false negative results can reach 50% (Huebner et al., 1992). False positive results may occur in patients infected by other non-TB mycobacteria, e.g. *M. avium* complex (Nash et al., 1980). This test cannot differentiate between infection and disease.

Figure: 2.3 A patient with TB presenting at one of the hospitals in Abuja



2.6 Laboratory Diagnosis

Microscopy

After much speculation about the cause of TB, Robert Koch in 1882 isolated the tubercle bacilli in the laboratory. Koch used an alkaline-based staining technique, which took 20-24 hours at room temperature to perform (Koch 1882). He was awarded the Nobel Prize for medicine and physiology for his work on TB in 1905. Paul Erlich, a medical assistant, soon developed a more rapid staining procedure exploiting the acid-fast property of the mycobacteria (Erlich 1882) and further improvement on the technique was made in the following years by Franz Ziehl and Friedrich Neelsen (Ziehl 1982), and it is their modifications that are still in use today.

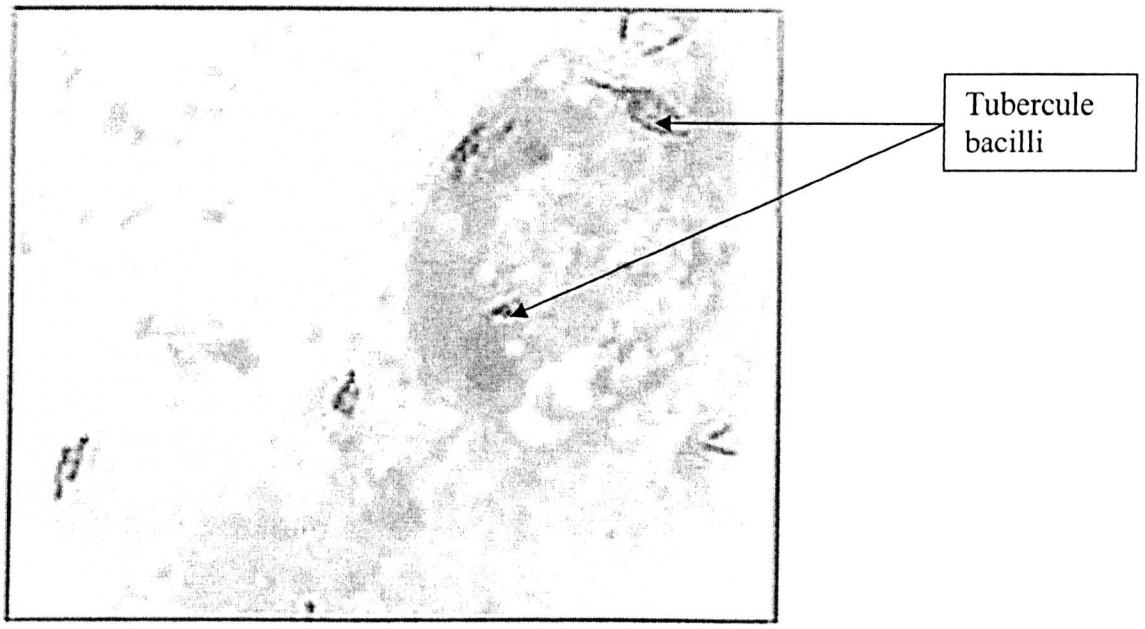
The acid-fast smear technique remained until recently the only rapid laboratory technique for the diagnosis of TB. The principle is based on an arylmethane stain, such as carbol-fuchsin or auramine, followed by an acid or acid-alcohol de-colouring solution, which fails to penetrate the acid-fast mycobacteria and therefore leaves them stained. A counter stain such as methylene blue or malachite green allows the red bacilli to be seen more easily under the microscope.

Ziehl-Neelsen staining

The identification of the tubercle bacilli on examination of a direct smear of sputum is the most frequently used method of making a diagnosis worldwide. The standard

practice requires staining at least three smears. An example of a smear with the ZN stain is shown in figure 2.4.

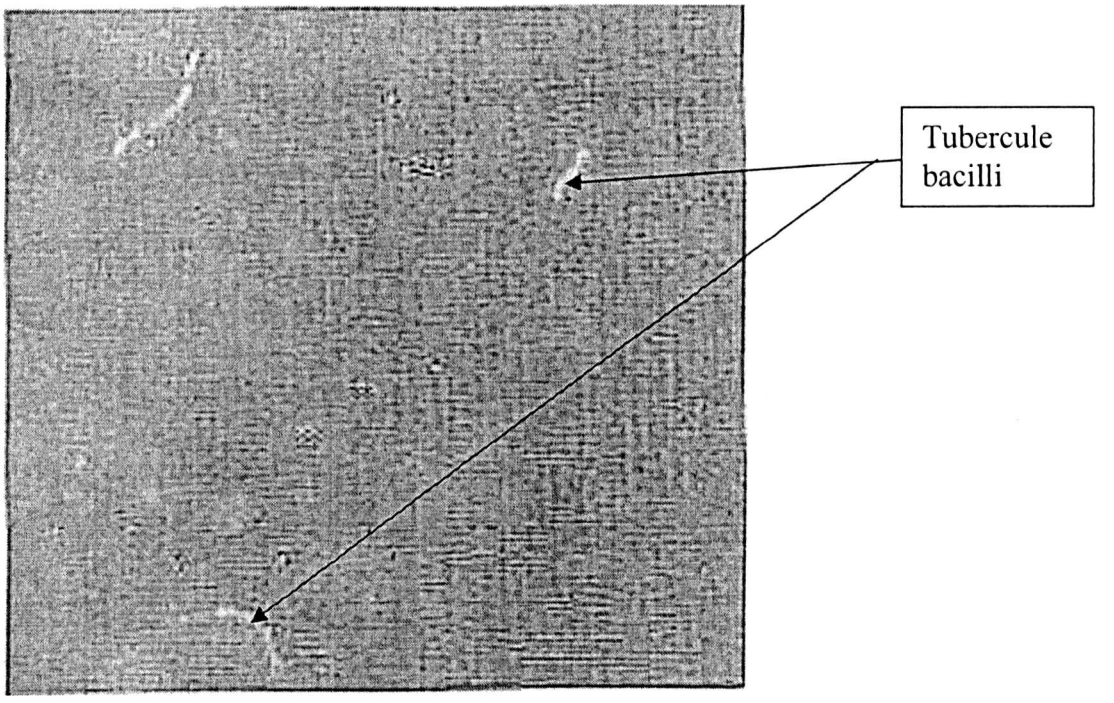
Figure: 2.4 Sputum specimen stained with ZN stain



Fluorescence staining

This technique is also based on the acid-fast principle. The auramine-phenol fluorochrome staining technique can be used to detect *M. tuberculosis* in sputum and other specimens when facilities for fluorescence microscopy are available. This technique is used by many laboratories because it permits a more rapid reading of smears as bacilli can be detected at the 40-x objective. The auramine-phenol staining technique is likely to detect tubercle bacilli even where they are few. This method requires a flouorescent microscope and staining and is only recommended for laboratories with large workload.

Figure: 2.5 Auramine-phenol staining under fluorescence microscopy



2.6.2 Culture

Isolation of mycobacteria from clinical samples by culture represents the cornerstone or the gold standard on which a definitive diagnosis of TB can be made. Traditionally mycobacteria culture has been performed on conventional egg based solid medium such as Lowenstein Jensen (LJ), agar based media such as Middlebrook formulations (7H10 and 7H11) and liquid media such as Kirshner's or Middlebrook 7H9 broth.

The problem with conventional culture methods is the slow growth of the bacilli in the media with a mean growth period of four to six weeks and an additional two weeks if drug sensitivity is required (Kent et al., 1985).

The time to detection of growth of mycobacteria can be shortened greatly by the use of the automated or semi-automated liquid culture systems that can detect growth earlier than the naked eye. These include the BACTEC 460 radiometric system, BACTEC MGIT 960 system (Becton Dickinson), MB/BacT system (Organon Teknika), the mycobacteria growth indicator tube (MGIT) and the ESP II culture system (Heifets 2000; Cambau et al., 1999; Katila et al., 2000; Somoskovi et al., 2000; Liu et al., 1999). A major drawback for these systems is their cost, as for example, processing samples with MGIT can cost £50.00 per sample. These tests therefore are rarely available or affordable in developing countries.

2.7 Molecular techniques

Tests based in molecular techniques can be very rapid and are becoming more widely available in developed countries. Depending on the setting of the TB system, these tests can be performed within one day (Pai et al., 2004). Various methods have been developed.

Polymerase Chain Reaction (PCR)

This technique allows the amplification of sequences of *M. tuberculosis* DNA in vitro, such that the amount of amplified DNA can be detected and identified. PCR is rapid and the results can be available within one day of DNA extraction from the sample. The test can be automated and can rapidly detect as few as a single organism from sputum, blood, gastric lavage, cerebrospinal fluid, pleural fluids and tissue samples with sensitivity and specificity of almost 90% in pulmonary tuberculosis (Montenegro

et al., 2003). PCR tests however require of a relatively complex equipment and good isolation facilities to avoid cross-contamination. In addition, PCR tests for TB are not very sensitive for the diagnosis of TB in children (Khan et al., 1995; Neu et al., 1999).

Gene probes

These tests target mycobacteria ribosomal RNA by transcription-mediated amplification. DNA probes that are highly specific for *M. tuberculosis* species are used in patients in whom AFB smears are positive and culture is in progress. Specificity is usually less than 100% even in positive smear patients with occasional false positive in non-TB mycobacterium infections (Suffys et al., 2001).

2.8 Serodiagnosis

Various attempts at developing a sensitive and specific serological assay, for the diagnosis of MTB, have been elusive. There are now newer promising procedures using enzyme immunoassay, which may make serological testing possible in the nearest future. These tests will be useful in detecting mycobacteria particularly in children and in patients with extra-pulmonary infections, although their ability to differentiate between disease and infection is still unclear.

In the past decade, a major scientific advancement made was to identify antigen that are expressed by *M. tuberculosis*, but not by BCG or by most environmental mycobacteria. The most studied of these antigens is the early secreted antigenic target

6-kD protein (ESAT-6) (2-4) which has multiple epitopes that are recognised by persons of many different HLA types (Ulrichs et al., 1998).

Y-Interferon Blood Test (Quantiferon-TB)

Quantiferon TB is an in-vitro blood test developed to detect latent TB infection considering the shortcomings of the TST. The assay evaluates patients' TB infection status by measuring interferon gamma (IFN-γ) secreted from T-lymphocyte cells in whole blood incubated overnight with *M. tuberculosis* specific antigens such as the proteins ESAT-6, CFP-10 and TB 7.7 (Mazurek et al., 2003). The quantiferon-TB test is said to identify only those individuals truly infected with TB (Pai 2005; Pai et al., 2004).

ELISPOT

The enzyme-linked immunospot (ELISPOT) is a new blood test to diagnose latent TB infection (Shams et al., 2005; Liebeschuetz et al., 2004; Ewer et al., 2003). ELISPOT is able to detect IFN-γ-producing cells in persons with latent TB infection (Lalvani et al., 1998). Compared to TST, the ELISPOT test appears to be as sensitive for diagnosis of latent TB infection in contacts with TB (Shams et al., 2005; Liebeschuetz et al., 2004). ELISPOT is not yet suitable for widespread use due to cost and the requirement to isolate mononuclear cells, a procedure that is not routinely performed in clinical laboratories.

2.9 Routine Blood Examination

The routine blood examination is usually not diagnostic for TB. However these tests are useful for objective monitoring of response to treatment. Due to the chronic nature of the disease, patients with TB have a high erythrocyte sedimentation rate (ESR) and lymphocytosis. The packed cell volume (PCV) may be low in severe TB or in malnourished patients. White blood cells (WBC) are usually within normal limits, except where there are other acute inflammatory conditions and a relative increase in monocytes can occur. Mild abnormalities of the liver function tests (LFT) have been observed in some patients (Prasad et al., 2004; Guryleva et al., 1993; El'kin et al., 1992) and this are further monitored because of the potential for clinical hepatitis during chemotherapy (Gordin et al., 2004; Jasmer et al., 2003).

2.10 Radiographic diagnosis

Quoted vastly from a comprehensive review of TB radiographic appearance, by Woodring et al., (Woodring et al., 1986), and reproduced in Clinical Tuberculosis (Davies 2003a) it was stated that PTB could produce a wide range of abnormalities on chest radiography from normal to very bizarre. During the primary phase, consolidation usually presents in the middle or lower lobes or the anterior segment of the upper lobe, the latter being most common. Other radiological features include cavitations, TB bronchopneumonia, segmental or lobar atelectasis, pleural effusion, hilar and mediastinal lymphadenopathy and disseminated miliary disease.

Common chest abnormalities during the second phase include exudative and/or fibro-productive densities mainly in the apical and posterior segments of the upper lobe, cavities, marked fibrotic response in the lungs, pleural effusion, empyema and fibrosis. Occasionally, pneumothorax and intra-thoracic lymphadenopathy are seen.

Imaging techniques

Computed tomography (CT) is a more sensitive method of detecting chest pathology such as cavities, lymphadenopathy, miliary diseases and pleural effusion, than chest radiography, but these techniques are still expensive and require equipment not available at peripheral settings of developing countries.

2.11 Treatment

Early diagnosis of TB infection and prompt initiation of optimal treatment would not only enable a cure of the patient but will also stop transmission of infection and disease to others in the community. The WHO recommended DOTS programme, which includes short- course chemotherapy is currently the most effective treatment for most patients. Direct observation of the patients helps most of them to complete the 6-8 month treatment regimen (Bayer et al., 1995; Iseman et al., 1993). The Joint Tuberculosis Committee (JTC) of the British Thoracic Society (BTS) in 1998 published revised guidelines for the chemotherapy and management of TB in the United Kingdom (BTS 1998). The recommendations include among others: (1) Patients with TB should be notified to the local authorities. (2) Bacteriological confirmation and drug susceptibility testing should be sought wherever possible. (3) A

six-month short course regimen with four drugs in the intensive phase should be used for all forms of TB except TB meningitis. (4) Treatment of all patients should be supervised by trained personnel. (5) Advise is given on follow up after treatment. (6) The role of directly observed therapy is discussed. (7) The management of multi-drug resistance TB is explained in outline and (8) Infection control and segregation for such patients and for patients with dual infection with HIV and TB are covered under special conditions.

The aims of drug treatment of TB are:

1. cure the patient of TB by the shortest duration of drug administration with minimum interference with their living;
2. prevent death from TB or late effects of disease;
3. prevent relapse of disease;
4. prevent emergence of drug resistance;
5. reduce transmission of TB to people within or outside the community. (Yew et al., 1999);

In 1993, the WHO officially adopted the DOTS strategy as the global strategy for the control of TB (Walley et al., 2001). The five key components of DOTS include:

A. Political commitment

B. Microscopy services

C. Drug supplies

D. Good record keeping

E. Direct observation of treatment

The recommended six or 8 month drug treatment for PTB is divided into a two month intensive phase and four to six-month continuation phase. The DOTS programme in Nigeria uses the 8-month therapy. Drugs recommended for use in the intensive phase consist of: Isoniazid (INH), Ethambutol (E), Rifampicin (R) and Pyrazinamide (RZ). The drugs recommended for the continuation phase comprise Rifampicin and Isoniazid for four months treatment or Isoniazid and Ethambutol for four or six months. In Nigeria, the recommended continuation phase is 6 months with INH and Ethambutol.

In cases, where a positive smear or culture for *M. tuberculosis* is still obtained after two months of treatment, drugs for the intensive phase are continued for another month (NTBLCP 2004). A summary of the treatment scheme for Nigeria is shown in table 2.2.

Table: 2.2 Chemotherapy for newly diagnosed patients

			Daily dose
Drug	**Preparation**	**Adults**	**children**
Isoniazid	Tablet 50mg	300mg	10mg/kg*
(INH)	100mg	10mg/kg in military	
Rifampicin	Capsule 150mg,	<50kg: 450mg	10mg/kg
(R)	300mg	>50kg: 600mg	(max. 600mg
Pyrazinamide	Tablet 500mg	<50kg: 1.5g	35mg/kg
(RZ)		50-74kg: 2.0g	
Ethambutol	Tablet 100mg:	15mg/kg	If over 12years
(E)	400mg		of age, for adults
Streptomycin	Vial 1g	<50kg: 0.75g	20mg/kg
		>50kg: 1.0g	

* WHO recommendation

Table 2.3 shows the treatment scheme recommended for patients with multidrug resistance (MDR) TB. There is however, no study on the prevalence of MDR-TB in Nigeria and patients who fail the first treatment are put on the retreatment scheme, which consist of 3 months of intensive phase, with steptomycin given for 2 months and 5 months of continuation phase using the 4 fixed drugs combination.

Table: 2.3 Drugs and Doses used in the Treatment of MDR-TB

Drug	**Dose**	**Frequecy**
Pyrazinamide	20-30mg/kg of body weight	Daily
Ethambutol	15-25mg/kg	daily
Amikacin, Kanamycin, Strepto-mycin, or Capreomycin	15/kg (max. dose, 1g)	Five days a week
Aminosalicylic acid	12g	Daily
Clofazimine	300mg	Daily
Amoxicillin-clavulanate	2-4g	Daily
Clarithromycin	1000mg	Daily
Protionamide	750-1000mg	Daily
Cycloserine	750-1000mg	Daily
Ofloxacin, ciprofloxacin	400-800mg	Daily
Rifabutin	300mg	Daily

CHAPTER THREE

Study design and methods

3.1 Introduction

This chapter describes the objectives of the thesis, the overall study design and methods common to all components of the project. Subsequent chapters will address study issues of each objective individually.

3.2 Objectives

The specific objectives of the thesis are:

1. To validate the use of bleach digested sputum with ZN stain for the diagnosis of PTB against culture
2. To describe the sensitivity and specificity of sputum smears with scanty results
3. To describe the prevalence of HIV among patients with smear positive PTB
4. To develop algorithms for the selection of individuals with high risk of having PTB with and without HIV infection
5. To assess the efficacy of weekly zinc and weekly zinc plus weekly vitamin A as an adjunct for the treatment of patients with PTB

3.3 Materials and Methods

Logistical arrangements

Preparation for the project started in May 2003 with the formation and the equipping of a TB research laboratory in Zankli Medical Centre in Abuja, Nigeria. Zankli Medical Centre is a forty-bed private hospital with approximately 150 staff, including 8 consultants in different disciplines. The hospital was founded in Jos, Nigeria in 1990 and relocated to Abuja in 1997, where it has since grown to be one of the largest private hospitals in the city. This is a fee-for-service hospital that provides services to mostly middle and upper class citizens, diplomats, company executives and staff. The study was based in Zankli Medical Centre, as the Principal Investigator (PI) is the medical director of the centre. He has promoted research and teaching activities within Abuja.

Given the characteristics of the patients attending the centre, an agreement was sought with the Ministry of the Federal Capital Territory (FCT), through the Directorate of Health of that ministry, whereby patients with suspicion of TB attending selected district government hospitals would be allowed to participate in the study. In return, the project was to support the incipient DOTS programme of the government by providing free diagnostic tests for the patients (sputum smears, cultures, X-rays, HIV testing and counselling and blood tests such as ESR and liver function tests). The laboratory was equipped with a new BACTEC 960, a hood, two microscopes, incubators and centrifuges. New materials, reagents and consumables were purchased in readiness for the project. In addition, eight community health research nurses, were attached to the participating government hospitals and three laboratory

technologists/microbiologists, one laboratory technician, a data clerk and a driver were employed. An experienced clinician with an interest in TB was identified in each of the participating hospitals, and paid an honorarium for his/her assistance in helping to supervise the nurses in the hospitals.

Each staff was given a job description. The nurses explained the aim of the study and completed the consent form, recruited patients with symptoms suggestive of TB and assisted the patients in answering the questionnaires. The nurses were responsible for obtaining the sputum specimens (1^{st}-on-the-spot, morning and 2^{nd}-on-the-spot) in two consecutive days and blood samples from the patients. PPD-tuberculin (0.1ml) was administered intradermally to the patients, using the Monotest (MT). This was examined after 48 to 72hours. The driver collected these sputa and blood samples on a daily basis, Monday to Friday, and took them to the Zankli research laboratory. The driver also took the nurses to houses of patients who failed to turn up for their appointments to facilitate follow up. The laboratory staff prepared all the smears and cultured the sputum samples. Blood analyses were done with semi-automated machines in the hospital chemistry laboratory. Serum samples were frozen in safe-lock tubes and stored at -20°C until transported to Liverpool for micronutrient analysis. Blood was also collected for HIV testing after counselling of the patients. Patients not willing to participate in the study were also offered diagnostic tests.

Pregnant women, lactating mothers, patients on corticosteroids or supplements containing vitamins A or D during the previous month, diabetic and renal failure patients, patients with extra-pulmonary TB, those residing outside Abuja's metropolitan area and those who could not attend follow-ups were excluded from the

study. Nurses at the participating hospitals were notified of all positive sputum results for AFB in order to enroll patients willing to participate in the micronutrient study.

Figure 3.1 Research investigators and TB research staff (Visit to project site by Dr Luis Cuevas)

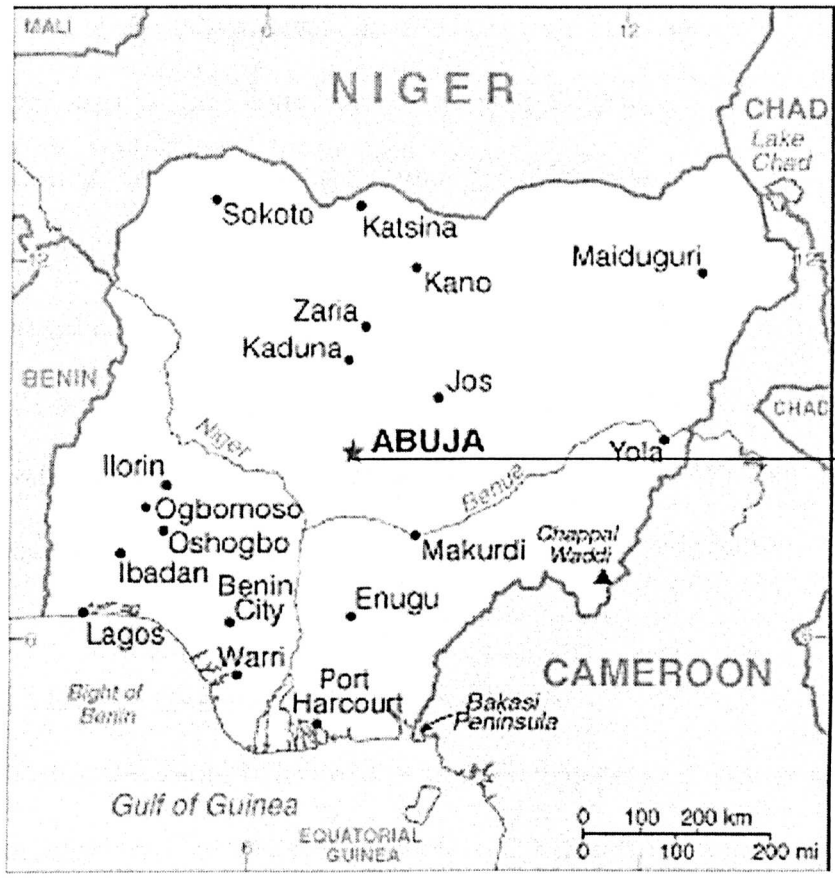
3.3.1 Project site

All field and most laboratory work was carried out in Abuja, Nigeria. Abuja is the capital of Nigeria. It is located within the Federal Capital city. It is the fastest growing city in Africa and the first planned city in Nigeria. The city was created in the 1980s after the decision to move the Nigerian capital from Lagos to its present location in the centre of the country. The capital was finally moved in 1991 and there is still construction going on in the city. It occupies an area of 3000-sq mi (7,770-sq km) and it is located at 9^{o} 10' north and 7^{o} 10' East. The climate is pleasant with the two main weather variations, typical for sub-Saharan tropical climate, the dry season (October-March) and the rainy season (March- October). It has an annual rainfall of 1632mm and between November and February the dry Harmattan winds are prevalent with occasional haze with cold nights and mornings (Abujacity.com et al., 2004).

The population of Abuja has increased from 1.5 million a few years ago to an estimated 4-5 million in 2005 with a large number of people living in satellite settlements around the city. There is a large influx of male unskilled workers, who come in for the construction jobs and a large civil service community.

There are 13 general district hospitals, several mission, many private and two reference hospitals, the National Hospital and the Gwagwalada Specialist Hospital. In addition, there are 156 primary health centres (PHC) in the FCT.

Figure 3.2 Map of Nigeria with the location of Abuja



3.3.2 The participating hospitals

Eight hospitals in Abuja were selected because of their size, number of beds, number of out patient visits and the convenience of being able to recruit patients for the study. The FCT runs six of these hospitals, as government owned district hospitals, one is owned by the Federal government and run as a specialist hospital and the eighth is owned and run as a general hospital by the Roman Catholic mission. The following sections describe each of these hospitals:

Wuse District Hospital (115 beds)

This is one of the largest hospitals in Abuja with 115 beds. It is located at a highly populated area in the Wuse district catering for mainly middle and lower class population. It was commissioned in the mid 1980s and has a very busy out-patient-department (OPD) with an average attendance of 230 to 250 patients daily (i.e. 6,500 to 7,000 patients/month). Apart from the OPD, there are other departments such as, obstetrics and gynaecology, surgery, medical and pharmaceutical. It has a laboratory that conducts most of the tests required in the hospital. Attached to the OPD is a DOTS treatment centre where upward of 20 patients are seen daily.

Asokoro District Hospital (80 beds)

Asoko District Hospital is one of the large Federal Capital City general hospitals. It was commissioned in December 2000 and serves the middle and lower class population of Asokoro, which is a low-density area. It has 80 beds for medical, surgical, paediatrics, obstetrics and gynaecological admission. It has a large outpatient department, which caters for approximately 6,400 patients a month. It has a complementary number of medical and non-medical staff, and a DOTS clinic which has in attendance 10 to 20 patients daily. The hospital is being used as a HIV vaccine research centre with funding from CDC and NIH from the USA.

Maitama General Hospital (80 beds)

This is also a large district general hospital, located at the centre of Abuja, the Maitama District. It is a well attended hospital by all class of patients with an average attendance of 6,000 patients per month. It was also commissioned in December 2001 and it is situated in a medium density area. This 80 bedded hospital has a well

equipped diagnostic laboratory and pharmacy. It has an outpatient and in-patient departments which include, surgical, medical, paediatrics, obstetrics and gynaecology and pharmacy. It runs DOTS and HIV/VCT clinics with average attendance of over 20 patients for counselling daily.

Kubwa General Hospital (30 beds)

Kubwa general Hospital is relatively a small hospital located in a fast growing Kubwa satellite town at the outskirt of Abuja. It a well attended hospital by the inhabitant and patients from the neiboughoring villages. It has an average attendance of 3000 patients per month and a DOTS programme, which caters for 5 to10 patients a day. It has 30 in-patients' beds and has an OPD department. Major medical or surgical cases are referred to the bigger hospitals or the National Hospital.

Gwarinpa General Hospital (30 beds)

Gwarinpa General Hospital is a 30-bedded general hospital located at an area formally used by construction workers who built up Abuja, called the Life Camp. It caters for people in this area and the nearby high density settlements of Du and Karimu. It has a DOTS programme that caters for 5 to 10 patients daily and an OPD that treats about 2000 patients monthly.

Nyanya General Hospital (20 beds)

Nyanya general Hospital is a small hospital with 20 beds and located in a densely populated satellite town at the southern outskirt of Abuja. Ten of the beds are allocated to male and 10 to the female patients and children. It has an average OPD

attendance of 3,500, a DOTS and NPI units, although patients in this area tend to attend the larger district hospitals in Abuja.

Gwagwalada Specialist Hospital (500 beds)

This is one of the oldest hospitals built in the FCT (1991). It is a 500 bedded specialist hospital, built with the intention of converting it into a teaching hospital when the University of Abuja starts a medical school. It was a very well equipped hospital but with time has deteriorated and most of the equipment is no longer functioning. It is well staffed with 29 consultants, 50 senior medical officers, 48 house officers and over 350 nurses. Its DOTS programme attends to over 300 patients daily and over 20,000 patients go through the OPD monthly.

St Mary's Hospital (30 beds)

St Mary's Hospital is a private hospital run by the Catholic mission in Abuja. It is located in the satellite town of Gwagwalada. It has 30 beds and sees an average of 650 patients at the OPD monthly. It has a DOTS programme, which was started at the initiation of this study.

Figure: 3.3 Map of the Federal Capital Territory, Abuja, Nigeria

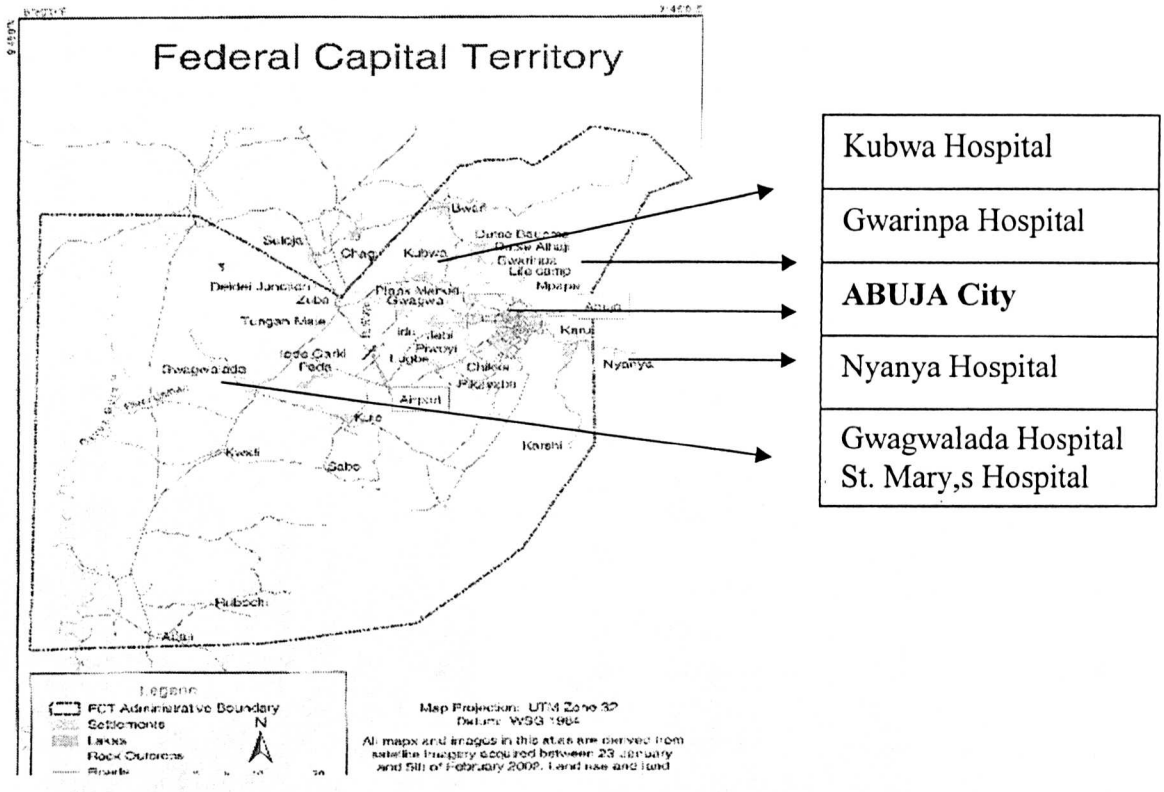


Figure: 3.4 Abuja, First phase of the Federal Capital City

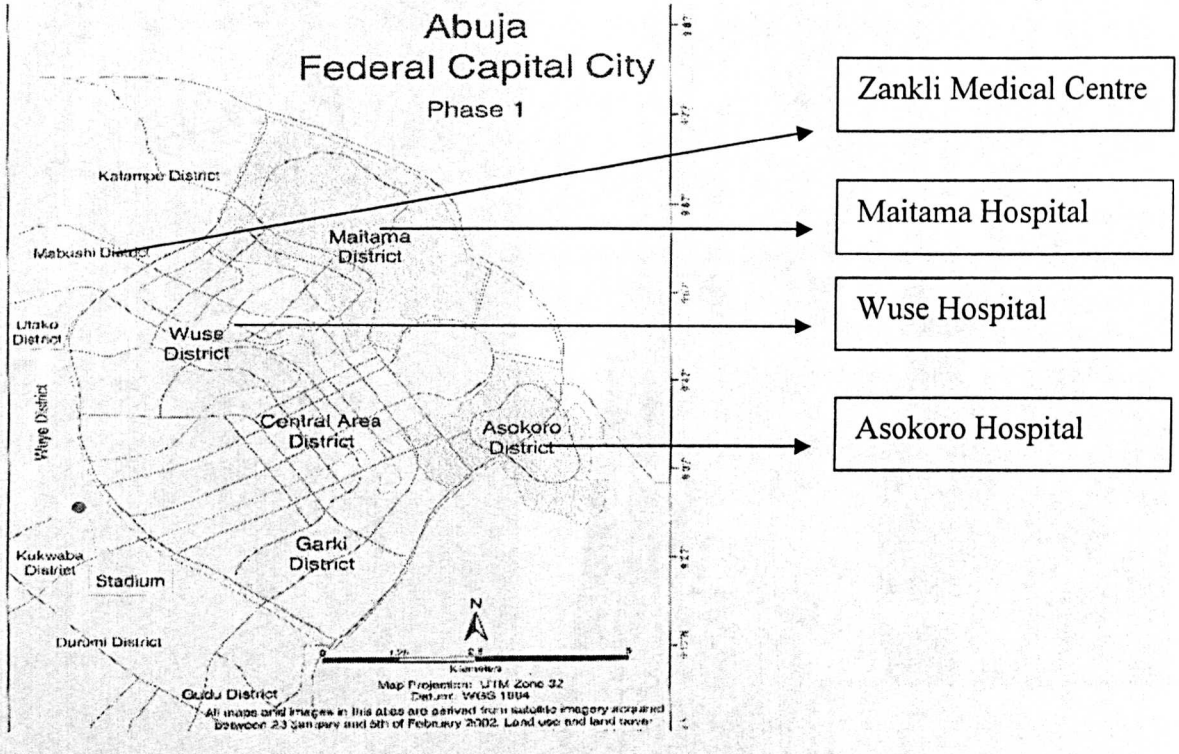
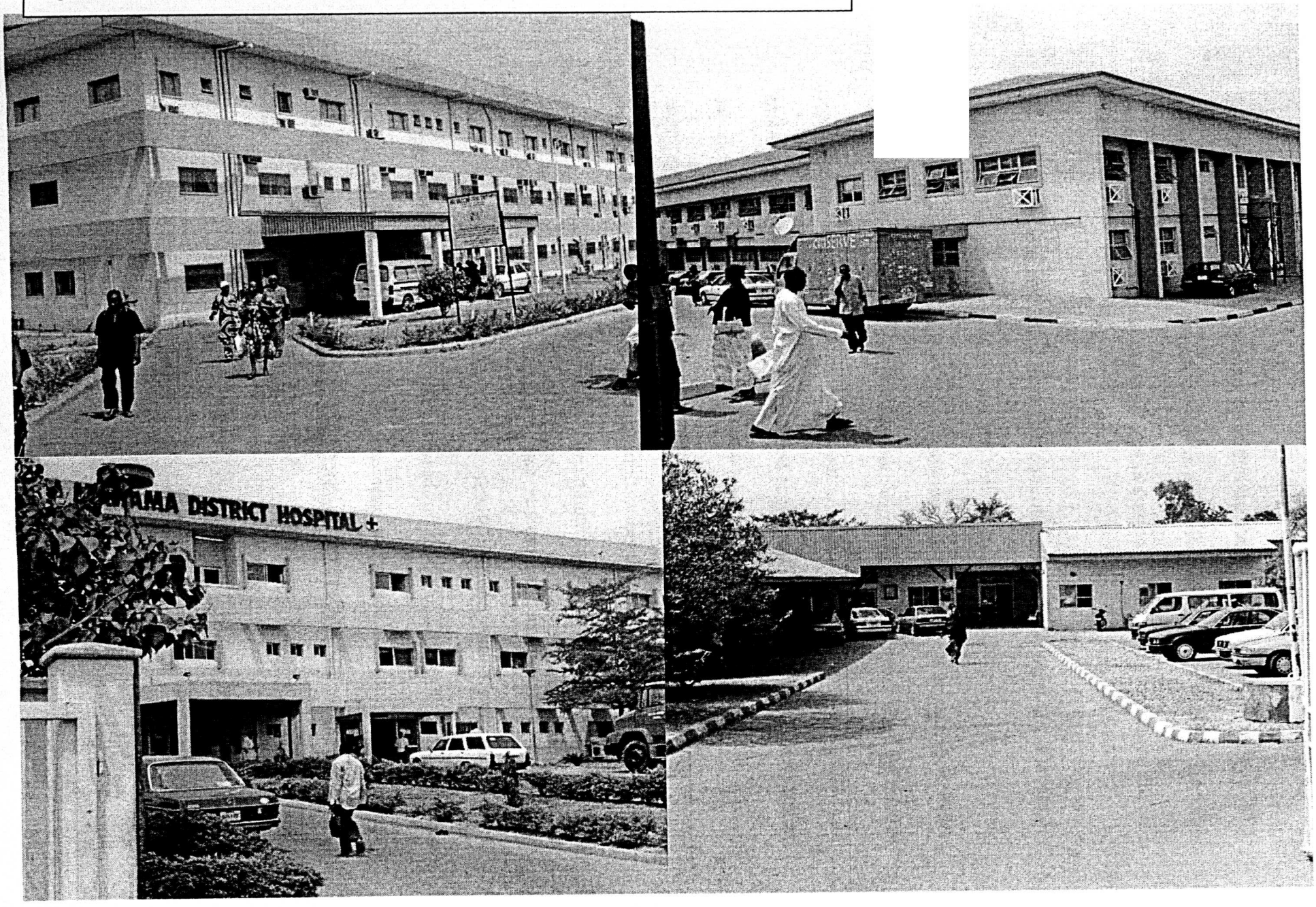


Figure 3.5 Some participating hospitals (a. Asokoro; b. Wuse; c. Maitama; d. Gwarinpa)

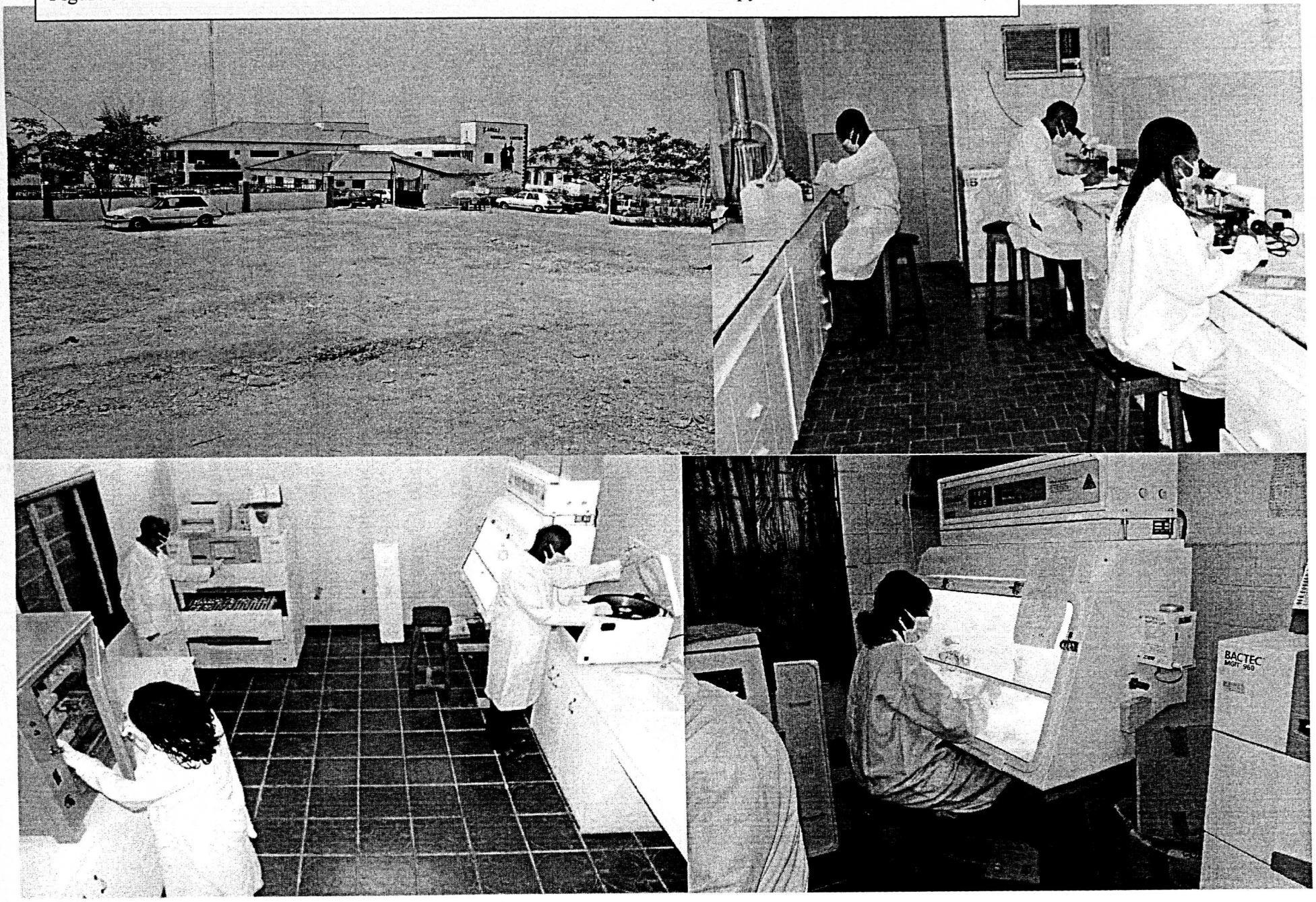


Zankli Research laboratory

Zankli research laboratory was set up within the Zankli Medical Centre in May 2003, primarily to handle this project, as it was difficult to obtain support from any other laboratory in the bigger institutions in Abuja that was capable of or willing to handle a project of this magnitude.

The laboratory was staffed by three microbiologists, a haematologist and a laboratory technician. The laboratory was equipped with a computer dedicated to data entry and managed by a data manager. The air-conditioned laboratory was equipped with a BACTEC 960 system for culture of sputum samples, two moderate sized incubators, microscopes, a safety cabinet, water bath, water distiller and facilities for ZN microscopy including reagents. The blood samples were handled in the haematology laboratory within the medical centre. The laboratory and the equipment were cleaned every day. Samples brought from the hospitals were received by the laboratory and registered. The data manager entered information on each patient into the database including results from the laboratory. The sputum samples collected from each patient were stained using the ZN technique. One randomly chosen sample from these was bleach-digested and stained by the ZN method and one further randomly selected sample was decontaminated and cultured in the BACTEC 960. All ZN slides including the bleach-digested smears were read and graded according to International Union Against Tuberculosis and Lung Disease (IUATLD) scale as shown in table: 3.1.

Figure 3.6 Zankli Medical Centre and Zankli Research Laboratories (Microscopy and Culture Laboratories)



3.3.3 Data processing

Data were collected through face to face interviews and using a standard questionnaire. Clinical, laboratory and radiological information were all entered daily into a database created in Epi-Info version 3.2 (Centre for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA, 30333) by a data manager. A second database was created to enter information collected on side effects of treatment in the patients. All data entered into the database were regularly backed up in a USB portable flash-disk and a personal laptop belonging to the principal investigator.

Data were regularly checked for typing errors and corrected where mistakes were detected. Data out of range were rechecked from the original data and correction made when necessary. Double entry of records and other inconsistencies were regularised. Epi-Info does not include missing data in the analysis.

3.3.4 Follow up

Patients were followed up at the clinics on weekly bases in the first two months; when they received the anti-TB drugs for the intensive phase, and were asked to swallow the weekly micronutrient pills on the spot (See chapter 10 for a full description of the micronutrient clinical trial design). After the intensive phase, patients were seen monthly for the continuation phase of the TB treatment and were given a monthly supply of micronutrients at each visit. Patients were given a bottle of Lucozade at each visit to encourage their return for the follow-up visits but were not given financial incentives. All patients in the micronutrient study had a chest X-ray taken at

the time of enrolment, at the end of the second month and at the end of six months of therapy.

3.4 Treatment

At each of the participating hospitals, there was an established DOTS programme where all smear positive patients were given free drugs and managed according to the DOTS programme. All patients received the standard TB treatment approved by the Federal Ministry of Health of Nigeria and used in the participating hospitals. This treatment is divided into an intensive phase of 2 months and a continuation phase of 6 months. For the Intensive phase patients were given Rifampicin, 450-600mg daily; Isoniazid, 300mg daily; Ethambutol 1gm daily (15mg/Kg); and Pyrazinzmide, 1.5- 2g daily and for the continuation phase, they were given Isoniazid and Ethambutol.

In addition to the standard treatment, patients participating in the clinical trial (described in chapter 10) were randomly assigned to receive micronutrient supplements. The randomisation process is described in chapter 10. The three supplement groups were:

a. one group received 90mg elemental Zn weekly (as zinc sulphate), in a lactose matrix, in form of a tablet plus weekly 1500 retinol, which is equivalent to 5000IU of vitamin A (as retinyl acetate) in a capsular form.

b. a second group received 90mg of elemental Zn weekly plus a placebo that looked similar to vitamin A

c. a third group received weekly placebos similar to the zinc tablet and weekly placebo capsules similar to vitamin A

All capsules and tablets were prepared in Liverpool, packed, coded and sent from LSTM. The capsules were indistinguishable to both researchers and patients. Because some patients were lost to follow up very early in the study, a further 50 patients were recruited the trial and allocated into the groups by a coded arrangement from LSTM.

The DOTS programme was strengthened by employment of the 8 community health nurses who were responsible for the follow up and supervision of the patients being treated for TB.

A two-month pilot study (July to September 2003) was set up to identify and address the problems involving the project. These were discussed weekly with all TB staff. These meetings continued to the end of the follow up.

A trial monitoring committee was set up to receive fortnightly data sets to monitor side effects of the interventions. This committee was also to supervise an interim analysis of the data. However, as the sample size of the study was cut down to 50% of the original plan, this interim analysis did not take place.

External quality control and technical advice was provided by Dr A Ramsay, research fellow at the LSTM who visited the premises and advised on standardisation of procedures.

Figure 3.7 Project supervisors visit Abuja (Dr Luis Cuevas, Professor Peter P.D. Davies and Dr Tom Thacher

3.5 WHO case definition for TB

The internationally accepted case definitions for TB by WHO is based solely on the principles of three direct smears and culture. The definitions as stated by WHO are;

1. Definite: $\geq$ 2 positive direct smears or one positive direct smear plus positive culture
2. Very likely: three negative direct smears but positive culture
3. Less likely: one positive direct smear and negative culture
4. Unlikely: three negative direct smears and negative culture

3.6 Laboratory Methods

This was a cross sectional survey of patients attending 8 district hospitals in Abuja, Nigeria, from September 2003 to July 2004 with a clinical suspicion of PTB. After signed informed consent, patients 15 years old or older were asked to submit 3 sputum samples (on-the-spot, early-morning and second-on-the-spot), as recommended by the International Union Against Tuberculosis and Lung Diseases (IUATLD) (Enarson 1995) and the National TB and Leprosy Control Programme of Nigeria. (NTBLCP, 2003) revised as a workers' manual 4th edition in 2004 (NTBLCP 2004).

Standard direct smears were prepared from the sputum samples using the ZN staining method. In addition, one of these three sputum samples was selected at random and an equal amount of a locally produced household bleach (Jik, 3.5% NaOCL, Reckitt Benkiser Nig LTD, Industrial Estate Agbara, Ogun State, Nigeria) was added and left

to digest for 30-45 min at a slant of 45° angle as described by Yassin et al (2000). A drop was taken from the bottom of the container of the digested specimen, smeared on a slide, and stained using the ZN technique. All four slides were read by two technicians unaware of the results from each other and graded according to the IUATLD scale. A third dedicated technician read the slides with discrepant results and the readings were discussed to reach an agreement. All sputum samples were processed in the Zankli Medical Centre laboratory. Sputum samples were collected on the day of submission and processed within 4 hours. One of the sputum samples was randomly selected from each patient and cultured on a BACTEC 960 system.

All patients were screened for HIV using a ImmunoComb HIV1 & 2 BiSpot kit (ORGENICS, P.O. Box 360 Yavne 70650, Israel) after written informed consent and pre- and post-test counselling. HIV positive patients who completed their anti-TB treatment were referred to the Gede foundation (http://www.gedefoundation.org), a non-governmental organisation providing anti-HIV treatment at subsidized price in Abuja. The results of all HIV tests were kept in a separate anonymous database that was linked with the main database.

3.6.1 ZN staining procedure

A drop of sputum was smeared (1cm x 2cm) on a new, clean and grease-free slide and left to air-dry. The slide was placed on a hot plate for about 5min to fix after which it was flooded with carbol fuchsin. The slide was left to stand for 15min at room temperature and was then steamed gently with flame from underside for 1min. The stain was allowed to stand on the slide for a further 5 min, washed with distilled water

and tilted to drain. The preparation was decolorized with 3% acid alcohol, rinsed with distilled water and tilted to drain. The slide was then flooded with malachite green for 1 min, rinsed with distilled water and placed on draining rack to air-dry.

3.6.2 Bleach/ZN staining procedure

An equal amount of locally produced household bleach (Jik, 3.5% NaOCL, Reckitt Benkiser Nig LTD, Industrial Estate Agbara, Ogun State, Nigeria) was added to a randomly selected sputum sample from each patient. The mixture was left to digest for 30-45 min at a slant of 45° angle as described by Yassin et al (2003) (Yassin et al., 2003) with the difference that the bleach concentration used was 3.5% as opposed to the 5% in Yassin's study. A drop was taken from the bottom of the digested specimen, smeared on a slide, and stained using the ZN technique. The slide was dried using a wall drier and then read together with the three standard ZN slides by the two-trained microscopists.

Table 3.1 **IUATLD sputum smears grading system (Enarson et al., 2000)**

IUATLD sputum smears grading system	
AFB Count	**Score/Reading**
No AFB seen (in at least 100 fields	Negative
1-9 AFB in 100 fields	Actual AFB count (Scanty)
10-99 AFB in 100 fields	+
1-10 AFB in at least 50 fields	++
>10 AFB per field in at least 20 fields	+++

3.6.3 Culture on BACTEC 960

One of the three sputum samples was randomly selected from each patient, decontaminated and cultured on the BACTEC 960 system. The procedures for decontamination were carried out in a safety cabinet.

Modified Petroff decontamination method

To an Xml of sputum (depending on the quantity available), twice that volume of NaOH (4%) was added and shaken properly to digest the sputum. The mixture was allowed to settle for 15min at room temperature with occasional shaking and then centrifuged at 3000g for 15min. The supernatant was poured off and 15mls of sterile saline was added and the sediment re-suspended. The mixture was again centrifuged for 15mins, the supernatant decanted and inoculated on to the culture medium (MGIT tube) immediately.

BACTEC procedure

A lyophilized vial of BBL MGIT PANTA antibiotic mixture was reconstituted with 15mls of BACTEC MGIT growth supplement (All from Becton Dickinson International, Distribution Centre, Laagstraat 57, 9140, Temse, Belgium). 0.8ml of the mixture was aseptically added to a labelled MGIT tube (Contains 7mls of modified Middlebrook 7H9 Brith base). 0.5ml of the decontaminated sputum specimen was also added to the MGIT tube. The tube was recapped properly, mixed thoroughly and placed into the BACTEC 960 at an incubation temperature of 37°C. The bottle was

automatically monitored every 60min by the instrument for increasing fluorescence. Culture bottles that remained negative after 42 days were removed from the instrument and classified as a negative culture. A fluorescent compound is embedded in silicon, which is placed at the bottom of the 16x100mm round bottom MGIT tube. The fluorescent compound is sensitive to the level of oxygen in the broth. The broth is well enriched with oxygen and this suppresses emission of the florescent compound. As the microorganism respires, it consumes the oxygen in the tube and allows the florescence to be detected and read by the BACTEC as positive. An instrument positive tube contains approximately 10^5-10^6 CFU per ml. The MGIT tube can not differentiate species of mycobacteria, as such, positive test result can not be said to be *M. tuberculosis*. Samples positive in the BACTEC system were sub-cultured onto LJ slopes to confirm a growth rate and morphology consistent with *M.tuberculosis* complex..

3.6.4 Follow up laboratory tests

Patients who were positive for TB by the WHO definition ($\geq$ 2 smear positives) were enrolled for the micronutrient clinical trial. First on-the-spot and morning sputum samples were collected from the patients during their weekly visit to the hospitals and were examined at the Zankli Research Laboratory. The weekly sputum examination of the patients continued until such a time that three consecutive samples were negative.

Apart from those taken at enrolment, blood samples were collected at the first, second, third and sixth month for chemistry and micronutrient studies. Samples for

micronutrient study were frozen in safe-lock tubes and stored at -20°C until transported to Liverpool.

Materials and methods for each specific objective will be further expanded in the appropriate chapters.

Monotest (MT) (PPD-tuberculin)

The Monotest was supplied as Monovac (Institute Merieux, 17, rue Bourgelat 69002, Lyon, France). It is a PPD-tuberculin test and 0.1ml is administered intradermally, preferably at the flexor surface of the forearm and examined after 48 to 72 hours. Induration greater than or equal to 2mm are considered positive (Son, 1977). The Monotest shows significantly more positive readings after 72 hours and is better tolerated than the routine Mantoux test or the Tine test. As regard to application and skin reaction, Herzog and Birkhauser (Herzog et al., 1980) recommended that the Monovac test be substituted for the Mantoux test and used as a screening test for general practice. Despite planning to apply the test to all participants, only few patients were inoculated due to sudden unavailability and increased cost of the Monotest during the study.

3.7 Sample size calculations for each objective

3.7.1 Sample size for objectives 1, 2 and 3

We expected that the prevalence with TB was about 85%. To compare the agreement between standard and bleached smears, we then set to find the Kappa statistics with types 1 and 2 errors of 90% and 95% respectively. A sample size of 250 ZN positive patients, were recruited based on its expected level of agreement between case definition and bleach (Kappa).

3.7.2 Sample size for objective 5

Sample size for the clinical trial was calculated to replicate the proportion of patients who were positive by ZN, two, four and eight weeks after treatment observed in an initial trial in Indonesia (Karyadi, 2002). This is fully discussed in chapter 10.

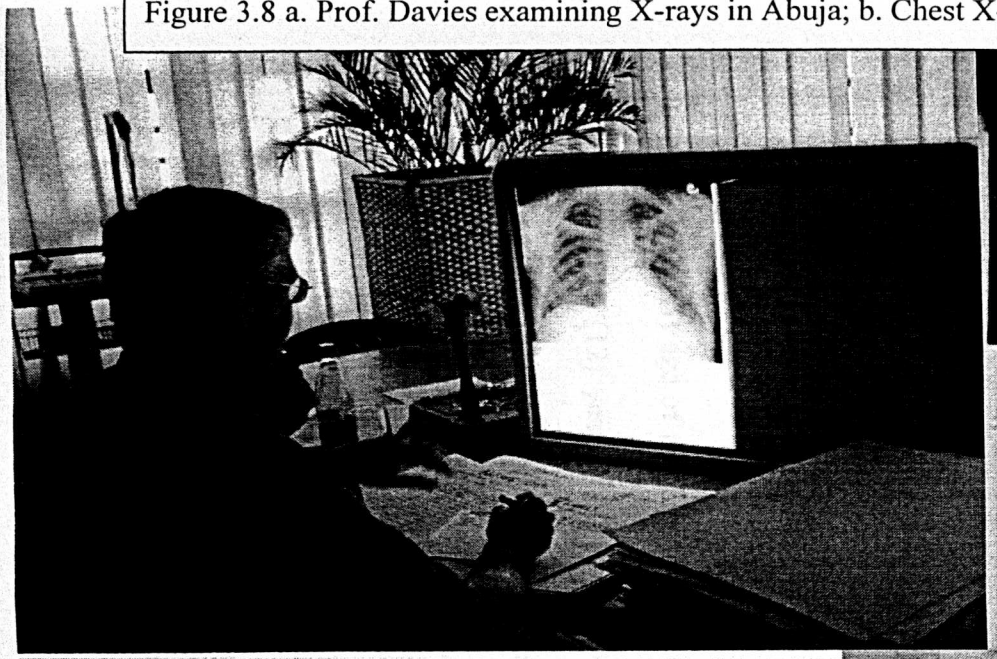
3.8 Quality Control

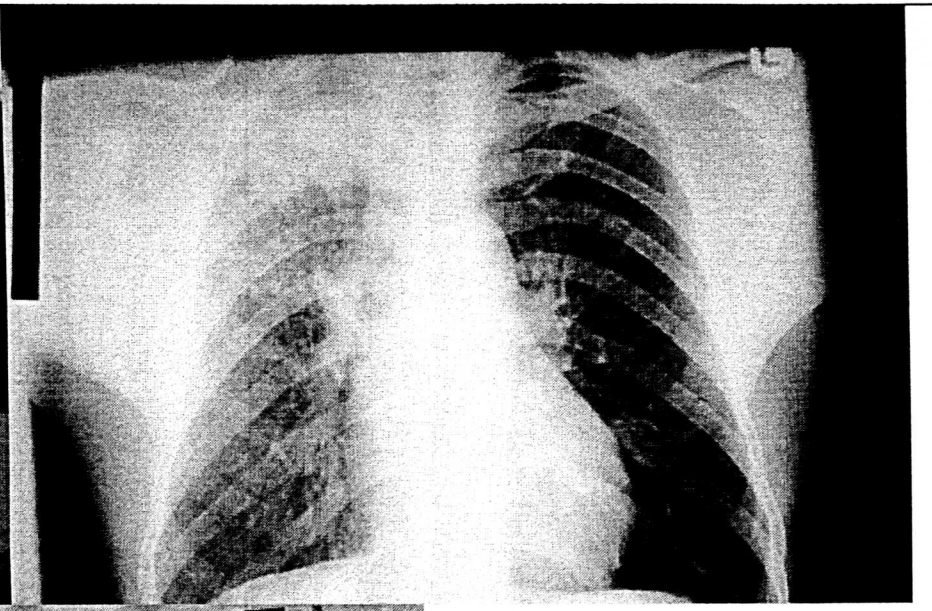
All reagents used during this project were purchased from local reputable companies and all solutions were prepared in the Zankli laboratory using standard procedures. The reagents were checked for expiry dates. At regular intervals and everytime when new reagents were purchased, sputum smears were prepared with known positive and negative samples to determine the quality of the reagents. Records were kept for the date, quality and type of reagents. One external assessor from LSTM visited the laboratory and conducted an external quality assessment. The PI supervisors visited the study site on several occassion.

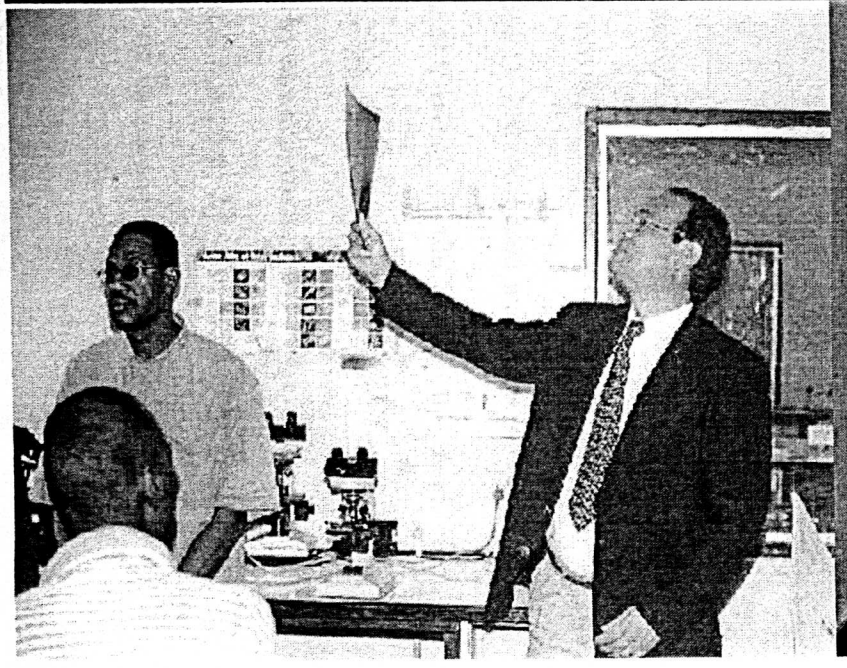
3.9 Ethical considerations

Ethical approval for the study was obtained from the ethics committees of the Liverpool School of Tropical Medicine, UK; Gwagwalada Specialist Hospital, St Mary's Hospital and the Department of Health Services of the Federal Capital Development Authority (FCDA), which is responsible for the management of all the participating district hospitals.

Figure 3.8 a. Prof. Davies examining X-rays in Abuja; b. Chest X-ray of a patient; c. Dr Cuevas in Abuja; d. BACTEC 960







CHAPTER FOUR

General characteristics of the study population

4.1 Background

During the study period from September 2003 to April 2005, 1,321 patients who attended the eight participating district hospitals with a history of cough of more than three weeks or symptoms suspicious of PTB, were screened. The procedures consisted of screening for compliance with inclusion and exclusion criteria for enrolment into the project, discussion and completion of the consent form and referral to the attending physician of the hospital if excluded. The inclusion and exclusion criteria are listed in appendix 1. This section describes the characteristics of the 1321 patients for background information.

General characteristics

The mean (SD) age of the1321 patients was 34 (12) years and was 35 (11) years for males and 33 (12) years for females. Seven hundred and seventy-four (59%) were male and 547 (41%) were female (table 4.1). Five hundred and sixty (42%) of the patients were between 25 to 34 years old, which is the age group most often associated with TB and HIV (Raviglione 2003; Aerts et al., 2004; Prasad et al., 2000; Anteyi et al., 1996). The age distribution by sex is shown in figure 4.1. The peak age group among both male and female patients was 25 to 34 years with 39% being male and 47% being female. The presence of more male patients in the study is

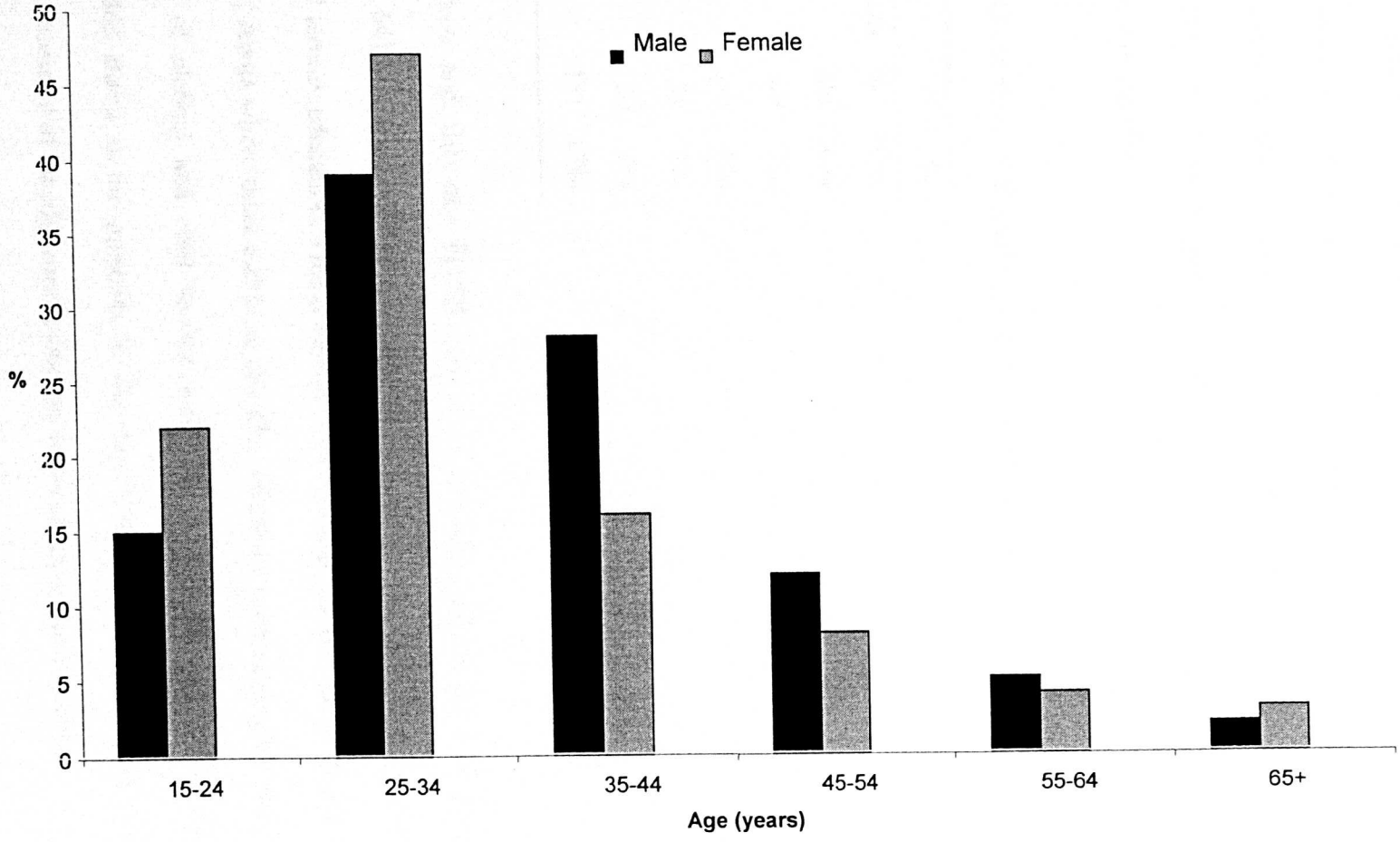
understandable as Abuja is a new city with many job opportunities for men. Few patients above the age of 65 years attended the hospital with symptoms suspicious of TB.

Table: 4.1 Characteristics of the population screened for TB

		N= 1321
Age in years	All	34 (12) [15-92]
	Male	35 (11) [15-92]
	Female	33 (12) [15-90]
Male: Female (% male)		774: 547 (59%)
Activities at the time of consultation	Working	912 (69%)
	Not working	95 (7%)
	Studying	176 (13%)
	Housewife	138 (10%)
Education	None	150 (11%)
	Primary	256 (19%)
	Secondary	643 (49%)
	Tertiary	272 (21%)
Number of people per room		3 (2) [1-26]
Number of smokers		291 (22%)
	Duration of smoking in years	10 (9) [1-54]
	Number of cigarettes per day	7 (7) [1-66]
Contact with person with cough of > 3wks		222 (17%)
	Mean length of contact in weeks	6 (8) [1-52]
Contact with person with fever of > 3 wks		100 (8%)
Contact with person with PTB within 2 years		205 (16%)
	Mean length of contact in years	5 (5) [1-28]
BCG in the past		920 (69%)

N (%) [range] unless stated

Figure: 4.1 Age of the participants screened by sex



4.2 Socioeconomic characteristics of the patients

Nine hundred and twelve (69%) patients were employed, 95 (7%) were unemployed, 177 (13%) were students and 138 (10%) were housewives. Three hundred and sixty-one (40%) of those working were civil servants and 237 (26%) businessmen (table 4.2). Most of the patients working in the civil service were mainly low-level civil servants and most of those in business were small traders. Of note is the small number of long distant drivers, as this group in other studies has been associated with high incidence of TB and HIV.

Table: 4.2 Occupation of the participants who were working

Activity	N	%
Civil servants	361	40%
Business	236	26%
Trader	60	7%
Farmer	56	6%
Driver	28	3%
Teacher	20	2%
Others	151	17%

One hundred and fifty-one (11%) had no education, 257 (19%) had primary education, 641 (49%) attended or were attending secondary school at the time and 273 (21%) had tertiary education. This is typical of the educational pattern in Nigeria and more so of Abuja where there had been an influx of people with secondary education in search of clerical and civil service work. Most of these individuals live in overcrowded slums at the outskirts of the city. The 151 (11%) patients without education consisted of mostly manual workers in the construction business.

4.3 History of contact

The mean (SD) number of people who shared a bedroom with the patients was 3 (2), with one of the patients sharing his bedroom with 26 persons. The majority shared bedrooms with between one and four persons (82%) and seven patients (0.5%) lived alone.

Only 291 (22%) of the 1321 patients smoked cigarettes. This was consistent with the smoking pattern of the low-income population in Nigeria, as most of them could not afford to buy cigarettes. It is estimated that 20 to 40% of the general population of Nigeria smoke cigarettes (Bandele et al., 1987). The patients who smoked had done so for a mean (SD) of 10 (9) years, with one patient having smoked for 54 years. These patients consumed a mean (SD) of 7 (7) cigarettes per day (range 1-54).

Two hundred and twenty-two of the patients (17%) indicated they had been in contact with other adults with cough of over three weeks. The mean (SD) time of contact with such patients was 6 (8) weeks. Only 100 patients (8%) indicated they had been in contact with other persons with fever. Two hundred and five patients (16%) had been in contact with persons that had been previously diagnosed with PTB with a mean (SD) time of contact of 5 (5) years.

Nine hundred and twenty (69%) of the patients had had BCG vaccination.

4.4 Prevalence of TB among the participants

Three hundred and ninety-nine (30%) of the 1321 patients screened fulfilled the WHO definition for positive TB (≥ 2 positive direct smears) as shown in table 4.3. Of the smear positive patients, 262 (66%) were males and 137 (34%) females, among smear negative patients, 512 (56%) were males and 410 (44%) females (P= 0.001).

Table: 4.3 Smear results of the patients screened according to the WHO case definition of smear positive TB

	ZNSmear	
Sex	**Positive**	**Negative**
Male	262 (66%)	512 (56%)
Female	137 (34%)	410 (44%)
Total	399 (30.2%	922 (69.8)

Table: 4.4 Smear results of the patients screened according to WHO case definition by hospitals

Hospitals	screened	Smear positive (%)
Asokoro	239 (18%)	68 (28%)
Gwagwalada	113 (9%)	44 (39%)
Gwarimpa	85 (6%)	38 (45%)
Kubwa	133 (10%)	42 (32%)
Kuje	17 (1%)	1 (6%)
Maitama	195 (15%)	72 (37%)
Nyanyan	58 (4%)	23 (40%)
St Mary's	114 (9%)	26 (23%)
Wuse	367 (28%)	86 (23%)
		P= 0.001

The number of patients screened in each hospital depended on its size and location. The larger hospitals located in well-populated areas, such as Wuse, Maitama and Asokoro screened larger numbers of patients and had higher numbers of patients fulfilling the WHO case definition. Kuje Hospital which is only mentioned here, is located in Kuje near the Kuje Prison. This prison one of the largest prisons in Nigeria. For logistic reasons, recruitment of patients from this hospital was discontinued after one month. The catholic mission in Abuja runs St. Mary's Hospital and members of the church attached to the hospital who had chronic cough were asked to attend the hospital for diagnosis and treatment through announcements made during church services. Gwagwalada Specialist Hospital already had a TB control programme in place before this study and recruitment of patients was easier from the beginning of the study.

One sputum sample was cultured for each patient on enrolment and 1286 (97%) sputum samples were cultured on the BACTEC 960. Seven hundred and thirty-one (62%) of the specimens cultured were culture positive (table 4.5). Therefore about 50% of the patients who had TB were missed by the direct sputum smear microscopy.

Table: 4.5 BACTEC culture results of patients with symptoms of TB

BACTEC 960	Frequency	Percent
Positive	731	62%
Negative	455	38%
Total	1186	100%

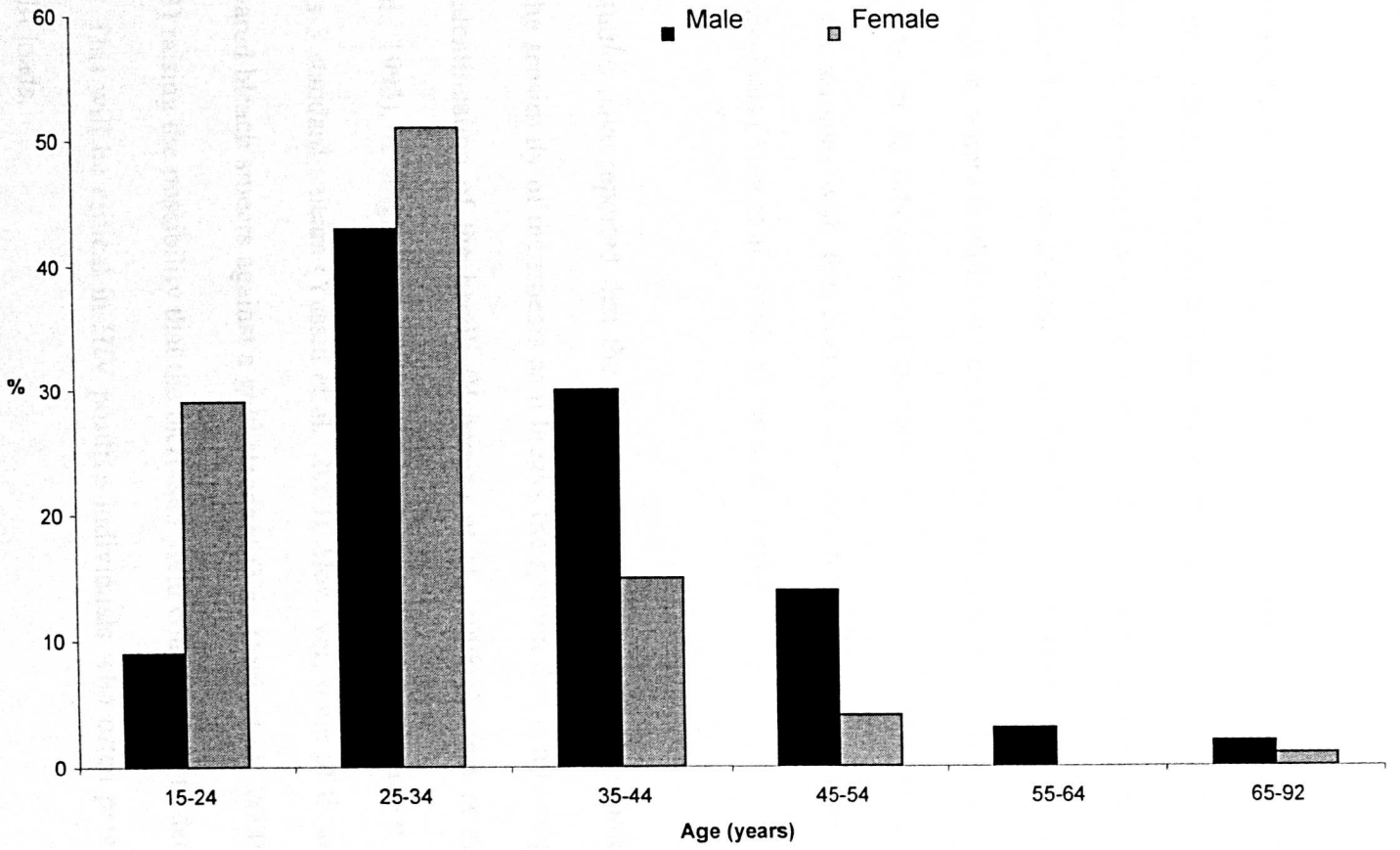
4.5 Prevalence of HIV among the participants

A total of 1045 (79%) of the 1321 patients were screened for HIV. Five hundred and sixty-six (54%) were positive for HIV and 479 (46%) were negative. Of those positive for HIV, 317 (56%) were male and 249 (44%) female (P= 0.003) (table 4.6)

Table 4.6 HIV prevalence by sex among participants

	HIV	
Sex	**Positive**	**Negative**
Male	317 (56%)	309 (64%)
Female	249 (44%)	170 (36%)
Total	566 (54%)	479 (46%)

Figure: 4.2 Proportion of patients who were HIV positive by age



CHAPTER FIVE

To validate the use of bleach digested sputum with Zeihl-Neelsen for the diagnosis of Pulmonary Tuberculosis against culture

5.1 Introduction

The cornerstone for TB control in resource-constrained settings is the rapid diagnosis and treatment of infectious patients. The standard practice requires the examination of 3 sputum smears, submitted over the course of 2 days, using ZN or other staining methods under the light microscope. The WHO case definition requires at least 2 positive smears or, where facilities are available, one positive smear together with one culture positive for *M. tuberculosis* (Enarson et al., 2000). This method however is insensitive for samples with few bacilli and seems to perform poorly in HIV co-infected individuals (Aber et al., 1980; Kemp et al., 1996).

Previous studies have reported that the digestion of sputum with household bleach improves the sensitivity of the smears as it breaks the sputum debris allowing for the enhanced identification of the bacilli (Habeenzu et al., 1998; Yassin et al., 2003; Gebre et al., 1995). A single smear using this technique has been reported to be as effective as 3 standard smears (Yassin et al., 2003). However, none of these studies have compared bleach smears against a gold standard (Van Deun et al., 2000; Yassin et al., 2003) raising the possibility that the increased yield could be due to decrease in specificity. This will be critical in HIV positive individuals who often present with lower bacilli loads.

This chapter describes a prospective survey of patients with PTB attending 8 district hospitals in Abuja, Nigeria, aiming to validate the bleach technique against culture.

5.2 Literature review

Although significant improvement have been made in improving mycobacteriological services in the developed world, little has been done in providing diagnostic tools utilizable in the resource poor settings. This is even more so in the development of diagnostic tools for TB, despite knowing about the aetiological agent for over a century.

The diagnosis of TB is the cornerstone of WHO global strategy for the implementation of the DOTS programme for the management of TB. The most widely used tool, the ZN method, though relatively inexpensive, rapid and able to detect those cases that are most infectious in areas of high TB prevalence, it is relatively insensitive (Perkins 2000; Aber et al., 1980; Kemp et al., 1996), detecting only about 20% to 40% of the world's eight million positive cases. The sensitivity of the technique is further undermined in areas with high HIV prevalence, probably through modifying the TB disease process, increases the proportion of patients with culture positive, smear negative TB (Elliott et al., 1993d). Problems associated with the 3 sputum specimens requirement include high drop out rates, since patients need to make repeated visits to the health facilities to submit specimens, obtain results and take treatment (Kemp et al., 1996). Consequently, some infectious cases will be missed, placing others at risk. In addition, the process of examination of 3 specimens

creates heavy workloads for the usually understaffed laboratories and this can impact upon the quality of the service (Mundy et al., 2003).

In the developed countries, new techniques are in the process of development or are fully developed and in use. These include nucleic acid amplification, phage replication, antibody detection, liquid culture, cellular immune recognition, antigen capture, chemical and physical detection, but none of these is available for use or has proved applicable in resource poor settings (Perkins 2000).

The global targets for TB control under DOTS include detecting 70% of new smear positive cases by 2005 (case detection). Current estimates suggest the current global detection rate is about (37%) and that this target will not be achieved (Dye et al., 2005). Health system weaknesses present challenges to improving case detection. Health staffing is in crisis in many countries and there is general lack of access to TB diagnostic services in resource-poor countries.

Various attempts to improve the sensitivity of the smear microscopy for the diagnosis of TB date as far back as 1908. Concentration of sputum in preparation for microscopy or culture was used in various forms (Hanks et al., 1938);(Petran et al., 1939). This was temporarily suspended because of the emergence of fluorescence microscopy and culture. It was revived in the nineties in the context of the developing countries when many studies showed impressive results in improving the sensitivity of smear microscopy using a variety of techniques (Gebre et al., 1995; Miorner et al., 1994; Habeenzu et al., 1998; Van Deun et al., 2000).

These methods generally involve an agent to digest or liquefy the sputum followed by sedimentation or centrifugation, prior to acid-fast staining. Agents used for sputum digestion include sodium hydroxide (Corper et al., 1949), N-acetylcysteine (Gilks et al., 1997), Chitin (Farnia et al., 2002) and sodium hypochlorite (household bleach 1%-5%) (Gebre et al., 1995; Van Deun et al., 2000; Habeenzu et al., 1998). Sodium hypochlorite (NaOCl) has the advantage of being readily available. It is an effective disinfectant, which kills off *M. tuberculosis* (Best et al., 1990) and HIV (Flynn et al., 1994), improving safety in laboratories that lack adequate bio-safety facilities.

Digestion of sputum with bleach, followed by centrifugation has been reported to show increased sensitivity over the direct smear (Miorner et al., 1996). Angeby et al., (Angeby et al., 2004), in their review of six studies where a gold standard was used, found a statistically significant ($P<0.05$) improvement in sensitivity with the bleach method compared to the direct smear method. Studies carried out in Ethiopia have Tabone et al., 1992reported that a single digested smear can equal the sensitivity of 3 direct smears (Yassin et al., 2003). However, Angeby et al., (Angeby et al., 2004) in their comparison of bleach microscopy with direct smear, found greater but not statistically significant increase in sensitivity using centrifugation (Gebre-Selassie 2003; Miorner et al., 1994). Most of the resource-poor countries however cannot afford the centrifugation technique due to the cost of purchasing centrifuges for their laboratories. Furthermore there is lack of or inconsistent electrical supply for the centrifuges to perform optimally and allow for wider use of the technique where it could be most useful.

Yassin et al., (Yassin et al., 2003) compared the diagnostic performance of the bleach sedimentation method to the standard WHO three-smear strategy and reported

increased sensitivity using this method over the direct smear and that a single digested smear can equal the sensitivity of three direct smears (Yassin et al., 2003). In this study however, the results were not validated against a culture, which is regarded as the gold standard.

Mycobacterial culture is a more sensitive method than direct smear microscopy for diagnosing PTB. We therefore compared bleach digested smear using the short-term method with the direct smear microscopy and culture in patients presenting with symptoms suggestive of PTB. If a single digested smear could replace the examination of 3 direct smears, and provide a one-stop diagnosis, it could make a significant contribution to improving case detection rates while reducing costs and workload.

5.3 Materials and Methods

5.3.1 Aims and objectives

The aim of this study was to validate the bleach technique against culture in a sub-Saharan African setting by comparing bleach digested sputum stained with ZN with smears prepared by the standard method and culture.

5.3.2 Study design

This was a cross sectional, prospective study of patients (aged ≥ 15 years) attending 8 district hospital in Abuja, Nigeria from September 2003 to June 2004 with clinical

suspicion of PTB and no previous history of TB treatment. The 8 community health nurses described in the general methods section (chapter 3) enrolled patients. Patients were asked to submit 3 sputum samples over 2 days (on-the-spot, early morning and second-on-the-spot) in sputum bottles. Samples were collected on a daily basis and submitted to the research laboratory at Zankli Medical Centre where they were processed within 24 hours.

5.3.3 Preparation of slides

Three standard direct smears were prepared from the sputum samples of each patient using the hot ZN staining method (Enarson et al., 2000). In addition, one sputum sample containing an adequate quantity of sputum (at least 5mLs) was selected from the three submitted and used to prepare a bleached-digested smear. A picture of a bottle of household bleach is shown in figure 5.1. A second randomly selected specimen was used for mycobacterial culture on the BACTEC 960. Preparation of the digested smear was already described in chapter 3.

The sputum smears (both digested and direct within each set) were examined under oil immersion lens (x 100) by two microscopists who were unaware of each others results and graded according to the IUATLD scale. A third dedicated technician read the slide with discrepant results and the readings were discussed to reach a consensus. Examples of digested and direct smears are shown in figure 5.2. For the purpose of this study, smears with less than 10 acid-fast bacilli (AFB) per 100 fields were grouped together under the description "scanty" and considered as positive (see chapter 7 for the validation of this procedure). Where results of digested smear were

Figure: 5.1 A JIK bottle containing 3.5% bleach

Figure: 5. 2 Bleach-digested and direct smear slides

Figure: 5. 3 Sputum specimen stained with ZN, demonstrating the typical appearance of AFB (Yassin, 2003)

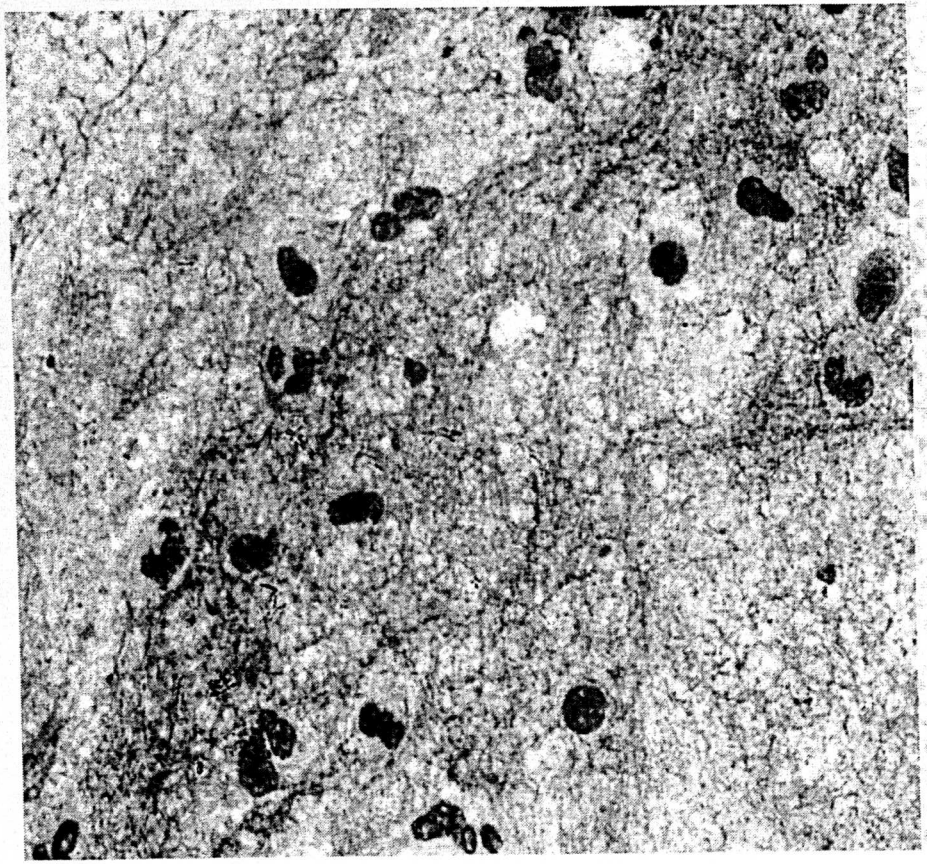
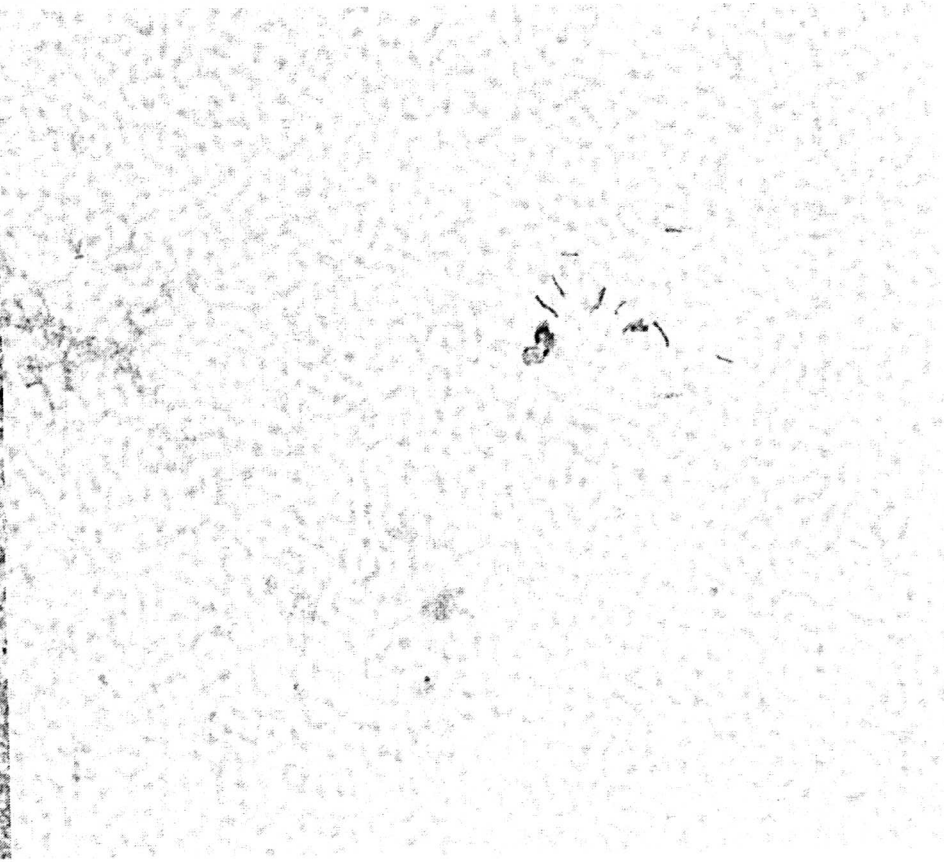


Figure: 5. 4 Bleach-digested sputum stained with ZN showing easily identifiable AFB (Yassin, 2003)

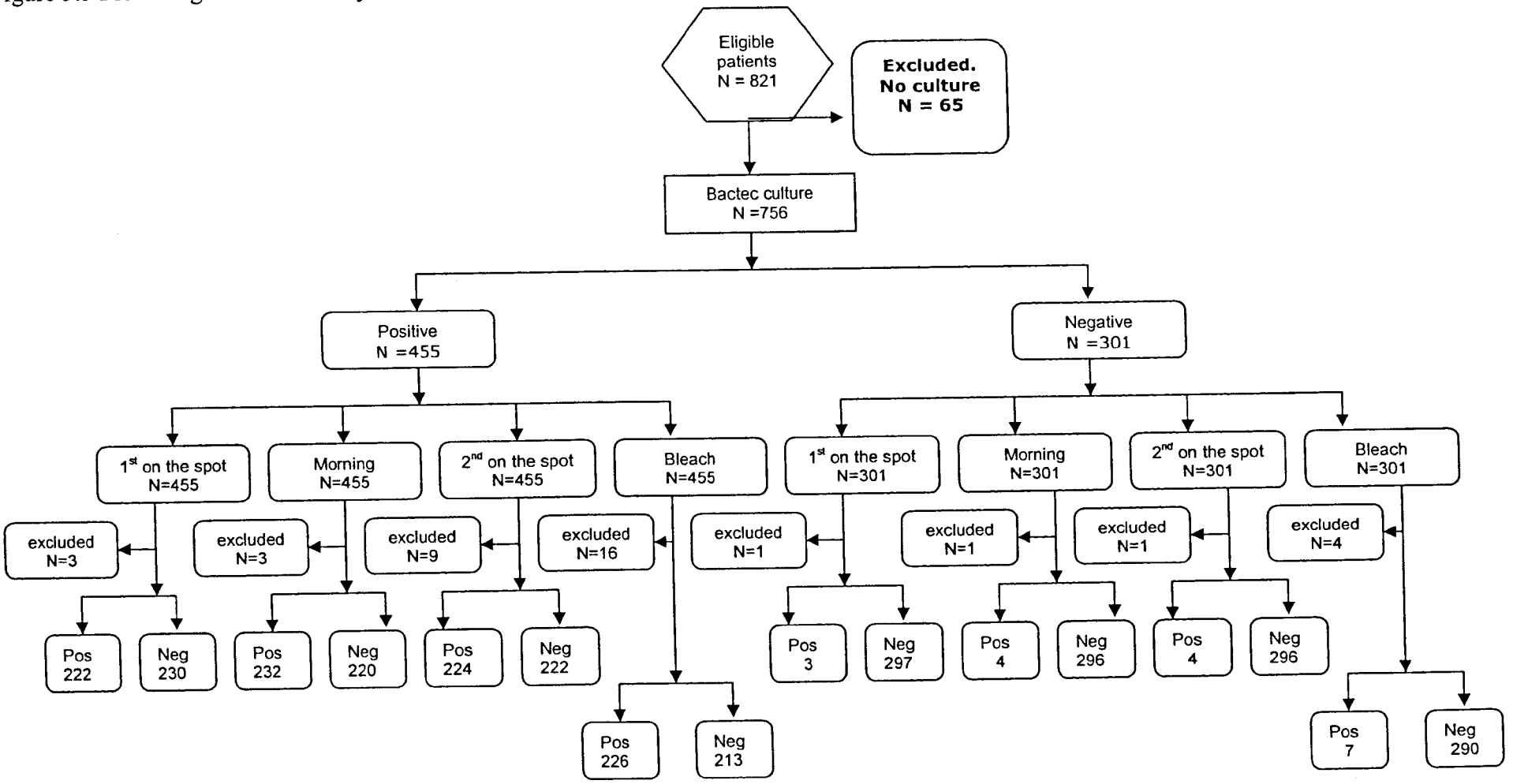


unavailable for analysis (due to non-submission of specimen, insufficient specimen, or mishap during laboratory processing) the results were recorded as missing and excluded from the comparison of the complete data sets. A flow diagram of the study is shown in figure 5.5.

5.3.4 BACTEC culture

The BACTEC 960 system was used for mycobacterial culture after specimens had been decontaminated using the Petroff's method as described in chapter 3. Facilities were not available at the time for definite confirmation of isolates as members of the *M. tuberculosis* complex, therefore the term "positive culture" here refers to the growth of AFB in culture.

Figure 5.5 Flow diagram of the study



5.3.5 Categorisation of patients

The categorisation of patients for this analysis was guided by the WHO case definitions for smear positive TB and based solely on the 3 direct smears and culture. The digested smear was not part of establishing cases to avoid incorporation bias The case definition was fully described in chapter 3. Briefly, the WHO considers any patient culture positive as a definite case. For the purpose of this study a "definite" sputum smear positive (SS+) PTB case was defined as patients with ≥ 2 initial sputum smear examinations positive for AFB; or one sputum smear examination positive for AFB plus sputum culture positive. Patients with negative smear microscopy but positive culture were considered "very likely" PTB cases but will be considered sputum smear negative (SS-). Patients with only one positive direct smear but negative culture were considered "less likely" to have PTB and those with negative direct microscopy and negative culture were considered as "unlikely" to have PTB.

5.3.6 Statistical methods

The results of single digested smears were compared to the WHO case definition. Sensitivity, specificity, positive and negative predictive values (PPV and NPV) were calculated. Comparison of the proportion of cases identified by direct smear microscopy and a single digested smear were tested using Chi-squares. The agreements of the digested smears with the direct ZN were compared using Kappa statistics.

5.4 Results and Analysis

The sample size for the study presented here is smaller than the sample size in subsequent chapters because the analysis was conducted before the project had finished recruiting patients. For this objective, 756 suspected TB patients were enrolled from 8 district hospitals. Of these, a total of 2251 (99% of the potential 2268)) direct smears, 736 (97%) bleached smears and 756 BACTEC cultures were prepared between September 2003 and June 2004. The results of 17 direct sputum smears and 20 digested smears were not available due to non-submission, insufficient volume of sputum specimen or mishap during laboratory processing. Four hundred and fifty-five (60%) of the 756 cultures were positive for TB and 301 were negative.

Of the 455 culture-positive patients, 222 (49%) of 1st-on-the-spot, 232 (51%) of morning and 224 (50%) of 2nd-on-the-pot specimens were positive by direct smear microscopy as shown in table 5.1. It should be noted that more smears prepared from early morning sputum samples were graded as "+++" than those prepared from 1st-on-spot ($P< 0.01$) and 2nd-on-spot ($P< 0.02$). Two hundred and twenty-six (51%) of the 455 culture positive patients had positive digested smears. Digested smears had more sputum samples graded "scanty" or "+" than the direct smears, consistent with the dilution effect of the digestion process. Both digested smears and all the three direct smears however had similar number of samples labelled negative.

Table: 5. 1 Microscopy results of standard direct smears and bleach digested smears by culture

	Unavailable	Negative	Scanty	+	++	+++
Positive culture						
1st on the spot	3 (1%)	230 (51%)	36 (8%)	83 (18%)	58 (13%)	45 (10%)
Morning	3 (1%)	220 (49%)	28 (6%)	72 (16%)	59 (13%)	73 (16%)
2nd on the spot	9 (1%)	222 (49%)	34 (8%)	72 (16%)	69 (15%)	49 (11%)
Bleach	16 (4%)	213 (47%)	30 (7%)	98 (22%)	55 (12%)	43 (10%)
Negative culture						
1st on the spot	1 (0.3%)	297 (99%)	0 (0%)	2 (0.7%)	1 (0.3%)	0 (0%)
Morning	1 (0.3%)	296 (99%)	1 (0.3%)	1 (0.3%)	1 (0.3%)	1 (0.3%)
2nd on the spot	1 (0.3%)	296 (99%)	2 (0.7%)	0 (0%)	1 (0.3%)	1 (0.3%)
Bleach	4 (1%)	290 (96%)	4 (1%)	1 (0.3%)	1 (0.3%)	1 (0.3%)

Of the 301 patients who were culture-negative, 3 (1%) of 1st-on-the-spot and 4 (1%) of morning and 2nd-on-the-spot direct smears were positive. In comparison, 7 (2%) of the digested smears of these patients were positive. Most of these positive digested smears were graded as scanty.

As per the study definition described above, a total of 235 (31%) patients were considered "definite" SS+ PTB cases (table 5.3). All these were diagnosed based on $\geq$ 2 direct positive smears (there were no cases of one positive direct smear plus positive culture). A further 223 (29%) were "very likely" to have SS- PTB, as they had positive culture but negative smear microscopy. Two patients had one positive direct smear but negative culture (considered "less likely" to have PTB) and 296 patients had 3 negative direct smears and negative culture (considered "unlikely PTB). Thus the WHO case definitions identified 51% of the cases with PTB (i.e. "definite" and "very likely" PTB). In comparison, a single digested smear identified

219 (93%) of the 235 patients with "definite" SS+ PTB and a further 10 who were "very likely" to have SS- PTB cases. A single digested smear therefore identified 229 (50%) of the cases with PTB. We can then justifiably say that the number of patients with PTB identified by a single digested smear is essentially the same as that identified by the 3-sputum smear strategy with confirmatory culture of singleton positives with similar specificity (294/298; 99%; 95%CI 97-100%)). The positive and negative predictive values (PPV, NPV) for the combined WHO case definitions for PTB were 100% (99%-100%) and 57% (53%-61%) respectively. In comparison, a single digested smear had a PPV and NPV of 98% (95%-99%) and 56% (52%-60%) respectively ($p>0.5$).

Table: 5.2 Bleach digested smears results by the case definitions

Certainty of PTB	N	Bleach digested smears						
		Unavailable	Negative	Scanty	+	++	+++	Total positive
Definite	235	12 (5%)	4 (2%)	28 (12%)	91 (39%)	56 (24%)	44 (19%)	219 (93%)
Very likely	223	4 (2%)	209 (94%)	3 (1%)	7 (3%)	0	0	10 (4%)
Less likely	2	0 (0%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Unlikely	296	4 (1%)	288 (98%)	3 (1%)	1 (0%)	0 (0%)	0 (0%)	4 (1%)

[1] Case definitions: Definite: ≥2 positive direct smears (positive/negative culture) or one positive direct smear plus positive culture; Very likely: three negative direct smears but positive culture; Less likely: only one positive direct smear and negative culture; Unlikely: three negative direct smears and negative culture.

5.5 Discussion

Direct sputum smear microscopy is considered the cornerstone of TB case finding in resource-poor countries. It is relatively cheap, simple to perform and identifies the most infectious cases amongst those presenting to health care facilities (i.e. those whose sputum contains the most TB bacilli). However, the requirement for repeated visits to submit specimens and receive a diagnosis is associated with considerable dropout rate from the diagnostic process, particularly of poorer patients. Furthermore, health service resources (including human resources) for TB control are increasingly constrained in many parts of the world, particularly those areas with high rates of HIV/TB dual infection. Sputum smear microscopy is considered one of the critical services provided by district health laboratories in Africa. Work in Malawi has shown that sputum smear microscopy using the 3-smear strategy can consume more than 40% of total staff time and, with the exception of blood transfusion services, consumes more of the annual laboratory budget than any other investigation (Mundy et al., 2003). The large numbers of sputum smears being examined in high prevalence settings also makes it difficult to adequately assess the quality of the service. Large samples of smears need to be rechecked (blind) by quality assessors if an assurance of minimum quality standards is to be statistically valid. Consequently, few national external quality assessment schemes function adequately in high prevalence countries.

The results presented here confirm the relatively low sensitivity of direct sputum smear microscopy: it identified 51% of the patients who were culture positive. Moreover, the sensitivity of the direct smear in this study is likely to be higher than that seen in most settings since all smears were examined twice in a research

laboratory, and all slides with 1-9 AFB per 100 fields were considered positive. The IUATLD/WHO grading system recommends a threshold of 10 AFB per 100 fields before a slide is regarded as a positive (i.e. only grades 1+ and above are considered positive). Recent work investigating the impact of lowering the positivity threshold in order to increase sensitivity of direct smear microscopy recommended a threshold of 4 or even 1 AFB per 100 fields as most of these smears are likely to be true positives in areas of high TB prevalence (Van Deun et al., 2004; Enarson et al., 2000) (see chapter 7). Most programmes require the collection of sputum specimens over 2 days to ensure the examination of an early morning specimen (the specimen considered most likely to be positive). Our findings indicate that, although morning samples from TB suspects are more likely to have higher smear grades, the proportion of slides classified as negative is independent of whether the sample was collected as an on the spot or as a morning sputum. Although this may be setting specific and be associated with later presentation and more advanced disease in our study population, such observations have prompted studies to examine the number of sputum specimens that are required to diagnose a patient as smear-positive in other locations (Van Deun et al., 2004; Yassin et al., 2003; Van Deun et al., 2002).

This study confirms earlier work in Ethiopia showing that a single digested smear is as sensitive as 3 direct sputum smears for the diagnosis of new cases of PTB (Yassin et al., 2003). The inclusion of mycobacterial culture in this study provides the validation of smear results, absent in previous work, and strengthens the evidence. It is likely that the method works, not so much through concentration of the TB bacilli in the specimen, but through the digestion of the material that forms the background of the microscopic field. The methylene blue-stained background of the microscopic

field appears paler and less distracting, and AFB are seen more clearly against it. See Figures 5.2 to 5.4 for examples of the smears. Thus, it may be more suitable for less-experienced microscopists.

The digestion method may benefit from some further development. During this study a number of digested smears were unavailable due to laboratory mishap. In every case, this mishap was the observed loss of the entire (heat-fixed) smear during the staining process. This is probably not an all-or-nothing phenomenon and it may be expected that all the digested smears are prone to some loss of material. It is possible that the bleach digests the proteinaceous material required for adherence of the clinical material to the glass slide. Bovine serum albumin has been used to promote adherence of sodium hydroxide treated sputum to microscope slides, but is likely to be a prohibitively expensive option for resource-poor settings (Ipuge et al., 1996). The use of alternatives such as sterile skimmed milk may be worth investigating. The effects of age and storage conditions of bleach on the performance of the method also need to be explored.

Advantages of using a single digested smear in place of the conventional 3 direct smears on specimens collected over 2 days include the potential of a same-day diagnosis and reduction of the drop-out rate during the diagnostic process (due to an anticipated reduction in patient costs and time involved). It allows for easier and safer preparation of smears, since digested sputum is easier to manipulate and is sterilised by the bleach treatment. This approach also reduces the laboratory load and costs as fewer specimens need to be processed resulting in a reduction in time spent on clerking specimens and writing reports, reduction in smears being fixed, stained and

examined and the clearer background to the microscopic field may be expected to make the screening of negative smears faster. Angeby et al (Angeby et al., 2004) recently reviewed studies of all methods using bleach for sputum digestion/concentration. They concluded that there is enough evidence and local concern to promote the introduction of a bleach method as a part of the DOTS strategy in countries where culture is not performed routinely, but they did not advocate any particular method of those reviewed.

5.6 Conclusion

This study provides evidence to support implementation, in an operational research setting, of a digested smear method for diagnosing new cases of TB in resource-poor countries. Household bleach is an almost universally available product even in rural areas of developing countries. A single digested smear equals the sensitivity and specificity of the 3 direct smear strategy. It can be expected to improve case-finding and lead to considerable savings in time and expenditure for health services and patients. Given the relatively low sensitivity of smear microscopy however, future work should focus on establishing how many additional patients would be detected if two or three smears are examined using the digested method.

CHAPTER SIX

Short-term bleach digestion of sputum for the diagnosis of PTB in patients co-infected with HIV/AIDS

6.1 Introduction

Globally, HIV infection is the single most important risk factor for the development of TB (Raviglione et al., 1997). Areas with high rates of dual TB and HIV infections, such as sub-Saharan Africa, are particularly hardest hit. Smear microscopy is the main diagnostic technique used in peripheral health centres in developing countries. These centres are where majority of the patients seek medical care (Enarson et al., 2000). Patients with TB and HIV co-infection however expectorate low numbers of AFB and direct smear microscopy has a lower sensitivity in these patients compared to patients without HIV (Elliott et al., 1993d). Techniques that could improve the detection of scanty bacilli are therefore needed to improve the performance of smear microscopy in patients with HIV.

The digestion of the sputum with household bleach prior to staining with ZN facilitates the preparation of clearer smears (Habeenzu et al., 1998; Gebre et al., 1995; Van Deun et al., 2000; Yassin et al., 2003). A single digested smear has been shown to result in the same yield as three direct undigested smears in patients suspected of having PTB (Yassin et al., 2003). Previous reports however have not validated its performance by culture in patients with HIV.

This chapter compares the performance of single a bleach digested ZN smear against standard direct microscopy and culture in patients with and without HIV co-infection.

6.2 Literature review

TB is a major cause of morbidity and mortality especially in the developing world and co-infection with HIV is a single risk factor for developing overt TB (Stanford et al., 1991; De Cock 1996); WHO 2000b). In Africa, especially in eastern and southern regions, HIV is one of the most important factors responsible for the increased incidence of TB in the past 10 years (Cantwell et al., 1996; De Cock 1996).

Even though microscopy performs well in cases of open PTB, this is not so in patients with low bacillary loads (Aber et al., 1980; Kemp et al., 1996) and in those co-infected with HIV (Perkins 2000), who have fewer cavities and produce fewer bacilli (Harries et al., 1998; Alarcon et al., 2003). This means that in HIV-positive patients co-infected with TB, the possibility of detecting AFB in sputum is reduced and the frequent occurrence of atypical and extra-pulmonary disease adds to the increasing problems of TB diagnosis and treatment in overburdened control programmes (Harries et al., 1998; Havlir et al., 1999).

Though sputum microscopy is specific and rapid, it has the flaw of being relatively insensitive, even before the advent of HIV, with sensitivity in the range of 30%-40% with a single sputum specimen and 65%-75% with repeated smear examinations (Daniel 1989). The digestion of sputum with household bleach (NaOCL) and sedimentation prior to ZN staining improves the sensitivity of smear microscopy in

several developing countries (Gebre et al., 1995; Wilkinson et al., 1997; Habeenzu et al., 1998; Angeby et al., 2004) but these reports did not relate the HIV status to the outcome of the smear test and not much was known of how the technique would perform in HIV patients co-infected with TB.

In 1996 in Addis Ababa, Ethiopia, Bruchfeld et al., evaluated the sensitivity of the concentration method in a large cohort of consecutive patients with suspected PTB and found that the overall sensitivity increased from 54.2% using the conventional direct microscopy to 63.1% after concentration (Bruchfeld et al., 2000) ($P<0.0015$). In HIV-positive patients, the sensitivity increased from 38.5% before to 50% after concentration ($P<0.0034$). They suggested that with the significant increase in yield of AFB in HIV-positive patients, the bleach/ZN method should have a place in routine diagnosis of PTB in countries with high prevalence of HIV. Douthwaite et al., (Douthwaite et al., 2006) suggested that the bleach technique could be useful in HIV-positive suspects with low bacillary counts in sputum, which will be especially important for TB control programmes with increasing numbers of HIV associated TB. Chapter 6 re-analyses the data in chapter 5 stratified by the HIV status of the patients.

6.3 Materials and Methods

6.3.1 Aim and Objective

The aim of this chapter is to compare the performance of a single bleach digested ZN smear against standard direct microscopy and culture in patients with and without HIV co-infection

6.3.2 Study Design

This was a cross sectional survey of patients with a clinical suspicion of PTB attending 8 district hospitals of Abuja, Nigeria as described in chapter 3. In addition to the laboratory work for the diagnosis of TB, patients were screened for HIV using an ImmunoComb HIV1 & 2 BiSpot kit (ORGENICS, P.O. Box 360 Yavne 70650, Israel) after written informed consent and pre- and post-test counselling. As culture results were unavailable on enrolment, all patients with positive smear microscopy were tested prospectively for HIV. All available blood samples which had been collected at enrolment from patients with negative smears but positive culture and those with negative smear and culture were tested retrospectively for HIV. Patients in whom a blood sample was unavailable (sample not taken or sample lost) were not tested for HIV but were included in the results as a separate group for completeness of the data. The smear microscopy results were reported in the previous chapter, which validated the bleach technique against culture.

6.3.3 Statistical Analysis

Digested smear results were compared to direct smears stratified by HIV status and the agreement of the smear readings were tested using kappa statistics. Patients were then classified into definite, very likely less likely and unlikely, using the WHO definition for TB, following the case definition described in chapter 3. Sensitivity, specificity, positive and negative predictive values (95% confidence intervals) and the yield of a single digested smear were calculated by defining cases with PTB as those with "definite" and "very likely" PTB.

6.4 Results

Seven hundred and fifty-six patients attending the eight participating hospitals between September 2003 and June 2004 with clinical symptoms of PTB had their sputum examined and cultured. Of these patients, 455 (60%) were culture positive. Four hundred and thirteen (91% of culture-positive patients) were screened for HIV. Of those screened 230 (56%) were HIV positive and 183 (44%) were HIV negative. The results of single sputum smears are shown in table 6.1. Among HIV positive individuals, 111 (48%), 116 (50%) and 113 (49%) out of 230 first-on-spot, early morning and second-on-spot direct smears had AFB (≥ scanty). Early morning samples were more often graded as "+++" (14%) than samples collected on the spot (8% and 9%; P=0.04). In comparison, 111 (48%) of their digested smears had AFB (≥ scanty). There was no difference in the proportion of slides graded as positive across the four smears. Among HIV-negative patients, 108 (59%), 113 (62%) and 108 (59%) of the 183 first-on-spot, early morning and second-on-spot direct smears had AFB. Early morning samples of HIV-negative patients also had more bacilli than on the spot specimens with 22% of the morning smears graded "+++" compared to 14% and 17% of the first and second on-the-spot specimens (P=0.05). In comparison 108 (59%) of the digested smears had AFB. Similarly the results of HIV-positive patients, showed no difference in the proportion of slides graded as positive across the four smears.

Table: 6.1 Direct and digested smear microscopy results by HIV status

	Missing	Negative	Scanty	+	++	+++	Graded as ≥ scanty
HIV Positive (N=230)							
First on the spot	1	118 (51%)	23 (10%)	41 (18%)	28 (12%)	19 (8%)	111 (48%)
Morning	1	113 (49%)	17 (7%)	43 (19%)	25 (11%)	31 (14%)	116 (50%)
Second on the spot	1	116 (50%)	22 (10%)	38 (17%)	33 (14%)	20 (9%)	113 (49%)
Bleach	9	110 (49%)	14 (6%)	49 (22%)	30 (13%)	18 (8%)	111 (48%)
HIV negative (N=183)							
First on the spot	1	74 (40%)	13 (7%)	40 (22%)	29 (16%)	26 (14%)	108 (59%)
Morning	1	69 (38%)	11 (6%)	29 (16%)	32 (18%)	41 (22%)	113 (62%)
Second on the spot	2	73 (40%)	11 (6%)	33 (18%)	34 (19%)	30 (17%)	108 (59%)
Bleach	6	69 (38%)	13 (7%)	45 (25%)	25 (14%)	25 (14%)	108 (59%)
HIV unknown (N=343)							
First on the spot	2	335 (98%)	0 (0%)	4 (1%)	2 (1%)	0 (0%)	6 (2%)
Morning	2	334 (97%)	1 (0%)	1 (0%)	3 (1%)	2 (1%)	7 (2%)
Second on the spot	2	334 (97%)	3 (1%)	1 (0%)	3 (1%)	0 (0%)	7 (2%)
Bleach	5	324 (94%)	7 (2%)	5 (2%)	1 (0%)	1 (0%)	14 (4%)

The Kappa Index of agreement between the direct and digested smears for complete pairs is shown in table 6.2. This showed a high level of agreement between the digested and the direct smears (Kappa ranged from 0.894 to 0.952 for all comparisons, p>0.5). Digested smears however identified more positive smears, with 50% of patients being HIV positive and 61% HIV negative and a number of specimens graded as AFB positive by the digested smears but negative by the direct smears. However, the difference between bleach and single direct smears was not statistically significant for any of the subgroups as shown in table 6.2. Both direct and digested smears were less sensitive in HIV positive than in HIV negative patients with a reduction in positivity of about 10%.

The yield of a single digested smear according to the PTB case definitions and HIV status is shown in table 6.3. Most patients with known HIV status had "definite" or "very likely" PTB reflecting the selection process, with HIV testing done in patients with smear-positive PTB or positive culture. HIV positive patients with PTB were more likely to be smear negative than HIV negative patients with PTB. Of the HIV positive patients, 116 (52%) had AFB and 109 (48%) were identified only by culture. In comparison, 113 (62%) of the 183 HIV negative patients with PTB had AFB in their smears and 68 (38%) had smear negative, culture positive PTB (P=0.03). A single digested smear identified 111 (49%) out of the 225 HIV positive and 108 (60%) out of the 181 HIV negative patients with "definite" or "very likely" PTB (P=0.04). In total, three direct smears identified 235 (51%) out of the 458 patients with "definite" or "very likely" PTB and a single digested smear identified 229 (50%) of these patients (p>0.5).

Table: 6. 2 Agreement between direct and digested smears by HIV status

			First spot		Morning		Second spot	
	Digested smear	N	Positive	Negative	Positive	Negative	Positive	Negative
HIV pos	Pos	111 (50%)	104 (47%)	7	107 (48%)	4	105 (48%)	6
	Neg	110 (50%)	1	109	2	108	2	108
HIV neg	Pos	108 (61%)	101 (57%)	7	106 (60%)	2	106 (60%)	2
	Neg	69 (39%)	2	67	2	67	2	67
HIV unknown	Pos	14 (4%)	6 (2%)	8	6 (2%)	8	6 (2%)	8
	Neg	324 (96%)	0	324	0	323	0	323

Pos = positive, neg = negative

Table: 6.3 Digested smear results by PTB case definitions and HIV status

PTB case definition[1]	N	Unavailable	Negative	Scanty	+	++	+++	Digested smear Smear graded ≥ scanty
HIV positive (N=230)								
Definite PTB	116 (50%)	7 (6%)	2 (2%)	13 (11%)	46 (40%)	30 (26%)	18 (16%)	107 (47%)
Very likely PTB	109 (47%)	2 (2%)	103 (95%)	1 (1%)	3 (3%)	0 (0%)	0 (0%)	4 (2%)
Less likely PTB	5 (3%)	0 (0%)	5 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
HIV negative (N=183)								
Definite PTB	113 (62%)	5 (5%)	2 (2%)	13 (12%)	43 (38%)	25 (22%)	25 (22%)	106 (58%)
Very likely PTB	68 (37%)	1 (1%)	65 (96%)	0 (0%)	2 (3%)	0 (0%)	0 (0%)	2 (1%)
Less likely PTB	2 (1%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
HIV unknown (N=343)								
Definite PTB	6 (2%)	0 (0%	0 (0%	2 (33%)	2 (33%)	1 (17%)	1 (17%)	6 (2%)
Very likely PTB	46 (13%)	1 (2%)	41 (89%)	2 (4%)	2 (4%)	0 (0%	0 (0%	4 (1%)
Less likely PTB	291 (85%)	4 (1%)	283 (97%)	3 (1%)	1 (0.3%)	0 (0%	0 (0%	4 (1%)

[1] Case definitions: Definite: ≥2 positive direct smears (positive/negative culture) or one positive direct smear plus positive culture; Very likely: three negative direct smears but positive culture; Less likely: only one positive direct smear and negative culture; Unlikely: three negative direct smears and negative culture.

6.5 Discussion

Direct smear microscopy of three sputum samples over the course of two days is the most frequently used test for the diagnosis of PTB. This process is however time consuming and costly to the patient. Avenues to reduce the amount of time and examinations required for diagnosis are urgently needed. Previous reports have suggested that the digestion of the sputum with household bleach – a cheap chemical available all over the world could improve the clarity of the smears, resulting in a higher yield of microscopy, even in the absence of concentration techniques. This approach would facilitate the development of a one-stop diagnosis of PTB in resource-poor settings, which is crucially important to increase the case detection rate in high prevalence countries. Although one digested smear resulted in the same yield as three direct smears in PTB suspects in Southern Ethiopia (Yassin et al., 2003), where 20% of patients are co-infected with HIV (Yassin et al., 2004), the performance of the technique still needed to be assessed in areas with higher HIV prevalence.

There are only scanty reports of the prevalence of HIV in Nigeria. It is estimated that about 5% of the population in Nigeria is infected with HIV, and according to a national sentinel survey for 2001, the prevalence of HIV among TB patients was 19.1% (NTBLCP 2004) and had gone up to 27% in 2004 (WHO 2005). The higher TB-HIV co-infection prevalence observed in our patients might reflect their origin, as a higher HIV prevalence is often observed in urban as opposed to rural areas (Yassin et al., 2004; Migliori et al., 1992; Palmieri et al., 2002). The recent implementation of the DOTS strategy in our area might have also prompted more seriously ill patients, who could be HIV co-infected to seek medical treatment. In addition, we cannot

exclude that the systematic testing of our patients at the time of their enrolment into a clinical trial may have resulted in a higher detection rate and further studies to confirm the prevalence of HIV-TB co-infection in our setting are urgently needed.

The results reported here confirm that direct smear microscopy has a relatively low sensitivity compared to culture, that HIV-TB co-infections decrease even further its sensitivity (Palmieri et al., 2002; Karstaedt et al., 1998), and that in our setting, microscopy was able to identify only 52% of the HIV positive compared to 62% of the HIV negative patients with PTB. The bleach digestion of sputum however followed the same pattern as the direct microscopy and the yield of bleach digested smears changes with the HIV status of the patients. One bleach digested smear identified 49% of the patient with TB who were HIV positive and 60% of those who were HIV negative. The overall yield of this approach however is the same as three direct standard ZN smears. These results confirm that patients who have TB and are co-infected with HIV are less likely to have positive sputum microscopy than patients without HIV. Although smear microscopy is less sensitive in patients with HIV co-infection, the bleach digestion of a single smear performs as well as three direct smears, even in patients with HIV infection. A single bleach digested smear therefore, would improve the efficiency of the diagnostic process of PTB, requiring only one visit for the submission of specimens. This improved efficiency could be obtained independently of the prevalence of HIV in the area. If these findings are corroborated by further studies, this technique could improve the number of sputum positive PTB cases identified.

CHAPTER SEVEN

Comparison of scanty AFB smears against culture in an area with high HIV prevalence

7.1 Introduction

Patients with symptoms suggestive of pulmonary PTB often have direct sputum smears graded as "scanty" (<10 acid-fast bacilli [AFB]/100 high power fields [HPF]). The WHO and the IUATLD international recommendations indicate that a single smear with scanty AFB should not be accepted as diagnostic unless confirmed by further smears (Enarson et al., 2000). This is however not uncommon as for example, Van Deun et al. (Van Deun et al., 2004) reported that sputum smears classified as "scanty" were observed in about 10% of their Bangladeshi patients. Although Van Deun et al., (Van Deun et al., 2004) documented that only 1.5% of these smears were false positive, very few recent studies have validated scanty smear results against culture, which is considered the gold standard. In sub-Saharan Africa, the number of patients presenting with scanty bacilli has increased due to the high incidence of HIV in the region (Elliott et al., 1993d; 1993; Karstaedt et al., 1998). Studies that compare the value of a single smear with scanty AFB against culture in areas of high HIV prevalence would be critical for the proper management of patients.

7.2 Study population and methods

We investigated all consecutive patients with symptoms suggestive of PTB attending 8 hospitals of Abuja, Nigeria from September 2003 to September 2004 as described in chapter 3. In the previous chapters we have shown that 31% of the patients in our

setting are considered 'definite' sputum smear positive cases based on 2 or more positive direct smears and a further 29% are smear negative, culture positive. A summary list of all scanty results is shown in table 7.1. Smear results were then tabulated according to the number of AFB seen in the scanty smears (< or ≥ 3 AFB per HPF) and whether they had single scanty smears or one scanty smear with further scanty, positive or negative smear permutations. These permutations are presented in the same order for all smears, independently of the timing of the sputum sample that was graded as scanty (spot or early morning specimens) to facilitate interpretation.

7.3 Results

A total of 1068 consecutive patients were screened during the study period and a total of 3204 direct smears results were available. Of these, 824 (25.7%) were graded as positive (+, ++ or +++), 137 (4.3%) as scanty and 2243 (70%) as negative. Forty eight (1.5%) first on-the-spot, 38 (1.2%) morning and 51 (1.6%) second on-the-spot specimens were graded as scanty. Six hundred and eighty (64%) of the 1068 BACTEC cultures were positive.

One hundred and thirty (95%), of the 137 scanty, and 809 (98%) of the 824 positive smears belonged to patients with positive cultures. Eighteen patients had only one scanty smear plus two negative smears as shown in the table. Of these, 10 had < 3 and $8 \geq 3$ AFB counts per 100 HPF. Six out of the 10 smears with < 3 AFB were culture-positive, compared to 6 out of the 8 with ≥ 3 AFB per 100 HPF ($p>0.1$).

Fourteen patients had two scanty plus one negative smears and only one of them had negative culture. A further 38 patients had three scanty or one scanty plus other positive smears. All of these patients were confirmed by culture.

Table: 7.1 Smear results of patients with scanty AFB grades by culture

Culture	N	**Smear result combinations** Scanty Negative Negative	Scanty Scanty Negative	Scanty Scanty Scanty	Scanty Positive Negative	Scanty Positive Positive	Scanty Scanty Positive
		< 3 AFB in all scanty smears					
Positive	15 (79)	6 (60)	5 (100)	0	3 (100)	0	1 (100)
Negative	4 (21)	4 (40)	0	0	0	0	0
		One or more scanty smears with ≥ 3 AFB					
Positive	48 (94)	6 (75)	8 (89)	9 (100)	3 (100)	0	22 (100)
Negative	3 (6)	2 (25)	1 (11)	0	0	0	0

(*)%

7.4 Discussion

Our findings confirm that in TB control programmes with adequate quality control, smears reported as "scanty" are more likely to be true than false positives. Our results are in agreement with previous studies that have demonstrated that the majority of scanty smears belong to patients with active PTB (Levy et al., 1989). Although about 50% of our patients with PTB are co-infected with HIV (chapter 8), accepting scanty smears as positive would result in less than 1% of the patients being wrongly classified as having PTB (7/1068) and a further 34 patients could have been identified for treatment. This would be particularly useful in African countries with high TB prevalence (where the test would have a high positive predictive value), which often care for populations with low accessibility to services and overburdened diagnostic facilities. Our results also have implications for the WHO case definition of smear-positive TB, which requires at least 2 positive smears. If a single smear has a high specificity, as shown in this study, the WHO case definition could be re-defined to 'at least' one positive smear. Further prospective studies should be conducted in high incidence countries to confirm these findings

CHAPTER EIGHT

Clinical Presentation and Risk Factors for Patients with and without TB by their HIV status

8.1 Introduction

The clinical presentation of TB has changed in countries with high HIV prevalence. It is estimated that worldwide over 40 million people are dually infected with HIV and TB, and of these, 95% live in resource-constrained countries (WHO 2000b). Nigeria has the highest estimated number of new TB cases annually and it is estimated that 27% of the 124 million people in the country are co-infected with TB and HIV (WHO 2005). HIV infection accelerates the natural progression of TB by diminishing CMI while the immune response to TB can enhance HIV replication and disease progression (Fauci et al., 1996). TB accounts for 11% of AIDS related deaths worldwide (WHO, 2000) and the combination of these two diseases has grave implications for already stretched public health services. Despite the magnitude of the problem, there are only a few documented reports on the prevalence, clinical presentation and risk factors associated with HIV infection among culture-positive TB patients due to the inaccessibility and high cost of culture facilities in the settings where these diseases are most prevalent.

We describe here the prevalence and clinical presentation of HIV in patients with culture-positive PTB and compare their presentation with patients who had cultures

negative for PTB. The analysis is based on the patients attending the district hospitals in Abuja, Nigeria as explained in chapter 3.

8.2 Literature Review

TB is the most common HIV-related complication in the world (Narain et al., 2004; Narain et al., 1992; Raviglione et al., 1992). The combined infection of TB and HIV has been christened "The cursed duet" (Chretien 1990). HIV has developed a complicated relationship with TB since its emergence in the 1980's, with both diseases creating a lethal combination, accelerating each other's progress. HIV has been identified as a predisposing factor to developing active TB in individuals harbouring the mycobacterium bacilli by impairing the CMI of the patient. An HIV patient harbouring *M. tuberculosis* has a 40 times greater risk of developing active TB than an HIV negative individual (Antonucci et al., 1995; WHO 2001). HIV infection rates in TB cases are correspondingly high, exceeding 60% in countries such as South Africa, Zambia and Zimbabwe (McLeod et al., 1988; Hira et al., 1990). In Africa, HIV is the most important factor responsible for the increased incidence of TB in the past 10 years (Cantwell et al., 1996; De Cock 1996). As HIV has fuelled the TB epidemic, so has TB affected significantly people living with HIV/AIDS. TB is a leading cause of death among people with HIV infection, accounting for 11% of AIDS-related deaths worldwide (Stanford et al., 1991; De Cock 1996); WHO 2000). It is estimated that 11% of new adult TB cases were infected with HIV in 2000 (Sudre et al., 1992; Raviglione et al., 1992; WHO 2000b). There are however marked variations within regions in the world, from 38% of HIV infection in TB patients in sub-Saharan Africa, 14% in industrialised countries to 1% in the WHO Western

Pacific Region such as Bangladesh, China and Indonesia (Dye et al., 1999; Dye et al., 2005). For example, 60% of newly diagnosed TB patients in Kampala, Uganda were HIV- seropositive in 1991 (Eriki et al., 1991). In contrast, Onorato et al., reported a mean of 3.4% of 3,077 TB patients in 14 USA cities as co-infected with HIV (Onorato et al., 1992) and in a sentinel surveillance study in New Delhi in 1999, Jain et al., reported a prevalence of 0.7% of HIV infection in 400 TB patients (Jain et al., 2000) (table 8.2.1). Variations in HIV seroprevalence among TB patients are also seen within the same region. For example in published reports from India, Solomon et al., found an HIV prevalence of 0.35% among TB patients (Solomon et al., 1995) and Banavaliker et al., reported that 0.5% of hospitalised TB patients were HIV positive (Banavaliker et al., 1997). Jayaswal et al., however reported a seropositivity of 4% among patients in a military hospital in India (Jayaswal et al., 1995). Similarly, in Africa 83 (67%) of 131 newly admitted patients into a TB ward in Ndola, Zambia, were HIV positive (Simooya et al., 1991). Elliot et al., in an out-patient TB clinic in the Teaching Hospital in Lusaka, Zambia, reported an overall HIV prevalence of 49% among TB patients (Elliott et al., 1990). A cohort study of 249 TB patients to investigate the interaction between TB and HIV, found that 76% of the cases were infected with HIV 1 (Elliott et al., 1993d) (table 8.2.2).

Table: 8.2.1 Variations in the prevalence of HIV among patients with TB in the world

Author and date	Country	Study Population	Prevalence of HIV in TB patients
(Eriki et al., 1991)	Uganda	Hospital	66%
(Onorato et al., 1992)	USA	Cities	3.4%
(Jain et al., 2000)	India	Hospital	0.7%
(Kritski et al., 1995)	Brazil	region	20%

Table: 8.2.2 Examples of intra-regional variation in the prevalence of HIV in patients with TB in India and Zambia

Author	State	Study Pop	HIV prevalence in TB patients
(Solomon et al., 1995)	India	Hospital	0.35%
(Jayaswal et al., 1995)	India	Hospital	4%
(Banavaliker et al., 1997)	India	Hospital	0.5%
(Elliott et al., 1990)	Zambia	Hospital	49%
(Simooya et al., 1991)	Zambia	Hospital	58%
(Elliott et al., 1993d)	Zambia	Hospital	73%

The prevalence of TB and HIV co-infection in Africa is heterogeneous. In Nairobi 18% of HIV-seropositive patients as opposed to 6% of HIV-seronegative patients were diagnosed with TB from 500 consecutive admissions to a hospital in Nairobi, (Gilks et al., 1990) while 60% of patients with HIV admitted to a respiratory unit in Abidjan, Cote d'Ivoire had TB (Grant et al., 1998). Also in Cote d'Ivoire, 40% of HIV patients were found to have TB (Abouya et al., 1992) and the prevalence of active TB, among HIV-seropositive adult patients in the University College Hospital, Ibadan, Nigeria was 32.8% in 2000 (Awoyemi et al., 2002).

The prevalence of HIV among TB patients is relatively high in sub Saharan Africa as shown in table 8.2.3. This is more predominant in the central and southern regions of the continent. While Malawi, Kenya and Botswana have prevalences of over 50%, most studies in Nigeria found a prevalence of less than 20% (table 8.2.5), reflecting differences in the prevalence of HIV and TB in the general population, the stage of the HIV epidermic and the study population selected. In general, patients in countries who have high prevalence of HIV and whose epidermic have been established for longer periods are more likely to be co-infected than patients with TB than in

countries with low prevalence of HIV or where the virus has only recently been introduced.

Table: 8.2.3 HIV prevalence in TB patients in sub-Saharan Africa

Author	Country	Study Pop	HIV prevalence in TB patients
(Kool et al., 1990)	Malawi	Hospital	26%
(Kelly et al., 1990)	Malawi	Hospital	52%
(Harries et al., 1995)	Malawi	Hospital	75%
(Harries et al., 1995)	Malawi	Hospital	77%
(Colebunders et al., 1989)	Zaire	Hospital	36%
(Mukadi et al., 1993)	Zaire	Hospital	22%
(Ouattara et al., 1988)	Cote d'Ivoire	Hospital	51%
(De Cock et al., 1991)	Cote d'Ivoire	Hospital	40%
(Abouya et al., 1992)	Cote d'Ivoire	Hospital	40%
(Gnaore et al., 1993)	Cote d'Ivoire	Hospital	44%
(Ackah et al., 1995)	Cote d'Ivoire	Hospital	44%
(Grant et al., 1998)	Cote d'Ivoire	Hospital	60%
(Gilks et al., 1990)	Kenya	Hospital	18%
(Nunn et al., 1992)	Kenya	Hospital	38%
(Houston et al., 1994)	Zimbabwe	Hospital	45%
(Kenyon et al., 1999)	Bostwana	Hospital	51%
(Awoyemi et al., 2002)	Nigeria	Hospital	33%

Similarly, the setting of the study will confound these factors, as for example, patients in rural areas are less likely to have HIV than residents of metropolitan areas and to have less accessibility of services.

Only few data on TB and HIV co-infection are available from Nigeria and the West African region. In Nigeria, most of the published articles have described populations located in the south of the country. Nwobu et al., reported an 11% HIV-seropositivity

in 102 TB patients examined in Irua and 9% in 303 patients in Benin in the Midwestern part of Nigeria (Nwobu et al., 2004). Idigbe, reported an HIV-PTB co-infection rate of 5% from Lagos (Idigbe et al., 1994), while Onipede reported a prevalence of 13% from Ile-Ife in Ogun State (Onipede et al., 1999). Okogun et al., reported a prevalence rate of 5% from Abeokuta and environs in Ogun State (Okogun et al., 2002). The diagnosis of TB in most of these studies however was done by using direct sputum smear microscopy, which is less sensitive than culture. Their studies therefore selected individuals with high bacillary loads. This is important, as patients with HIV and TB often have low bacillary loads in sputum and their detection process would under-represent the incidence of HIV in TB. Accordingly Nwobu et al., noted that the low rate of HIV-PTB co-infection in these studies, could be due to the sampling method used (Nwobu et al., 2004). A summary of the studies from Nigeria is shown in table 8.2.5. Although these studies are hardly comparable to each other, hospital studies seem to be reporting a higher incidence of HIV among TB patients over time.

Table: 8.2.4 Prevalence of HIV in patients with TB in Nigeria

Author	State	Study Pop.	HIV prevalence in TB patients
(Idigbe et al., 1994)	Lagos	Community	5%
(Okogun et al., 2002)	Abeokuta	Community	5%
(Anteyi et al., 1996)	Jos	Hospital	6.1%
(Onipede et al., 1999)	Ile-Ife	Hospital	13%
(Moses et al., 2003)	Maiduguri	Hospital	19%
(Nwobu. et al., 2004)	Benin	Hospital	9%
(Nwobu. et al., 2004)	Irua	Hospital	11%
(Daniel et al., 2004)	Shagamu	Hospital	15%

8.2.1 Natural history of TB after HIV infection

The natural history of *M. tuberculosis* infection indicates that the emergence of the delayed-type hypersensitivity reaction to the mycobacterium and acquired resistance are associated with the control of the initial infection in 95% of cases while 5% develop progressive primary TB. Five to 10% of the infected individuals will reactivate latent pulmonary or extra-pulmonary infections years after (Narain et al., 1992; Raviglione et al., 1995). The clinical presentation of TB in a patient co-infected with HIV is strongly associated with low CD4 counts, which in turn depend on the severity of the HIV infection. The immune system has two main responses to the introduction of foreign antigens into the body, a CMI and a humoral response. TB and HIV primarily affect the CMI response. HIV/AIDS patients have a remarkable susceptibility to TB, thus increasing the risk of reactivation of TB manifolds (Villarino et al., 1992). Monocytes and macrophages are important target cells for both TB and HIV and play crucial roles in their pathogenesis (Meduri et al., 1992; Meltzer et al., 1990). Infection of the macrophages with TB and HIV results in decreased cell viability, increased bacilli multiplication and altered cytokine production in vitro (Newman et al., 1993). The released cytokines stimulate HIV replication (Fauci et al., 1996). The immunological resistance and susceptibility to intracellular infections also depends on T-lymphocytes and the macrophage regulatory cytokines. Susceptibility to TB is related to the type of cytokines produced by the T lymphocytes. Th1 lymphocytes produce interferon-γ (IFN-γ), and are central to antimycobacterial immune defences. The reduced Th1 response in HIV-infected patients contributes to the increased susceptibility to TB. TB decreases the number of CD4 T-lymphocytes, the best indicator of AIDS survivability (Katz et al., 1979). The

importance of this reductions in CD4 counts is that the infected CD4 T-lymphocytes are unable to control the immune response against TB and HIV.

The majority of TB infection occurs in the sexually active age group of 15 to 49 years and this is the age group when the majority of HIV infections also occur (Raviglione et al., 1992; Aerts et al., 2004; Prasad et al., 2004; Anteyi et al., 1996).

8.2.2 Other factors associated with increased risk of TB and HIV co-infection

Liberato et al., and Aerts in Brazil found that patients co-infected with TB and HIV were mostly male (Liberato et al., 2004; Aerts et al., 2004). Jain et al., in India also observed a significantly higher risk of co-infection among male TB patients (Jain et al., 2000). In contrast, Nwobu et al., in Nigeria (Nwobu et al., 2004) and Simooya in Zambia (Simooya et al., 1991) found a higher HIV seroprevalence in female TB patients.

Health workers are at greater risk than most other professions of being co-infected with TB and HIV (Beck-Sague et al., 1992). Ani et al., in Nigeria reported high rates of HIV infections among commercial sex workers (71% in one region), long haul truck drivers, police officers and professional blood donors (Ani et al., 1998) and Ahmed et al., in India, observed a high prevalence of co-infection in truck drivers and cleaners (Ahmed et al., 2003). Early onset of sexual activities, and high mobility are therefore major risk factors for TB and HIV co-infection (Alarcon et al., 2003; Anteyi et al., 1996).

In India, a highly significant association was observed between literacy level and HIV among TB patients (Dey et al., 2003; Jain et al., 2000). Individuals with less than 8 years of schooling were more co-infected with TB and HIV than those with higher education in southern Brazil (Aerts et al., 2004).

Blood transfusion is also an important source of HIV infection in developing countries (Alvarez-Suarez et al., 1989; Anteyi et al., 1996). Haemophiliacs, sickle cell and cancer patients are more prone to HIV because of their regular requirements for blood. Drug users are prone to co-infection with TB and HIV as a result of their communal living and sharing of infected syringes (Ahmed et al., 2003; al-Haddad et al., 1997; Alseda Graells Pere et al., 2004; Barnes et al., 1993).

8.3 Materials and Methods

8.3.1 Aims and Objectives

This chapter aims to describe the clinical presentation and risk factors for TB in patients with and without HIV

8.3.2 Study design

Patients, aged 15 years and above, attending the 8 participating district hospitals in Abuja, Nigeria, from September 2003 to June 2004, with a clinical suspicion of PTB were invited to participate in this study as described in chapter 3. Briefly, patients underwent a physical examination on enrolment and their Karnofsky performance scores were calculated (Karyadi et al., 2002). Blood samples were taken for biochemical studies and HIV serology and information on their medical history and socio-economic background was collected using structured questionnaires. Sputum samples were collected from the patients and processed for the diagnosis of TB as described in chapter 3. Both BACTEC culture-positive and BACTEC culture-negative patients were included in this chapter.

8.3.3 Karnofsky Score

The Karnofsky score was devised for the measurement of the quality of life of patients with cancer but it now has a wider use in clinical research in monitoring and grading the level of function of patients with other diseases. This scoring method classifies patients as to their functional impairment, allowing the comparison of the effectiveness of different therapies and the assessment of prognosis in individual patients. It is scored from 100 to zero (Schag et al., 1984) and the criteria used for the score is listed in table 8.3.1. A score of 100 is allocated to individuals who are able to carry on normal activities, requiring no special care with their routine daily lifestyle, have no complains and no evidence of disease. A score of zero is allocated to patients who die. In between these scores in a multiple of ten, are categories and criteria graded according to the state of health of the patient. The lower the Karnofsky score, the worst is the prognosis for survival. Its use in TB research has been limited. It was

first used in a double-blind, placebo-controlled study of vitamin A and zinc supplementation in patients with TB in Indonesia to review the progress of the patients (Karyadi et al., 2002) and for this reason, it was also included in the current study to allow for the comparison of the response to therapy. The Kanofsky score is shown in table 8.3.1.

Table: 8.3.1 The Karnofsky Score

General category	Index	Specific criteria
Able to carry on normal activity; no special care needed.	100	Normal, no complaints, no evidence of disease.
	90	Able to carry on normal activity, minor signs or symptoms disease.
	80	Normal activity with effort, some signs or symptoms of disease.
Unable to work, able to live at home and care for most personal needs, varying amount of assistance needed.	70	Cares for self, unable to carry on normal activity or to do work.
	60	Requires occasional assistance from others but able to care for most needs.
	50	Requires considerable assistance from others and frequent medical care.
Unable to care for self, requires institutional or hospital care or equivalent, disease may be rapidly progressing.	40	Disabled, requires special care and assistance.
	30	Severely disabled, hospitalisation indicated, death not imminent.
	20	Very sick, hospitalisation necessary, active supportive treatment necessary.
	10	Moribund.
	0	Dead.

8.3.4 HIV screening

Patients were screened for HIV1 and HIV2 after pre and post-test counselling using an ImmunoComb HIV1& 2 BiSpot kit (ORGENICS, Yavne, Israel). The sensitivity and specificity of this ELISA kit in a multi-centre study in Europe was reported to be 100% and 99.4%, respectively (Beelaert et al., 2002). In African studies, these were also reported as 100% and 98.4%, respectivel (Ndjoyi-Mbiguino et al., 2005) and Aghokeng repoted 98.9% and 99.3% respectively (Aghokeng et al., 2004). For quality control, samples were re-tested with the ImmunoComb II HIV 1 & 2 Combfirm (ORGENICS, Yavne, Israel). This is reported to have a sensitivity and specificity in a multi-centre study in Europe and Australia of 99.7% and 100% respectively (Groopman et al., 1986; Dax et al., 1993) and sensitivity and specificity of 100% and 100% in Africa (Dax et al., 1993). Only smear-positive patients were screened prospectively for HIV, while culture-positive and culture negative patients were screened retrospectively.

8.3.5 Statistical Analysis

The characteristics of the patients with culture-positive TB were compared to the characteristics of culture negative patients. This was followed by a stratified analysis by HIV status. Unadjusted odds ratios (OR) were calculated using univariate analysis. Factors with p values ≤ 0.20 in the univariate analysis were entered into a logistic regression analysis to obtain Adjusted Odds Ratios (AOR).

8.4 Results

8.4.1 Characteristics of patients with positive and negative BACTEC culture

For the purpose of this analysis, patients with positive BACTEC culture (for AFB) were assumed to have TB and patients with negative culture will be classified as not having TB. The characteristics of patient with and without TB are presented here. Section 8.6 presents a similar analysis stratified by HIV status. One thousand, three hundred and twenty-one patients suspected of having PTB infection were screened and 1186 had their sputum cultured on BACTEC 960. Of these 731 (62%) were culture-positive and 455 (38%) culture-negative. Of the 731 culture-positive patients, 353 (48%) were smear-positive and 378 (52%) smear-negative. Among culture-negative patients 8 (2%) were smear-positive and 477 (98%) were smear-negative (table 8.4.1).

The ages of the patients with positive culture ranged from 15 to 92 years with a mean (SD) of 33 (11) years. This was lower than the mean (SD) age of patients with negative culture, (35 (13) years, P= 0.02). The mean (SD) age for male patients with positive-culture was 34 (11) years and 31 (12) years for females, compared to 36 (12) and 34 (13) years for male and female culture-negative patients respectively (P=0.21 for males and 0.008 for females). The peak age of infection for both male and female patients was 25 to 34 years as shown in figure 8.4.1.

Table: 8.4.1 Sputum smear and culture among patients with symptoms of PTB

	Culture		
ZN smear	**Positive**	**Negative**	**Total**
Positive*	353 (48%)	8 (2%)	361 (30%)
Negative	378 (52%)	447 (98%)	825 (70%)
Total	731 (62%)	455 (38%)	1186 (100%)

* At least 2 positive smears

Age and sex characteristics of patients with and without TB by BACTEC culture

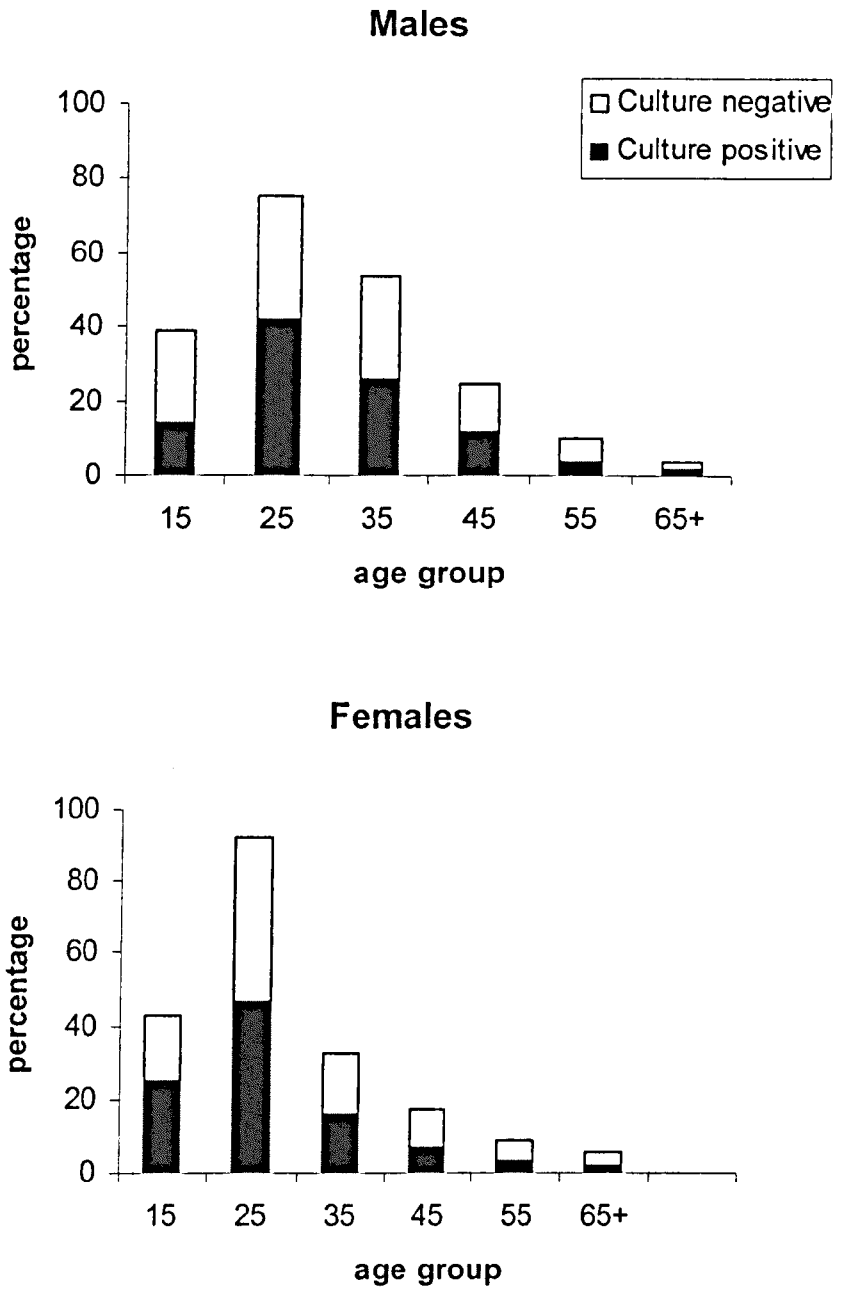
Four hundred and thirty-seven (60%) of the 731 culture-positive patients, were male and 294 (40%) female. Of the 455 culture-negative patients, 255 (56%) were male and 201 (44%) female (P= 0.09) (table 8.4.2). Among culture positive patients, males were older than females whereas such age difference was not observed in culture negative- patients (table 8.4.2 and figure 8.4.1).

Table: 8.4.2 Characteristics of patients with symptoms suggestive of PTB by BACTEC culture

	BACTEC culture			
	positive	**negative**	**OR**	**P value**
N	731 (62)	455 (38)		
Age (years) Mean (SD)				
All	33 (11) [15-92]	35 (13) [15-30]		0.02
Male	34 (11) [15-92]	36 (12) [15-80]		0.2
Female	31 (12) [15-90]	34 (13) [15-80]		0.008
Male: Female (% male)	437:294 (60%)	224:201 (56%)	1.18 (0.9-1.5)	0.09
Activities				
Working	488 (67%)	326 (72%)		
Not working	59 (8%)	29 (6%)		
Student	101 (14%)	60 (13%)		
Housewife	82 (11%)	41 (9%)		0.65
Education				
None	84 (12%)	50 (11%)		
Primary	146 (20%)	81 (18%)		
Secondary	363 (50%)	220 (49%)		
Tertiary	138 (19%)	103 (23%)		0.4
Persons per room*	3.1 (2) [1-19]	3 (2.2) [1-26]		0.2
Number of smokers	159 (22%)	96 (21%)	1.04 (0.8-1.4)	0.4
Length of time smoked (years)*	10 (9.1) [1-46]	10 (8.8) [1-54]		0.8
Cigarettes per day*	7 (6.8) [1-40]	7 (7.8) [1-66]		0.6
Contact with cough > 3 weeks	120 (17%)	80 (18%)		0.7
Length of contact (weeks)*	6 (7) [1-44]	6 (9) [1-52]		0.6
Contact with fever > 3 weeks	53 (8%)	39 (9%)		0.09
Contact with TB within 2 years	117 (16%)	64 (14%		0.4
Length of contact (years)*	5 (5) [1-25]	5 (5) [1-20		0.5

*Mean (SD) [range]

Figure 8.1 Age and sex characteristics of patients with positive and negative BACTEC culture



Socioeconomic characteristics

Four hundred and eighty eight (67%) of the culture-positive patients were employed, 59 (8%) unemployed, 101 (14%) were students and 82 (11%) housewives. A higher

percentage of the culture-negative than the culture-positive patients were employed, (326 (72%) out of 455 vs. 488 (67%) out of 731, as shown in table 8.4.2.

Illiteracy levels were low with only 84 (12%) of the culture-positive and 50 (11%) of the culture-negative patients not having attended school. One hundred and forty-six (20%) of the culture-positive and 81 (18%) of the culture-negative patients had attended or were attending primary school. Three hundred and sixty three (50%) of the culture-positive and 220 (49%) of the culture-negative patients were students in secondary school or had had secondary school education, and 138 (19%) of the culture-positive patients and 103 (23%) culture-negative patients attended or were attending university or tertiary institutions.

History of contact

A mean (SD) of 3 people shared the same room with both culture-positive and culture-negative patients. Most patients (82%) shared a room with between one and four other persons. Three patients lived alone while one shared a room with 19 other persons.

Only 159 (22%) out of 731 culture-positive and 96 (21%) of 455 culture-negative patients smoked cigarettes, with a mean (SD) length of time for smoking for both groups of 10 (9) years. The patients had smoked between one and forty-six years, smoking one to forty sticks of cigarettes a day, with a mean (SD) of 7 (7) cigarettes per day for culture-positive and 7 (8) for culture-negative patients.

One hundred and twenty (17%) of the culture-positive patients and 80 (18%) of the culture-negative patients had been in contact with persons with cough of over 3 weeks. The mean (SD) contact time reported by culture positive-patients was 6 (8) years and by culture negative-patients was 6 (9) years. Only 53 (7%) of the culture-positive patients and 39 (9%) of the culture-negative patients were in contact with persons with fever of over 3 weeks.

One hundred and seventeen (16%) culture-positive patients and 64 (14%) the culture-negative patients were in contact with other adults with PTB. The mean (SD) contact time with these individuals was 5 (5) years for both groups.

8.5 Clinical presentation

8.5.1 Clinical symptoms

Three hundred and twenty-nine (72%) of the 455 culture-negative patients and 496 (68%) of the 731 culture-positive patients had BCG (P= 0.02) as shown in table 8.5.1. The mean (SD) number of years since given BCG was 33 (11) years for culture-positive and 35 (12) years for culture-negative patients, (P= 0.06). Most of the patients had been vaccinated at birth.

Seven hundred and twenty-eight (99.6%) culture-positive and 451 (99%) culture-negative patients had cough of more than 3 weeks duration. The mean (SD) duration of the cough was 14 (15) weeks for culture-positive and 12 (14) weeks for culture-negative patients (P = 0.02).

Four hundred and seventy (64%) culture-positive and 281 (62%) culture-negative patients had fever. The mean (SD) duration of the fever was 11 (16) weeks for culture-positive and 10 (15) weeks for culture-negative-patients. These differences were not statistically significant. A minority of patients had haemoptysis in both groups, 188 (26%) for culture-positive compared to 135 (30%) for culture-negative patients (P= 0.07).

Four hundred and eighty-one (66%) culture-positive and 289 (64%) culture-negative patients complained of breathlessness and the mean (SD) duration of the breathlessness was 11 (16) weeks for the former and 9 (15) weeks for the later (P= 0.14). A larger proportion of culture-positive patients, (607, 83%) had chest pains than in culture negative patients (354, 78%, P = 0.01), but the mean (SD) duration of the chest pains was not significantly different.

A larger proportion of culture-positive patients (592 (81%) versus 319 (70%) for culture-negative patients) complained of weight loss (P= 0.001). The mean (SD) duration of their weight loss however was not statistically different in the two groups. Five hundred and thirteen (70%) culture-positive patients indicated they sweated at night compared to 298 (66%) culture-negative patients (P = 0.05). More culture-positive patients (465, 64%) complained of loss of appetite compared to culture negative-patients (246, 54%), (P= 0.001) and 23 (3.1%) culture positive and 8 (1.8%) culture-negative patients had enlarged cervical lymph nodes (P= 0.07).

Table: 8.5.1 Clinical presentations of patients with positive and negative BACTEC culture

	BACTEC culture				Mean (SD)[range] duration of symptoms		
Clinical symptoms	**Positive**	**Negative**	**OR (95%CI)**	**P value**	**Culture positive**	**Culture negative**	**P value**
N	731	455					
Received BCG	496 (68%)	329 (72%	0.7 (0.5-0.95)	0.02	33 (11)[2-90]	35 (12)[1-80]	0.06
Cough	728 (99.6%)	450 (98.9%)	2.7 (0.6-11.3)	0.09	14 (15)[1-80]	12 (14)[1-72]	0.02
Unexplained fever/ sweating	469 (64%)	281 (62%)	1.1 (0.9-1.4)	0.19	11 (16)[1-52]	10 (15)[1-52]	0.24
Haemoptysis	188 (26%)	135 (30%)	0.8 (0.6-1.1)	0.07	10 (16)[1-52]	10 (16)[1-52]	0.94
Breathlessness	481 (66%)	289 (64%)	1.1 (0.9-1.4)	0.21	11 (16)[1-52]	9 (15)[1-52	0.14
Chest pains	607 (83%)	354 (78%)	1.4 (1.0-1.9)	0.01	11 (15)[1-96]	10 (14)[1-96]	0.24
Weight loss	592 (81%)	319 (70%)	1.8 (1.4-2.4)	0.001	10 (15)[1-52]	9 (15)[1-52]	0.31
Night sweats	513 (70%)	298 (66%)	1.2 (.96-1.6)	0.05	11 (16)[1-88]	10 (15)[1-52]	0.25
Anorexia	465 (64%)	246 (54%)	1.5 (1.2-1.9)	0.001	10 (16)[1-52]	9 (15)[1-52]	0.38
Enlarged lymph nodes	23 (3.1%)	8 (1.8%)	1.8 (0.8-4.4)	0.07	NA	NA	
Finger clubbing	8 (1%)	3 (0.7%)	1.7 (0.4-7.9)	0.34	NA	NA	

NA= Information not available

Table 8.5.2 Logistic regression analysis for clinical variables with p values < 0.20

Clinical symptoms	P	OR	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	AOR
Received BCG	0.02	0.7 (0.5-0.95)	0.92									NS	
Cough	0.09	2.7 (0.6-14.3)	0.18	0.18	0.17	0.18	0.18					NS	
Fever	0.19	1.1 (0.9-1.4)	0.83	0.81								NS	
Haemoptysis	0.07	0.8 (0.6-1.1)	0.03	0.03	0.03	0.02	0.03	0.03	0.04	0.05	0.053	NS	
Chest pains	0.01	1.4 (1.0-1.9)	0.17	0.17	0.17	0.19						NS	
Weight loss	0.001	1.8 (1.4-2.4)	0.006	0.006	0.006	0.006	0.003	0.002	0.001	0.001	0.001	0.001	1.8 (1.4-2.4)
Night sweats	0.05	1.2 (.96-1.6)	0.68	0.69	0.60							NS	
Anorexia	0.001	1.5 (1.2-1.9)	0.11	0.11	0.11	0.13	0.09	0.10	0.10	0.10		NS	
Cervical lymph nodes	0.07	1.8 (0.8-4.4)	0.15	0.15	0.15	0.15	0.14	0.16				NS	
Sex	0.09	1.2 (0.9-1.5)	0.10	0.10	0.10	0.10	0.13	0.14	0.16			NS	

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1 to P10 = p values after runningthe backward logistic regression

In order to identify the factors associated with positive BACTEC culture, all personal characteristics and clinical symptoms of p value ≤0.20 were entered into a logistic regression. Table 8.5.2 shows the results of these analysis. Only one factor was positively associated with a positive TB culture after excluding the confounding effects, namely weight loss (AOR 1.9, 95%CI = 1.4-2.4, P= 0.001).

8.5.2 Clinical signs

It is often difficult to differentiate patients with PTB from those with other chronic pulmonary problems by clinical examination. This is because patients with TB show a myriad of symptoms and signs. Findings on clinical examination of the patients were not significantly different in both groups. A summary of the clinical findings of patients with and without TB is shown in table 8.5.3. The mean temperature for both culture-positive and negative patients was similar. Differences in PPD sizes for both groups were not significant.

Culture-positive patients had a mean (SD) BMI of 21 (4) which was statistically lower than that for culture-negative (22 (5), P= 0.001). Two hundred and seven (28%) culture-positive patients had a BMI <18.5% compared to 90 (20%) culture-negative patients (P= 0.001). The mean (SD) Karnofsky score for culture-positive patients was 59 (12) compared to 60 (11) for culture-negative patients (P= 0.15). Four hundred and sixty-one (63%) culture positive patients had a Karnofsky score < 60 compared to 264 (58%) culture negative patients (P= 0.04).

To identify the clinical signs associated with a patient having a positive BACTEC culture for TB, all clinical findings with p values ≤ 0.20 were analysed using logistic regression. Table 8.5.4 shows that only BMI was positively associated with a positive culture (AOR 1.6, 95%CI = 1.2-2.1, P= 0.001) with TB positive patients having a lower BMI. Though not statistically significant, patients with TB were also more likely to have a lower Karnofsky score (AOR 1.3. 95%CI = 1.0-1.6, P= 0.07).

Table: 8.5.3 Clinical findings of culture-positive and culture-negative patients

		BACTEC Culture			
Clinical Signs		**positive**	**negative**	**OR (95%CI)**	**P**
Fever	< 37°C	385 (53%)	245 (54%)		
	≥ 37°C	346 (47%)	210 (46%)	1.0 (0.8-1.2)	0.35
PPD	< 2mm	44 (57.1%)	19 (52.8%)		
	≥ 2mm	33 (42.9%)	17 (47.2%)	1.2 (0.5-2.6)	0.33
BMI*		21 (4) [11-38]	22 (5) [12-47]		0.001
	< 18.5	207 (28%)	90 (20%)		
	≥ 18.5	523 (72%)	364 (80%)	1.6 (1.2-2.1)	0.001
Karnofsky score*		59 (12)	60 (11)		0.15
	< 60	461 (63%)	264 (58%)		
	≥ 60	270 (37%)	191 (42%)	1.2 (.97-1.6)	0.04
Wheeze		173 (24%)	103 (23%)	1.1 (0.8-1.4)	0.35
Bronchial breath sounds		325 (45%)	188 (42%)	1.1 (0.9-1.4)	0.17
Rhonchi		255 (35%)	143 (32%)	1.2 (0.9-1.5)	0.12
Crepitations		380 (53%)	217 (50%)	1.2 (0.9-1.5)	0.12

* Mean (SD)

Table: 8.5.4 Logistic regression analysis for clinical variables with p values < 0.20

Clinical signs	P	OR	P1	P2	P3	P4	P5	AOR
BMI*	0.001	1.6 (1.2-1.6)	0.001	0.001	0.002	0.001	0.001	1.6 (1.2-2
Karnofsky score*	0.04	1.2 (1.0-1.6)	0.05	0.05	0.005	0.07	NS	
Bronchial sounds	0.17	1.1 (0.9-1.4)	0.72				NS	
Rhonchi	0.12	1.2 (1-1.5)	0.33	0.28	0.23		NS	
Crepitations	0.12	1.2 (0.9-1.5)	0.42	0.41			NS	

OR = Odds ratip, AOR =Adjusted odds ratio, NS = Not significant, P1 to P5 = p values after runningthe backward logistic regression. * BMI < 18.5 and Kanofsky score < 60

8.5.3 Laboratory results of culture-positive and culture-negative patients

The laboratory results of the patients are summarised in table 8.5.3. Patients with positive culture had lower mean (SD) haemoglobin (P= 0.001) and a higher proportion had anaemia (Hb <11gm/dl) than culture negative-patients (P= 0.001).

Culture-positive and negative patients had similar WBC counts (P= 0.3). The mean (SD) granulocytes for culture-positive patients (61 (13)) was significantlly higher than that for culture-negative patients (59 (13), P= 0.03), while the mean (SD) lymphocyte count for culture-positive patients (39% (13%)) was significantly lower than that for culture-negative patients (41% (14%), P= 0.05). A larger proportion of culture-negative patients were within the normal granulocyte range compared to culture-positive patients (P= 0.005).

The mean (SD) ESR for culture positive patients was 78 (44) mm/hr and for culture negative patients 70 (46) mm/hr (P= 0.005). The mean (SD) total bilirubin was significantly higher for culture-negative patients compared to that for culture-positive

patients (P= 0.03). A larger proportion of culture-positive patients had normal levels of total and conjugated bilirubins, and this was statistically significant for conjugated bilirubin (P= 0.01) but not for total bilirubin (P= 0.28).

The mean (SD) serum albumin and the proportion of patients with normal albumin were significantly greater in culture-negative patients than in culture-positive patients (P= 0.002 and 0.04 respectively) (table 8.5.3) and a larger proportion of culture-positive patients had hypo-albuminaemia. The mean (SD) SGOT and SGPT were 7 (6) U/L and 12 (10) U/L respectively for culture positive patients and 7 (8) U/L and 13 (13) U/L for culture negative patients respective (P> 0.5 for both). The mean (SD) alkaline phosphatase was higher in culture-positive patients (221 (128)) versus to 193 (106) for culture-negative patients, P= 0.001). The proportion of patients with normal alkaline phospatase was higher in culture-negative patients (P= 0.04).

All laboratory results with p values ≤ 0.20 were entered into a logistic regression in order to identify the factors that might be associated with positive TB after controlling for confounding factors. Table 8.5.6 shows that two factors were positively and one negatively associated with TB. TB patients were more likely to be anaemic than non-TB patients (AOR = 1.6, 95%CI = 1.2-2.1, P= 0.001), to have normal conjugated bilirubin (AOR = 1.3, 95%CI = 1.0-1.7, P= 0.03) and less likely to have high granulocyte counts (AOR= 0.7, 95%CI = 0.6-1.0, P= 0.04).

In summary, the factors significantly associated with an increased risk of culture-positive TB were weight loss, a BMI < 18.5, the presence of anaemia and conjugated

bilirubin ≥ 0.2 mg/dl and granulocyte count < 65%. Those marginally associated were the presence haemoptysis and a Kanorfsky score < 60.

Tab: 8.5.5 Laboratory results for BACTEC culture-positive-and culture-negative patients

	BACTEC Culture			
Laboratory test*	**positive**	**negative**	**OR**	**P**
Haemoglobin level	11 (2) [5-16]	12 (2) [5-18]		0.001
< 11g/dl	264 (41%)	108 (29%)		
≥ 11g/dl	388 (60%)	267 (71%)	1.7 (1.3-2.2)	0.001
White blood count	8 (4) [3-38]	8 (4) [2-34]		0.3
< 10 x 10^9L	491 (76%)	302 (81%)		
≥ 10 x 10^9L	158 (24%)	72 (19%)	0.7 (0.5-1.0)	0.03
Granulocytes	61 (13) [6-92]	59 (13) [18-96]		0.03
< 65%	401 (62%)	261 (70%)		
≥ 65%	251 (39%)	114 (30%)	0.7 (0.5-0.9)	0.004
Lymphocytes (%)	39 (13) [8-94]	41 (14) [4-96]		0.05
< 45%	436 (67%)	233 (62%)		
≥ 45%	216 (33%)	142 (38%)	1.2 (0.9-1.6)	0.06
ESR mm/hr	78 (44) [1-167]	70 (46) [1-165]		0.005
< 7mm/hr	31 (5%)	20 (5%)		
≥ 7mm/hr	614 (95%)	353 (95%)	0.9 (0.5-1.6)	0.4
Total bilirubin	0.49 (0.4) [0.1-6]	0.54 (0.4) [0.1-4.8]		0.03
<1.0mg/dl	631 (94%)	350 (90%)		
≥ 1.0mg/dl	43 (6%)	37 (10%)	1.6 (1.0-2.5)	0.03
Conjugated bilirubin	0.2 (0.28) [0.01-4.5]	0.2 (0.3) [0.01-3.8]		0.1
< 0.2mg/dl	427 (63%)	218 (56%)		
≥ 0.2mg/dl	249 (37%)	170 (44%)	1.3 (1.0-1.7)	0.01
Serum albumin	3 (0.9) [1.2-6.9]	4 (0.9) [1.4-6.4]		0.002
< 3.8g/dl	461 (68%)	236 (60%)		
≥ 3.8g/dl	216 (32%)	154 (40%)	1.4 (1.1-1.8)	0.006
SGOT U/L	7 (6) [1-70]	7 (8) [1-96]		0.8
< 12U/L	587 (87%)	337 (86%)		
≥ 12U/L	90 (13%)	54 (14%)	1.0 (0.7-1.5)	0.4
SGPT U/L	12 (10) [2-150]	12 (13) [2-176]		0.7
< 12U/L	354 (52%)	207 (53%)		
≥ 12U/L	323 (48%)	184 (47%)	1.0 (0.8-1.3)	0.42
Alk phosphatase	221 (138) [4-940]	193 (106) [18-846]		0.001
< 279iu/l	535 (79%)	327 (84%)		
≥ 279iu/l	141 (21%)	60 (16%)	0.7 (0.5-1.0)	0.02

Values are mean (SD)[range], followed by frequencies (%) for values above/below selected cut off point

Figure 8.5.6 Logistic regression analysis for laboratory variables with p values < 0.20

Laboratory tests	P	OR	P1	P2	P3	P4	P5	P5	AOR
Haemoglobin ≤ 11g/dl	0.001	1.7 (1.3-2.2)	0.003	0.003	0.002	0.001	0.002	0.001	1.6 (1.2-2.1)
WBC ≤ 10 x 10^9	0.03	0.7 (0.5-1.0)	0.45	0.45	0.41			NS	
Granulocytes ≤ 65%	0.004	0.7 (0.5-0.9)	0.13	0.07	0.07	0.05	0.04	0.04	0.7 (0.6-1.0)
Lymphocytes ≤ 45%	0.06	1.2 (0.9-1.6)	0.95					NS	
Bilirubin T ≤ 1.0mg/dl	0.03	1.6 (1.0-2.5)	0.26	0.26	0.26	0.25		NS	
Bilirubin C ≤ 0.20mg/dl	0.01	1.3 (1.0-1.7)	0.09	0.09	0.08	0.09	0.04	0.03	1.3 (1.0-1.7)
Serun Albumin ≤ 3.8g/dl	0.006	1.4 (1.1-1.8)	0.85	0.85				NS	
Alk Phosphatase ≤ iu/l	0.02	0.7 (0.5-1.0)	0.13	0.12	0.12	0.11	0.17	NS	

OR = Odds ratip, AOR =Adjusted odds ratio, NS = Not significant, P1 to P5 = p values after runningthe backward logistic regression

8.6 Clinical presentation and risk factors for HIV infection in patients with positive and negative BACTEC culture

Of 1186 patients who had sputum BACTEC culture, 1002 also had blood tests results for HIV I and HIV II. Of these, 546 (54%) were positive for HIV I and 9 (1%) for HIV II. Six hundred and twenty-five of the patients (62%) had positive culture, and of these, 329 (53%) were positive for HIV I and 6 (1%) for HIV II. In comparison 217 (58%) out of the 377 culture-negative patients were positive for HIV I and 3 (1%) for HIV II as shown in table 8.6.1.

There were no age differences among culture-positive patients with and without HIV (mean age 33 (10) and 33 (12) years respectively) or among culture-negative patients who were HIV positive or negative (mean (SD) age of 35 (11) and 36 (15) years, respectively) as shown in table 8.6.1. Male patients with a positive cultures had a mean age of 35 (10) years if they were HIV positive and 33 (12) years if HIV negative (P= 0.001) while males that were culture-negative had a mean (SD) age of 36 years independently of their HIV status (P= 0.52). Female patients with positive culture had a mean (SD) age of 29 (8) years among patients with HIV and a mean (SD) age of 32 (13) years in patients without HIV (P= 0.1) while female culture-negative patients had a mean (SD) age of 33 (10) years among patients co-infected with HIV and 37 (17) in patients without HIV (table 8.6.1). Males who were HIV positive in both culture-positive and culture-negative patients were significantly older than HIV positive females (P= 0.001). There were no significant differences in the ages of male and female patients, who were HIV negative in both culture-positive and negative groups. Patients in the age group 24 to 35 years old had the highest infection rates for HIV in

both sexes in both culture-positive and culture-negative groups (table 8.6.2 and figures 8.2 and 8.3). Male patients were infected with HIV at older ages (P= 0.01) than female patients (P= 0.03) (figures 8.4 and 8.5). More females (58%) than males (50%) were infected with HIV in BACTEC culture-positive patients (P= 0.05), also more females (63%) than males (54%) were infected among culture-negative patients (P= 0.001) (table 8.6.2).

Table: 8.6.1 Age distribution of patients with positive and negative BACTEC culture by HIV status

		Culture positive		Culture negative	
Age	**Sex**	**HIV+**	**HIV-**	**HIV+**	**HIV-**
15-24	M	16 (9%)	43 (22%)	9 (8%)	24 (25%)
	F	40 (29%)	27 (27%)	18 (17%)	12 (19%)
25-34	M	81 (43%)	82 (43%)	45 (40%)	30 (31%)
	F	72 (51%)	43 (42%)	49 (47%)	26 (42%)
35-44	M	57 (30%)	39 (20%)	37 (33%)	18 (18%)
	F	21 (15%)	15 (15%)	23 (22%)	6 (10%)
45-54	M	26 (14%)	17 (9%)	14 (12%)	13 (13%)
	F	6 (4%)	9 (9%)	9 (9%)	7 (11%)
55-64	M	6 (3%)	8 (4%)	5 (4%)	10 (10%)
	F	0 (0%)	5 (5%)	4 (4%)	5 (8%)
≥ 65	M	3 (2%)	4 (2%)	3 (3%)	3 (3%)
	F	1 (1%)	3 (3%)	1 (1%)	6 (10%)
Total	M	189 (50%)	193 (50%)	113 (54%)	98 (46%)
	F	140 (58%)	102 (42%)	104 (63%)	62 (37%)

8.6.1 Socio-economic characteristics

There was no significant difference in the occupation of the patients with positive cultures irrespective of their HIV status (table 8.6.1). In comparison a higher proportion of culture-negative patients infected with HIV were employed, (163, 75%) while a lower proportion were students (15, 7% versus 33, 21%, P= 0.01) (table 8.6.1). The educational level of the participants is shown in table 8.6.1. There were no statistical differences in the educational pattern between culture-positive and culture-negative patients or between HIV co-infected and HIV negative patients.

8.6.2 History of contact

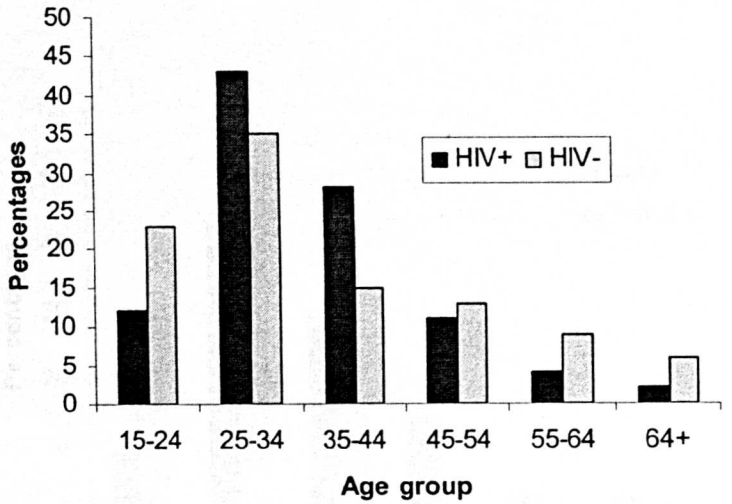
Most patients with both positive and negative culture with and without HIV shared their bedrooms with between 1 and 4 other individuals and there was no difference in the mean (SD) number of people sharing the bedroom with the patients (table 8.6.1). Similarly, there was no difference in the proportion of smokers or the number of cigarettes smoked between the groups.

The history of contact with other individuals with cough for more than 3 weeks was not significantly different across the groups. Similarly, between 6% and 11% of the patients said they had been in contact with other individuals with fever > 3 weeks and between 13% and 20% had been in contact with other persons with TB, but the proportions were similar across the groups and not associated with HIV.

Table: 8.6.2 Characteristics of culture-positive and culture-negative patients by HIV status

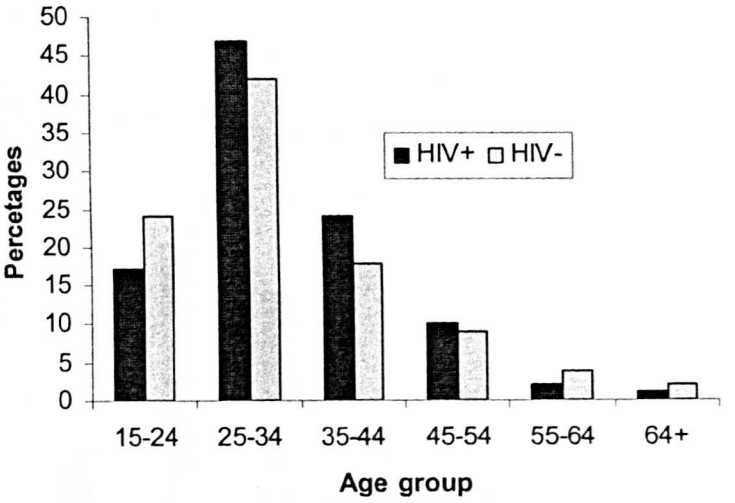
Risk factor	BACTEC culture					
	Positive N= 625 (62%)			Negative N=377 (38%)		
	HIV status					
	positive	negative	P value	positive	negative	P value
N	329 (53%)	296 (47%)		217 (58%)	160 (42%)	0.07
Age (years) Mean (SD)						
All	33 (10)[15-70]	33 (12) [15-92]	0.9	35 (11)[16-79]	36 (15)[15-80]	0.2
Male	35 (10)[15-70]	33 (12) [15-92]		36 (11)[16-79]	36 (14)[16-80]	
Female	29 (8)[15-70]	32 (13) [15-75]		33 (10)[16-78]	37 (17)[15-80]	
Sex: Male: Female (% male)	189:140 (57%)	193:102 (65%)	0.02	113 (104 (52%)	98:62 (61%)	0.04
Activities						
Working	227 (68%)	193 (65%)		163 (75%)	107 (67%)	
Not working	32 (10%)	17 (6%)		22 (10%)	6 (3%)	
Student	40 (12%)	54 (18%)		15 (7%)	33 (21%)	
Housewife	34 (10%)	31 (11%)	0.4	17 (8%)	14 (9%)	0.01
Education						
None	34 (10%)	38 (13%)		21 (10%)	23 (14%)	
Primary	73 (22%)	50 (17%)		40 (18%)	31 (19%)	
Secondary	159 (48%)	151 (51%)		111 (51%)	70 (44%)	
Tertiary	63 (19%)	56 (19%)	0.4	45 (21%)	36 (23%)	0.4
Persons per room*	3 (2) [1-10]	3 (2) [1-19]	0.2	3 (2)[0-10]	3 (3)[0-26]	0.2
Number of smokers	81 (25%)	61 (21%)	0.2	46 (21%)	34 (21%)	1.0
Time smoked (years)*	11 (9) [1-46]	9 (9) [1-43]	0.2	8 (7)[1-30]	12 (11)[1-54]	0.08
Cigarettes per day*	7 (7) [1-40]	7 (7) [1-40]	0.8	7 (10[1-66]	6 (5)[2-20]	0.7
Contact with cough of > 3 weeks	54 (16%)	52 (18%)	0.3	45 (21%)	22 (14%)	0.2
Length of contact*	5 (6) [1-31]	7 (9) [1-44]	0.6	6 (10)[1-52]	8 (9)[1-34]	0.4
Contact with fever of > 3 weeks	26 (8%)	24 (8%)	1.0	23 (11%)	10 (6%)	0.3
Contact with PTB within 2 years	46 (14%)	58 (20%)	0.1	34 (16%)	20 (13%)	0.7
Length of contact*	5 (5) [1-20]	5 (5) [1-25]	0.8	5 (5)[1-20]	6 (5)[1-18]	0.42

Figure 8.2 Age distribution of culture-positive patients by HIV status



P=0.05

Figure 8.3 Age distribution of culture-negative patients by HIV status



P=0.01

Figure 8.4 Sex distribution of culture-positive patients with HIV

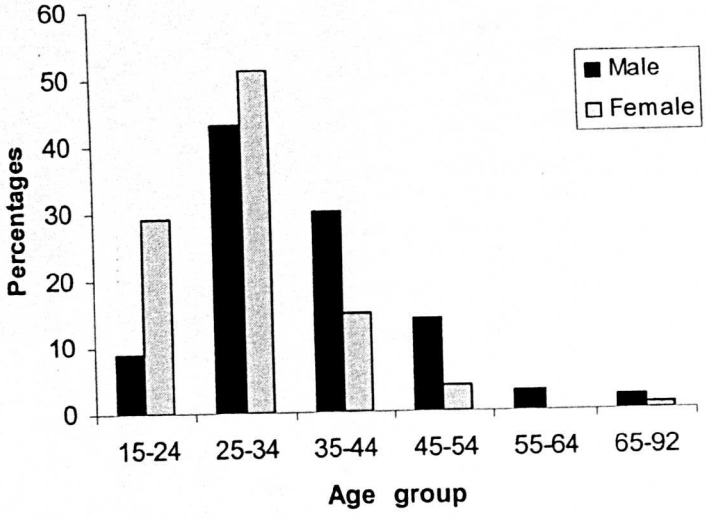
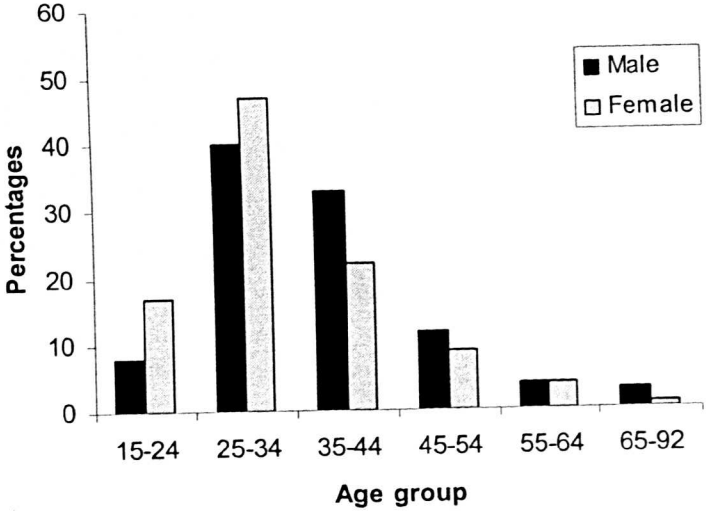


Figure 8.5 Sex distribution of culture-negative patients with HIV



8.6.3 Clinical symptoms of patients with PTB with and without HIV co-infection

The clinical symptoms of patients with positive and negative BACTEC cultures at the time of their first presentation to hospital by HIV status are summarized in table 8.6.3. Among culture positive-patients, those co-infected with HIV were more likely to have unexplained fever (OR= 1.35, 95%CI = 0.97-1.89), breathlessness (OR= 1.33, 95%CI = 0.96-1.86), weight loss (OR= 1.87, 95%CI = 1.21-2.90), night sweats (OR= 1.42, 95%CI = 1.0-2.01) and anorexia (OR= 2.2, 95%CI = 1.58-3.12) than patients without HIV. The duration of the fever and breathlessness was shorter in patients co-infected with HIV (mean (SD) of 10 (16) and 10 (16) weeks for fever and breathlessness respectively) than in patients without HIV (mean (SD) of 12 (16) and 11 (16) weeks respectively). The presence of cervical lymph nodes was also more frequent in HIV positive patients (OR= 2.31, 95%CI = 0.9-6.0, P= 0.04).

Among culture-negative patients, those co-infected with HIV were more likely to report breathlessness (OR= 1.45, 95%CI = 0.95-2.23) and weight loss (OR= 2.16, 95%CI = 1.37-3.39) than patients without HIV. The duration of these symptoms however was similar in both groups (P> 0.1 for both).

There was no statistically significant association between the presence or duration of cough, unexplained fever, night sweats or anorexia among culture-positive or culture-negative patients, irrespective of their HIV status (P>0.1 for all). Two hundred and twenty-three (68%) HIV positive and 197 (67%) HIV negative patients who were culture-positive had received BCG vaccination (P= 0.62), while among culture-

negative patients, 160 (74%) of the patients with HIV and 115 (72%) of those without HIV had received BCG (P= 0.92).

In order to identify the factors associated with a positive HIV status among patients with and without TB, all personal characteristics and clinical symptoms with a P value ≤ 0.20 were analysed using logistic regression. Table 8.6.4 shows these factors. Only one factor was positively associated with positive HIV among culture-positive patients and two factors among culture-negative patients. Culture-positive patients co-infected with HIV were more likely to suffer from anorexia (AOR= 2.2, 95%CI=1.6-3.1, P= 0.004), than culture-positive patients without HIV. Culture-negative patients infected with HIV were less likely to present with chest pains (AOR= 0.5, 95%CI = 0.3-0.8, P= 0.05), but more likely to have increased weight loss (OR= 2.6 95%CI= 1.6-4.2, P= 0.006) than culture-negative patients without HIV. Though not statistically significant, HIV patients in both culture-positive and culture-negative groups were less likely to be male (AOR= 0.72, 95%CI = 0.52-1.0, P= 0.056 for culture-positive patients and AOR= 0.66, 95%CI =0.43-1.0, P= 0.053 for culture-negative patients).

Table: 8.6.3 Clinical presentation of HIV positive and HIV negative patients by culture

	BACTEC CULTURE					
	Positive			Negative		
	HIV status					
Clinical Symptoms	**positive**	**negative**	**P value**	**positive**	**negative**	**P value**
BCG at birth or any other time	223 (68%)	197 (67%)	0.6	160 (74%)	115 (72%)	0.9
Mean BCG size (mm)	1 (1) [0-5]	1 (1) [0-5]	0.7	1 (1) [0-4]	1 (1) [0-5]	0.7
Cough	328 (99.7%)	295 (99.7%)	0.5	215 (99.1%)	159 (99.4%)	0.4
Cough duration*	13 (14) [0-80]	14 (15) [0-52]	0.3	12 (14) [1-52]	11 (13) [1-72]	0.4
Unexplained fever or sweating	228 (69%)	185 (62)	0.04	143 (66%)	98 (61%)	0.2
Fever/sweat duration*	10 (16) [1-52]	12 (16) [1-52]	0.4	10 (16) [1-52]	9 (14) [1-52]	0.6
Cough with bloody sputum	79 (24%)	82 (28%)	0.2	64 (30%)	56 (35%)	0.2
Bloody cough duration*	7 (12) [1-52]	11 (17) [1-52]	0.8	12 (19) [1-52]	6 (11) [1-52]	0.8
Breathlessness	229 (70%)	187 (63%)	0.05	150 (69%)	97 (61%)	0.04
Breathlessness duration*	10 (16) [1-52]	11 (16) [1-52]	0.6	9 (15) [1-52]	8 (13) [1-52]	0.4
Chest pains	280 (85%)	245 (83%)	0.2	163 (75%)	131 (82%)	0.06
Chest pains duration*	10 (15) [1-96]	11 (14) [1-52]	0.8	10 (14) [1-52]	8 (12) [1-52]	0.3
Loss of weight	289 (88%)	235 (79%)	0.002	168 (78%)	99 (62%)	0.001
Loss of weight duration*	10 (15) [1-52]	11 (15) [1-52]	0.4	10 (15) [1-52]	8 (13) [1-52]	0.4
Night sweats	245 (75%)	199 (67%)	0.02	149 (69%)	102 (64%)	0.2
Night sweats duration*	10 (15) [1-52]	11 (17) [1-88]	0.3	10 (16) [1-52]	8 (13) [1-52]	0.4
Loss of appetite	245 (75%)	168 (57%)	0.001	123 (57%)	82 (51%)	0.2
Loss of appetite duration*	9 (15) [1-52]	11 (16) [1-52]	0.4	10 (17) [1-52]	7 (13) [1-52]	0.9
Enlarged cervical lymph-nodes	15 (5%)	6 (2%)	0.04	6 (3%)	2 (1%)	0.2
Finger clubbing	5 (2%)	3 (1%)	0.3	2 (1%)	1 (1%)	0.4

* Duration of all symptoms expressed as mean (SD) and range

Table 8.6.4 Logistic regression analysis for clinical variables of culture-positive and culture-negative patients by HIV status with p values < 0.02

Culture-positive	P	OR	P1	P2	P3	P4	P5	P6	P7	P8	AOR
Fever	0.04	1.4 (1.0-1.9)	0.60	0.59	0.58					NS	
Haemoptysis	0.15	0.8 (0.6-1.2)	0.31	0.31	0.31	0.34				NS	
Breathlessness	0.05	1.3 (1.0-1.9)	0.91							NS	
Lost of weight	0.002	1.9 (1.2-2.9)	0.25	0.23	0.23	0.19	0.20			NS	
Night sweats	0.02	1.4 (1.0-2.0)	0.98	0.98						NS	
Anorexia	0.001	2.2 (1.6-3.1)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	2.2 (1.6-3.1)
Cervical lymph nodes	0.04	2.3 (0.9-6.0)	0.13	0.13	0.13	0.12	0.14	0.13		NS	
Sex	0.02	0.7 (0.5-1.0)	0.08	0.08	0.08	0.08	0.06	0.06	0.06	NS	0.7 (0.5-1.0)
Culture-negative	**P**	**AOR**	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**AOR**
Fever	0.18	1.2 (0.8-1.9)	0.67	0.67						NS	
Haemoptysis	0.14	0.8 (0.5-1.2)	0.12	0.12	0.13	0.13	0.14	0.18		NS	
Breathlessness	0.04	1.5 (0.9-2.2)	0.20	0.20	0.18	0.20	0.21			NS	
Chestpains	0.06	0.7 (0.4-1.1)	0.01	0.01	0.01	0.008	0.007	0.01	0.01	0.01	0.5 (0.3-0.8)
Loss of weight	0.001	2.2 (1.4-3.4)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	2.6 (1.6-4.2)
Night sweats	0.16	1.2 (0.8-1.9)	0.93							NS	
Anorexia	0.15	1.2 (0.8-1.9)	0.62	0.60	0.61					NS	
Cervical lymph nodes	0.17	2.3 (0.4-11)	0.39	0.38	0.40	0.38				NS	
Male sex	0.04	0.7 (0.5-1.0)	0.08	0.08	0.08	0.08	0.06	0.06	0.05	NS	0.7 (0.4-1.0)

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1 to P4 = p values after running the logistic regression

8.6.4 Clinical Signs

The clinical signs of the patients at the time of presentation are described in table 8.6.5. Patients with positive cultures co-infected with HIV had similar clinical signs than patients without HIV with the exception of their BMI (OR= 1.51, 95%CI = 1.06-2.14) and the presence of bronchial breath sounds (OR = 1.32, 95%CI = 0.96-1.82). Patients co-infected with HIV had a mean (SD) BMI of 20 (6) compared to a BMI of 21 (4) for HIV negative patients (P< 0.001).

Regarding patients with negative cultures, only the BMI was different between HIV co-infected and HIV negative patients. The mean (SD) BMI of patients co-infected with HIV was 22 (4) compared to 24 (5) for the HIV negative patients. Patients with TB and HIV had the lowest BMI, followed by patients with TB but no HIV and those with HIV but no TB. Patients without TB or HIV had the highest BMI (F statistics= 31.99, P= 0.001).

The mean (SD) Karnofsky score for patients with TB co-infected with HIV was 59 (12) which was similar to the score observed for patients without HIV (60 (11), P= 0.3). Similarly, among culture-negative patients, HIV infected patients had a mean (SD) Karnofsky score of 59 (12) and patients without HIV had a mean score of 61 (10), (P= 0.1).

Clinical variables with P values ≤ 0.20 for HIV-positive and negative patients were entered into logistic regressions to identify the clinical signs of culture-positive and culture-negative patients that were associated with HIV infection after allowing for

confounding effects between variables. The factors selected are shown in tables 8.6.6 and 8.6.7. In both culture-positive and culture-negative patients, a higher BMI was associated with a lower likelihood of HIV infection (OR= 1.5, 95%CI = 1.0-2.1, P= 0.03 for culture positive and OR = 2.7, 95%CI = 1.5-4.8, P= 0.001 for culture negative patients).

Table: 8.6.5 Clinical characteristics of patients with positive and negative culture by HIV status

	BACTEC CULTURE					
	Positive			Negative		
	HIV status					
Clinical Signs	**positive**	**negative**	**P value**	**positive**	**negative**	**P value**
Temperature*	37 (1) [35-40]	37 (1) [34-47]	0.7	36.7 (1)[35-40]	36.8 (1)[35-40]	0.2
< 37.5°C	263 (80%)	240 (81%)		187 (86%)	131 (82%)	
≥ 37.5°C	66 (20%)	56 (19%)	0.4	30 (14%)	29 (18%)	0.1
PPD size*	2 (1) [0-5]	1 (1) [0-3]	0.2	2 (1)[0-3]	1 (1)[0-3]	0.1
PPD neg < 2mm	17 (47.2%)	17 (60.7%)		11 (50%)	5 (83.3%)	
pos ≥ 2mm	19 (52.8%)	11 (39.3%)	0.2	11 (50%)	1 (16.7%)	0.09
BMI*	20 (6) [11-37]	21 (4) [12-38]	0.001	22 (4)[12-35]	24 (5)[13-47]	0.001
< 18.5	107 (33%)	72 (24%)		53 (24%)	17 (11%)	
≥ 18.5	221 (67%)	224 (76%)	0.01	164 (76%)	143 (89%)	0.001
Karnofsky score*	59 (12) [20-100]	60 (11) [20-100]	0.3	59 (12)[40-100]	61 (10)[40-90]	0.1
< 60	212 (64%)	191 (65%)		137 (63%)	91 (57%)	
≥ 60	117 (36%)	105 (35%)	0.5	80 (37%)	69 (43%)	0.1
Presence of : Wheeze	79 (24%)	72 (25%)	0.4	47 (22%)	33 (21%)	0.4
Bronchial breath sounds	160 (50%)	122 (42%)	0.04	95 (45%)	63 (41%)	0.2
Rhonchi	121 (37%)	101 (35%)	0.2	70 (33%)	44 (28%)	0.2
Crepitations	173 (53%)	157 (54%)	0.4	96 (46)	72 (47%)	0.4

*Mean (SD) [range

Table 8.6.6 Logistic regression analysis for clinical variables with p values <0.20 to identify patients with HIV among culture-positive patients

Culture positive		P	OR	P1	P2	P3	AOR
BMI	≤ 18.5	0.01	1.5 (1.1-2.1)	0.03	0.03	0.03	1.5 (1.0-2.1)
Bronchial sounds		0.04	1.3 (1.0-1.4)	0.14	0.15	NS	

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1 to P3 = p values after running the backward logistic regression

Table 8.6.7 Logistic regression analysis for clinical variables with p values <0.20 to identify patients with HIV among culture-negative patients

Culture negative		P	OR	P1	P2	P3	AOR
BMI	≤ 18.5	0.001	2.7 (1.5-4.9)	0.001	0.001	0.001	2.7 (1.5-4.8)
Karnofsky	≤ 60	0.11	1.5 (1.0-2.3)	0.37	0.37	NS	
Rhonchi		0.18	1.2 (0.8-1.9)	0.70		NS	

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1 to P3 = p values after running the backward logistic regression

8.7 Laboratory results of patients with positive and negative cultures by HIV status.

The results of the routine laboratory exams by culture and HIV status are summarised in table 8.7.1. The mean (SD) haemoglobin concentration in culture-positive patients was 11 (2) g/dl for those co-infected with HIV and 12 (2) gm/dl for those negative for HIV (P= 0.001). Similarly in culture-negative patients, the mean (SD) haemoglobin for those infected with HIV was 11 (3) gm/dl and for those not infected was 13 (2) gm/dl (P= 0.001). A larger proportion of patients with culture-positive TB were anaemic if they were HIV positive (174, 56%) than if they were HIV negative (67,

24%, P= 0.001). Similarly, in patients without TB, a larger proportion of patients with HIV (83 (42%) were anaemic compared to those without HIV (P= 0.001).

The WBC count and differentials (granulocytes and lymphocytes) were similar across the four groups. All groups had mean granulocyte counts ranging from 61% in culture-positive patients to 59% in culture-negative patients and lymphocyte counts ranging from 39% in culture-positive to 41% in culture-negative patients. The differences between HIV-positive and HIV-negative patients were not satistically significant.

The mean (SD) ESR was significantly higher in culture-positive patients who were HIV-positive (91 (42) mm/hr) compared to HIV negative patients (66 (42) mm/hr, P= 0.001). This pattern was also observed in culture-negative patients with a mean (SD) ESR of 85 (46) mm/hr for HIV positive and 50 (39) mm/hr for HIV negative patients (P= 0.001).

The mean (SD) total and conjugated bilirubins and the proportion of patients with normal values were similar across the 4 groups.

In both culture-positive and culture-negative patients, HIV positive patients had significantly lower mean serum albumin than HIV negative patients (P= 0.001 for both) (table 8.7.1) and a larger proportion of HIV positive patients in both culture-positive and culture-negative patients had low serum albumin levels than in HIV negative patients (P= 0.001 for both culture groups).

Among culture-positive patients the mean (SD) SGOT was 8 (6) for HIV positive and 7 (7) for HIV negative patients (P= 0.06) and for culture-negative patients the means were 7 (5) for HIV positive and 8 (10) for HIV negative patients respectively (P= 0.62). The proportion of patients with high SGOT was significantly larger for HIV positive compared to HIV negative patients in the culture-positive group (P= 0.02).

HIV positive patients in both culture-positive and culture-negative groups had higher mean (SD) alkaline phosphatase (240 (161) and 197 (112) respectively) than HIV negative patients (204 (114) and 176 (78) respectively) (P= 0.002 and 0.05). The proportion of patients with high alkaline phosphatase was only significantly higher among the culture-positive patients who were HIV positive (P= 0.005).

Table: 8.7.1 Laboratory results in culture-positive and culture-negative patients by HIV status

	BACTEC CULTURE					
	Positive			Negative		
	HIV status					
Laboratory test (Mean)	**positive**	**negative**	**P value**	**positive**	**negative**	**P value**
Haemoglobin level g/dl	11 (2) [6-10]	12 (2) [5-12]	0.001	11 (3) [5-17]	13 (2) [7-18]	0.001
> 11g/dl	174 (56%)	67 (24%)		83 (42%)	19 (13%)	
≥ 11g/dl	135 (44%)	211 (76%)	0.001	117 (59%)	130 (87%)	0.001
White blood count	8 (4) [3-31]	8 (4) [4-38]	0.7	8 (5) [2-34]	7 (3) [2-18]	0.09
< 10 x 10^9	224 (73%)	208 (75%)		155 (78%)	125 (83%)	
≥ 10 x 10^9	83 (27%)	70 (25%	0.3	43 (22%)	25 (17%)	0.1
Granulocytes (%)	61 (14) [6-92]	61 (13) [9-92]	0.5	59 (15) [18-96]	59 (11) [26-84]	0.8
< 65%	184 (59%)	170 (61%)		133 (67%)	108 (72%)	
≥ 65%	125 (41%)	108 (39%)	0.4	66 (33%)	42 (28%)	0.2
Lymphocytes (%)	39 (14) [8-94]	39 (13) [8-91]	0.5	41 (16) [4-81]	41(12) [16-74]	0.9
< 45%	211 (68%)	189 (68%)		123 (62%)	97 (65%)	
≥ 45%	98 (32%)	89 (32%)	0.5	76 (38%)	53 (35%)	0.3
ESR mm/hr	91 (42) [3-167]	66 (42) [1-160]	0.001	85 (46) [3-162]	50 (39) [1-165]	0.001
< 7mm/hr	3 (1%)	22 (8%)		6 (3%)	11 (7%)	
≥ 7mm/hr	304 (99%)	255 (92%)	0.001	194 (97%)	138 (93%)	0.03
Total bilirubin mm/dl	0.5 (0.5) [0.1-6.30]	0.5 (0.4) [0.01-3.3]	0.9	0.5 (0.5) [0.1-5]	0.6 (0.4) [0.1-3]	0.5
< 1.0mg/dl	303 (94%)	268 (94%)		187 (90%)	139 (90%)	
≥ 1.0mg/dl	19 (6%)	18 (6%)	0.4	21 (10%)	15 (10%)	0.5
Conjugated bilirubin mm/d	0.2 (0.3) [0.01-4.50]	0.2 (0.3) [0.01-2.1]	0.8	0.2 (0.3) [0.01-4]	0.2 (0.3) [0.01-2]	0.9
< 0.2mg/dl	208 (65%)	175 (61%)		122 (59%)	81 (52%)	
≥ 0.2mg/dl	114 (35%)	113 (19%)	0.2	86 (41%)	74 (48%)	0.1
Serum albumin g/dl	3 (2) [1-7]	4 (0.8) [2-7]	0.001	3 (1) [1-6]	4 (1) [2-6]	0.001
< 3.8g/dl	261 (81%)	165 (57%)		147 (71%)	73 (47%)	

	≥ 3.8g/dl	61 (19%)	123 (43%)	0.001	61 (29%)	83 (53%)	0.001
SGOT U/L		8 (6) [1-58]	7 (7) [1-70]	0.06	7 (5) [1-39]	8 (10) [1-96]	0.6
	< 12U/L	269 (83%)	258 (90%)		176 (85%)	137 (88%)	
	≥ 12U/L	53 (17%)	30 (10%)	0.02	32 (15%)	19 (12%)	0.2
SGPT U/L		13 (13) [2-150]	12 (8) [2-90]	0.03	13 (15) [2-176]	12 (11) [2-120]	0.5
	< 12U/L	157 (49%)	162 (56%)		104 (50%)	90 (58%)	
	≥ 12U/L	165 (51%)	126 (44%)	0.03	104 (50%)	66 (42%)	0.07
Alkaline phosphatase U/L		240 (161) [4-940]	204 (114) [33-825]	0.002	197 (112) [46- 846]	176 (78) [18-498]	0.05
	< 279U/L	241 (75%)	241 (84%)		174 (84%)	137 (88%)	
	≥ 279U/L	80 (25%)	47 (16%)	0.005	32 (16%)	19 (12%)	0.2

Table 8.7.2 Logistic regression for laboratory variables with p values < 0.02 to identify patients with HIV infection in culture-positive patients

Laboratory tests		**P**	**OR**	**P1**	**P2**	**P3**	**P4**	**AOR**
Haemoglobin	≤ 11g/dl	0.001	4.1 (2.8-5.8)	0.001	0.001	0.001	0.001	3.0 (2.1-4.4)
ESR	≤ 7mm/hr	0.001	8.7 (2.6-29.3)	0.01	0.01	0.009	0.01	9.5 (2.8-32.4)
Bilirubin C	≤ .2mg/dl	0.16	1.2 (0.8-1.6)	0.80			NS	
Albumin	≤ 3.8g/dl	0.001	3.2 (2.2-4.6)	0.001	0.001	0.001	0.001	2.3 (1.6-3.5)
SGOT	≤ 12U/L	0.02	1.7 (1.0-2.7)	0.01	0.02	0.01	0.009	2.0 (1.2-3.3)
SGPT	≤ 12U/L	0.03	0.7 (0.5-1.0)	0.51	0.52	0.53	NS	
Alkaline Phosphatase	≤ 279U/L	0.005	0.6 (0.4-0.9)	0.63	0.61		NS	

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1 to P4 = p values after running the logistic regression

Table 8.7.3 Logistic regression for laboratory variables with p values < 0.02 to identify patients with HIV infection in culture-negative patients

Laboratory test		P	OR	P1	P2	P3	P4	P5	P6	P7	P8	AOR
Haemoglobin	≤ 11g/dl	0.001	4.9 (2.8-8.5)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	4.1 (2.3-7.2)
WBC	≤ 10 x 10^9	0.12	0.8 (0.4-1.4)	0.70	0.71						NS	
Granulocytes	≤ 65%	0.15	0.8 (0.5-1.2)	0.55	0.54	0.62					NS	
ESR	≤ 7mm/hr	0.03	2.6 (0.9-7.1)	0.40	0.39	0.38	0.38	0.37			NS	
Bilirubin C	≤ 0.20mg/dl	0.11	1.3 (0.9-2.0)	0.12	0.12	0.12	0.13	0.15	0.20	0.23	NS	
Albumin	≤ 3.8g/dl	0.001	2.7 (1.8-4.2)	0.01	0.01	0.009	0.01	0.01	0.01	0.009	0.008	1.9 (1.2-3.0)
SGOT	≤ 12U/L	0.19	1.3 (0.7-2.4)	0.29	0.27	0.26	0.27	0.23	0.24		NS	
SGPT	≤ 12U/L	0.07	0.7 (0.5-1.1)	0.89							NS	
Alkaline Phosphatase	≤ 279U/L	0.18	0.8 (0.4-1.4)	0.64	0.64	0.61	0.61				NS	

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1 to P4 = p values after running the backward logistic regression

In order to identify the laboratory tests that were significantly associated with HIV among culture-positive and negative-patients, all laboratory variables with P value ≤ 0.20 were entered into backward logistic regressions. Tables 8.7.2 and 8.7.3 show these analyses. Four factors were independently associated with HIV infection in culture-positive patients and two variables in culture-negative patients. HIV positive patients with TB were more likely to have anaemia (AOR= 3.04, 95%CI = 2.08-4.44, P= 0.001) and hypoalbuminaemia (AOR= 2.34, 95%CI = 1.56-3.50, P= 0.001) but more likely to have a high ESR (AOR= 9.5, 95%CI = 2.8-32.4, P= 0.01) and SGOT (AOR= 2.0, 95%CI = 1.2-3.3, P= 0.009). Patients with negative cultures who had HIV infection (i.e. negative BACTEC culture) were more likely to have anaemia (AOR= 4.06, 95%CI = 2.29-7.20, P= 0.001), and hypoalbuminaemia (AOR= 1.89, 95%CI = 1.18-3.01, P= 0.008) than patients without HIV.

8.8 Discussion

This chapter discribes the prevalence, clinical presentation, risk factors and bacteriological features of patients with and without TB attending district hospitals in Abuja, Nigeria. Despite the importance of TB and HIV, few reports have been published on this topic in West Africa. The prevalence of TB is increasing in many countries and it is estimated to be the leading cause of death worldwide among communicable diseases, killing nearly 3 million people each year (Narain et al., 2004; Raviglione et al., 1995; Raviglione et al., 1996; WHO 2002). The association of TB with HIV presents a public health catastrophe and a socioeconomic threat, with ominous medical implications particularly in the developing world. One of the most commonly cited explanations for the increased rate of infection is the delayed recognition and isolation of patients with active PTB (Mathur et al., 1994; Kramer et al., 1990; Ellner et al., 1993). And the prompt recognition of PTB patients and treatment should be a high priority in TB control policies (Ellner et al., 1993; Blumberg 1997; McGowan 1995).

A major finding of our study was the nearly double prevalence of PTB identified by culture compared to smear microscopy and the significant number of patients who are co-infected with HIV among those attending the hospitals. Of the 1185 patients cultured, 62% were culture-positive as against 30% who were positive by smear microscopy. This showed that over 50% of cases would have been missed if only sputum smears were performed. Of the 624 patients that were culture positive, 53% were also HIV positive. Our results are similar to other prevalence studies of TB and TB and HIV co-infection in other developing countries (Harries 1997; 1998; Grant et al., 1998; Kelly et al., 1990). Culture however, is usually not a diagnostic tool for PTB

in most resource poor countries and not many reports of the prevalence of TB by culture are available. The estimated prevalence of sputum smear-positive TB worldwide in 2001 was 426/100,000 and for all cases, 963/100,000 population (Dye et al., 2005). The Nigerian TB incidence (all cases/100,000 pop) for TB was estimated to be 548/100,000 population in 2002 and 27% of the patients with TB were co-infected with HIV (WHO 2005). Several factors can explain these differences. These estimated Nigerian figures are lower than the results presented here. The government estimates are based on extrapolated data from WHO and our results are research-based and reported on a more sensitive test (culture). Secondly, our results were based on patients from an urban area with high mobility who were already suspected of having TB on the basis of symptoms, whereas the government data came from sentinel studies with a mixture of rural and urban centres. Abuja is the new capital of Nigeria and has an uncontrolled influx of population from all over the country. Most new arrivals live in squalor in the shanty settlements in the outskirts of the city. In addition, the DOTS programme was only recently introduced into Abuja. As previously observed in Ethiopia, when a TB control programme is first introduced, the number of cases of TB increases because of the introduction of the programme.

Like most studies, we found that patients with PTB were younger than those without TB. In Brazil for example (Liberato et al., 2004), Turkey (Aktogu et al., 1996), India (Prasad et al., 2004) and Benin (Nwobu et al., 2004) the greatest number of patients with TB occur in patients between 25 and 44 years of age. A study in Jos, Nigeria, found that TB predominantly affected individuals below 40 years of age with a peak frequency between 21 and 30 years (Anteyi et al., 1996). This was also observed in a prospective, descriptive French multicentre study which found a mean age of 40.8

years for culture proven TB patients as against 47.4 years for non-TB patients (Tattevin et al., 1999). Also, patients with PTB who were co-infected with HIV were younger than those without HIV. This is not unexpected as this is the age group when people are more socially and sexually active. In a prevalence study of TB among HIV-infected persons in Phnom Penh, Cambodia, patients had a mean (SD) age of 34 (8) years for co-infected patients, with most of the infection occuring in the age group 25-34 years (Kimerling et al., 2002). Similarly, in Taiwan, the mean age of patients co-infected with TB and HIV was 37 years (Hsieh et al., 1996), which was younger than the age of patients who had TB but no HIV. This pattern has also been observed in many other countries including Brazil (Liberato et al., 2004), India (Prasad et al., 2004) and Benin (Nwobu et al., 2004).

Female patients in our study were younger than their male counterparts. Among patients infected with HIV, female patients were also younger than male patients. These findings agree with observations in Africa and other developing countries (Aerts et al., 2004; Liberato et al., 2004; Anteyi et al., 1996; Kimerling et al., 2002). Younger women are more often co-infected with TB and HIV because of cultural, societal and socioeconomic conditions in most developing countries, which expose women at an earlier age to various detrimental risk factors.

More culture-positive and culture-negative male patients visited the participating hospitals and more male than female patients had TB. However, more females than males were co-infected with TB and HIV. Other authors have reported similar findings in their studies. Kimerling et al., (Kimerling et al., 2002) found that males were slightly at higher risk of having TB and Prasad et al., in a demographic study of

PTB cases found that male patients were more frequently infected than their female counterparts (Prasad et al., 2004). In Pernambuco, Northeast Brazil, there was a higher frequency of male individuals among PTB patients and among those co-infected with HIV (Liberato et al., 2004) and in Paris and Guyana, Tattevin et al., observed a male prevalence of 72% in a multicentre study (Tattevin et al., 1999). In Turkey, Aktogu et al., reported that TB was more common among males (81%) living in large urban centres (Aktogu et al., 1996) and a study in Benin and Irua, Nigeria found that more males than females had PTB but that females were more frequently co-infected with TB and HIV (Nwobu et al., 2004). Although most of these observations agree with the findings in our study, Nwachokor et al., in Nigeria however found that more females were infected with TB which reached twice the rate of males in some regions (Nwachokor et al., 2000).

Most of the patients in employment were civil servants and are likely to attend the participating hospitals, which are government official hospitals for civil servants. Most of these patients are low-level civil servants and construction site labourers. There was no association between the positivity of the sputum and the activities of the patients. A high proportion were attending secondary schools. Like civil servants, these are hospitals frequently visited by secondary school students for medical treatment. All groups were equally affected with TB irrespective of their literacy level.

Our study observed that patients with and without TB had similar history of exposure to TB and did not identify any association between cigarette smoking and PTB nor contact with persons with chronic cough, fever or treated for PTB. These results are in

agreement with Kimerling et al., who did not find any association between household crowding and smoking with TB (Kimerling et al., 2002). Smoking and history of overcrowding of are very frequent in Nigeria and may explain why these factors are not reliable to identify adults with TB in our environment.

In Nigeria, as in most developing countries, BCG is the first vaccine used in the routine vaccination schedule of the National Expanded Programme on Immunizations and most children receive BCG at birth. This accounts for the high proportion of patients who had received BCG. A significantly higher proportion of culture-negative patients however had been vaccinated with BCG than culture-positive patients. This was however not independently predictive after controlling for confounding factors. Tattevin et al., also described in a series of 211 cases that BCG status was associated with the presence of culture proven TB but that this factor was not significant after multivariate analysis (Tattevin et al., 1999). Though given routinely, the significance of BCG has recently been questioned as an efficient method of prevention of TB. It has a low efficacy in preventing infectious TB in some countries with high disease burden (Colebunders et al., 1989; Collins et al., 2001; Paterson 2001) and is a subject of continuos debate.

Most patients with TB are diagnosed on the basis of their clinical presentation, sputum microscopy and chest X-ray findings where available. Given the local scarcity of culture facilities for the diagnosis of TB, culture for the identification of *M. tuberculosis* is rarely used in most resource-poor countries. This chapter thus aimed to evaluate if the clinical presentation of the patients could be used as predictors of TB in places where diagnostic facilities are limited (Cohen et al., 1996; Scott et al., 1994;

Blumberg 1995). In a similar approach, the European guidelines for identification of TB define 'other than definite' cases of TB as those meeting two conditions: (1) a clinician's judgement that the patient's clinical, and/or radiological signs and/or symptoms are compatible with TB and (2) a clinician's decision to treat a patient with a full course of chemotherapy. Due to the protean clinical and radiological manifestations of TB, the American Thoracic Society and CDC also recommended that pulmonary diagnosis should always be included in the differential diagnosis of persons with pulmonary signs or symptoms and appropriate diagnostic measures should be instituted (American Thoracic Society, (ATS 1992)). A diagnosis that is only based on clinical information however may have different positive and negative predictive values in areas with high and low TB incidence and these diagnostic approaches need to be evaluated in high TB incidence areas. In addition, it is important to identify predictors for TB in patients co-infected with HIV, to be able to identify patients who should be referred for HIV testing and treatment.There is very limited information in this field.

Patients with chronic cough of more than 3 weeks duration are suspected to have TB. Almost all the patients presenting to the TB clinic had cough, and was one of the criteria for inclusion into the study. Patients with culture-positive TB however had cough for a longer duration than those without TB and this was independent on whether the patient had HIV. Kimerling et al., also found that cough of more than 3 weeks duration was moderately sensitive to identify TB but that this was not independently predictive (Kimerling et al., 2002). In contrast, Cohen et al., found that symptoms of cough, sputum production and lost of weight were independent risk factors for TB (Cohen et al., 1996).

Fever in TB patients is normally low grade, rarely rising above 40°C, characteristically being low in the morning, and peaking towards evening (Davies 2003 978). Our study observed relatively low-grade fever in most patients rarely rising to 40°C. A large proportion of culture-positive patients had a history of recent fever but this was not statistically different to culture-negative patients. Similarly, culture-positive patients with HIV were more likely to have fever than HIV-negative patients but this was not independently predictive after multivariate analysis. This was expected, as patients with pulmonary infections other than TB often have fever of recent or long duration.

Haemoptysis is a major diagnostic symptom in the classical TB patients. Other pulmonary diseases such as acute bacterial pneumonia, cancer and mitral stenosis however can also present as acute or chronic cough with haemoptysis. Our study did not show any association between haemoptysis and TB or TB plus HIV co-infection. This may be due to the presence of a large proportion of patients with HIV as HIV patients have fewer chest cavities and less haemoptysis (Raviglione et al., 1992). Other respiratory symptoms such as dyspnoea and chest pains had limited predictive values and were not significant in the multivariate analysis.

Among the factors that were significant, a history of weight loss was a significant predictor for TB, this has been reported in other studies (Aktogu et al., 1996) and patients co-infected with TB and HIV were more likely to have more significant weight losses than those with TB alone. As expected, we observed a significantly lower BMI in patients with TB compared to those without TB, and patients with TB co-infected with HIV had lower BMI than patients with TB alone. El-Sony et al.,

reported that, in Sudan, symptoms with the greatest predicted significance were weight loss, tiredness and night sweats (El-Sony et al., 2003). We observed a significant proportion of culture positive patients with night sweats and anorexia, but these factors were not independently predictive after multivariate analysis. Cohen et al., in the state of Illinois, USA, reported that fever, night sweats dyspnoea, anorexia and haemoptysis were not different between TB and non TB patients (Cohen et al., 1996).

Our study did not find significant differences in the clinical signs between patients with and without TB.

Clinical features of HIV-associated PTB are frequently atypical, resembling those of primary TB (Raviglione et al., 1992), with symptoms and signs of extrapulmonary TB especially in the immunocompromised. More typical post-primary TB is seen during the early stages of infection (Harries 1990; Barnes et al., 1991). In our study, TB patients co-infected with HIV had significantly more fever, haemoptysis, dyspnoea, weight loss, night sweats, anorexia and enlarged cervical lymph nodes than patients with TB but no HIV infection. However, on multivariate analysis, only haemoptysis, weight loss and anorexia remained independently predictive for patients co-infected with HIV. Several reports on TB and HIV co-infection have described the clinical features of these patients. These studies showed that most HIV seropositive patients from Africa would have experienced significant weight loss (Eriki et al., 1991; Colebunders et al., 1989; Kelly et al., 1990), had fever of shorter duration (Colebunders et al., 1989), dyspnoea (Colebunders et al., 1989; Elliott et al., 1990) and lymhadenopathy (Colebunders et al., 1989; Elliott et al., 1990). Haemoptysis

results from caseous necrosis of the bronchial arteries inside chest cavities. The observation made in our study which showed haemoptysis less common in HIV-seropositive patients compared to seronegative patients had also been reported in other studies (Eriki et al., 1991) indicating that cavities are less likely to form in HIV-infected patients with TB.

Our laboratory results show that compared with culture-negative patients, culture positive-patients were more likely to be anaemic and to have increased conjugated bilirubin but less likely to have leukocytosis. TB patients co-infected with HIV were more likely than patients without HIV to have anaemia and hypoalbuminaemia but less likely to have lower ESR and SGOT. These results though can not discriminate those who have HIV or TB from those who do not (Brandli 1998).

In conclusion, clinical sign of a BMI < 18.5 plus symptom of weight loss and the presence of anaemia and conjugated bilirubin ≥ 0.2 mg/dl and granulocyte count $\leq$ 65% are independent prediction of TB. In addition, being a female, having anorexia plus a BMI < 18.5, in the presence of anaemia, hypoalbuminaemia, raised ESR, and SGPT are independent predictors of TB and HIV. These factors could be used to develop algorithm for the identification of patients with TB or TB and HIV.

CHAPTER NINE

Clinical presentation of patients with smear-positive and smear-negative TB with or without HIV

9.1 Introduction

With the resurgence of TB in the mid 1980s, attention has focused on its early identification and treatment (Fairchild et al., 1998). Early identification of patients with TB, whether they are smear-positive or smear-negative is necessary, both to enable appropriate control procedures and to provide a basis for early therapy. The cornerstone of a WHO-led control programme depends on the detection of a large proportion of smear-positive pulmonary TB cases as these are the most infectious individuals in the community with capacity to spread infection (Shaw et al., 1954; Rose et al., 1979). Sputum smear examination for AFB can only diagnose between 30% to 60% of cases of TB in well-equipped laboratories (Aber et al., 1980). In countries with high prevalence of TB and HIV, the detection rate is even lower owing to the paucibacillary nature of PTB in patients with HIV (Hargreaves et al., 2001a; Elliott et al., 1990; Hargreaves et al., 2001b; Long et al., 1991). In countries with low prevalence of PTB, smear-negative TB is said to pose less of a threat to public health compared to smear-positive PTB, based on its low infectivity potential, lesser extent of disease, and good prognosis even without treatment (Long 2001). However, smear-negative patients who are diagnosed with TB by culture, radiology or other means are also capable of transmitting the infection (Dutt et al., 1994). Most publications on the clinical features of PTB have not differentiated between smear-positive and smear-

negative disease as these patients may have different clinical and radiological findings (Kanaya et al., 2001). Pulmonary TB with a positive smear signifies larger bacterial population in a diseased lung lesion whereas several negative smears suggest a smaller bacterial load (Dutt et al., 1994; Hargreaves et al., 2001a). PTB patients whose smears are negative for AFB represent a diagnostic dilemma and pose challenges to clinical services and public health programmes in countries where culture facilities are not available (El-Sony et al., 2003). Many of these smear-negative patients yield positive cultures for *M.tuberculosis* (Dutt et al., 1994). Based on studies performed prior to the advent of the HIV pandemic, smear-negative PTB patients had generally been thought to have mild disease with good prognosis (Narain et al., 1968), however, this is not so anymore. The contribution of HIV infection to the apparent increasing incidence of TB especially in the developing world is immense and their epidemic in sub-Saharan Africa has been associated with an increase in the proportion of smear-negative PTB cases, where such cases are not confirmed by culture (Harries 1997; Hargreaves et al., 2001b; Elliott et al., 1993c). Even though patients with sputum smear-negative TB are less infectious than smear-positive patients, both theoretical and empirical evidence suggest they can still transmit the infection (Behr et al., 1999; Elwood et al., 2005; Grzybowski et al., 1975).

Previous studies have suggested that patients with smear-negative PTB usually have less clinical symptoms and signs compared to smear-positive patients (Kobashi et al., 1995). Tsao et al., observed that smear-negative patients were significantly older and had less clinical symptoms e.g., cough, fever, and body weight loss (Tsao et al., 2004). Smear-negative patients however had significantly more underlying disease,

which may account for the high mortality rate seen in this group. Other authors had made similar observations (Kobashi et al., 1995 77; Hargreaves et al., 2001a).

Considering that DOTS programmes in most developing countries are run at the level of health centres (Enarson 1995), it is important to aid the health care workers identify patients most likely to be suffering from TB but are smear-negative with little or no manifestation of the disease.

9.2 Materials and methods

The methods for this chapter are as described in chapter eight. Patients were identified by smear microscopy and the clinical presentation and risk factors for patients with smear-positive and smear-negative, culture-positive TB were compared, stratified by HIV.

9.2.1 Radiological examination

Patients who had positive smear microscopy had their chest X-rays routinely taken. The same radiologist (Dr. Olatunji in Nigeria) and a chest specialist (Professor Davies, in Liverpool), reviewed the X-rays. The two met several times to agree on the scoring method. The X-rays were scored as 0 to 6, depending on the radiological extent of disease according to the number of abnormalities in the six lung zones. Each lung zone was classified as a 0 (normal) or 1 (affected). Cavities were also scored from 0 to 3 depending on the total diameter of the cavities in the lung fields. No cavity present was graded zero, cavities with a total diameter of less than 2 cm were

graded as 1, cavities with diameter between 2 and 4 cm were graded as 2 and those greater than 4 cm were graded as 3. The results of each X-ray reviewer were entered independently into a database. X-rays with disagreement between the two readers were re-read by the readers to standardise the scores. It was agreed that the lower value should be taken when there was a difference in scoring of ≥ 2 for extent of disease and of ≤ 1 for cavities. The two readers met to review the remaining X-rays where there were discrepancies and came to an agreement. A third set of results were entered into the database based on their agreement.

9.3 Results

9.3.1 Characteristics of patients with smear-positive and smear-negative TB

On thousand, three hundred and twenty-one patients with suspicion of TB were screened. Of these, 731 were culture-positive. Three hundred and fifty-three (48%) of the 731 culture-positive patients were smear-positive and 378 (52%) smear-negative. Two hundred and twenty-eight (65%) of the patients with smear-positive and 208 (55%) of those with smear-negative TB were male. Patients with smear-negative TB were older (mean (SD) of 35 (12) years) than those with smear-positive TB (mean (SD) 31 (10) years) (P= 0.001) in both sexes.

This analysis compares the characteristics of patients with smear-positive TB (n=353 and patients with smear-negative TB (n=378).

The occupational status and education of the patients were not significantly different between smear-positive and smear-negative TB. Similarly there was no significant difference in the number of patients who shared their rooms or the number of patients who smoked cigarettes (table 9.3.1). Smear-positive and smear-negative patients had similar frequency of contact with other patients with cough or fever. Contact with patients with PTB however was more frequently reported in patients with smear-positive TB (table 9.3.1) and this was predictive for TB when entered into multivariate analysis (AOR = 1.4, 95%CI = 1.1-1.7, P= 0.01) (9.3.2). However, such contact was reported in only a minority of patients (18% and 14% of smear-positive and smear-negative patients, respectively P= 0.003).

Table: 9.3.1 Characteristics of patients with smear-positive and smear-negative TB

		Culture positive		
		ZN positive	ZN negative	P
N=731		353 (48%)	378 (52%)	
Age (years) Means	All	31 (10) [15-92]	35 (12) [15-90]	0.001
	Male	33 (10) [19-92]	36 (11) [15-70]	0.001
	Female	29 (9) [15-70]	34 (13) [15-90]	0.001
Sex: M:F (%male)		229:124 (65%)	208:170 (55%)	
% among groups	Males	52%	48%	
	Females	42%	58%	0.003
Activities	Working	231 (65%)	257 (68%)	
	Not working	28 (8%)	31 (8%)	
	Student	56 (16%)	46 (12%)	
	Housewife	38 (11%)	44 (12%)	0.4
Education	None	36 (10%)	48 (13%)	
	Primary	79 (22%)	67 (18%)	
	Secondary	166 (47%)	196 (52%)	
	Tertiary	71 (20%)	67 (18%)	0.2
Persons per room*		3 (2) [1-19]	3 (2) [1-12]	0.9
Number of smokers		82 (23%)	77 (20%)	0.2
Time smoked (years)*		10 (10) [1-46]	10 (8) [1-35]	0.7
Cigarettes per day*		8 (7) [1-40]	6 (6) [1-40]	0.08
Contact with cough (3 wks)		52 (15%)	68 (18%)	0.5
	Duration*	5 (8) [1-44]	6 (7) [1-33]	0.4
Contact with fever (3wks)		26 (7%)	27 (7%)	0.9
Contact with PTB (2 yrs)		64 (18%)	53 (14%)	0.003
	Duration*	5 (5) [1-25]	5 (5) [1-20]	0.8

*Mean (SD) [Range]

Table 9.3.2 Logistic regression analysis for characteristic variables with p values < 0.20

Characteristics of patients	P	OR	P1	P2	P3	AOR
Sex	0.003	1.5 (1.0-2.0)	0.55		NS	
Number of smokers	0.20		0.12	0.60	NS	
Contact with PTB	0.003		0.01	0.01	0.01	1.4 (1.1-1.7)

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1-P3 = p value after running the backward logistic regression

9.3.2 Clinical symptoms of patients with smear-positive and smear-negative TB

A lower proportion of smear-positive patients (227, 64%) had received BCG vaccinations in the past than patients with smear-negative TB (269, 71%, P= 0.04). The mean (SD) number of years since BCG vaccination was longer in smear-negative patients (P= 0.002) as shown in table 9.3.2.

As expected nearly all patients with smear-positive and smear-negative TB had cough. However, the duration of the cough was significantly longer in smear-positive patients (P= 0.001) (table 9.3.2). Patients with smear-positive TB also complained more frequently of fever, dyspnoea, chest pains, weight loss, night sweats and anorexia than patients with smear-negative TB (P= 0.001 for all). Smear-positive patients also had longer duration of cough, fever, breathlessness, loss of weight and night sweats. Enlarged cervical lymph nodes, normally associated with extra-pulmonary TB, were more frequent in smear-negative patients (14, 4%), than in those with smear-positive TB (9, 3%), but this was not statistically significant. Similarly, more smear-positive patients had finger clubbing (6, 2% and 2, 0.5% respectively) but this was not statistically significant.

Table: 9.3.3 Clinical symptoms of patients with smear-positive and smear-negative TB

	ZN Smear			
Clinical Symptoms	**Positive**	**Negative**	**OR (95%CI)**	**P value**
N	353 (48%)	378 (52%)		
BCG at birth	227 (64%)	269 (71%)		0.04
Weeks since BCG*	32 (10) [2-82]	35 (12) [14-90]		0.002
Cough	352 (99.7%)	376 (99.5%)	1.9 (0.2-20.7)	0.5
Mean duration*	15 (15) [1-80]	12 (13) [1-52]		0.001
Unexplained fever	241 (69%)	228 (60%)	1.4 (1.1-1.9)	0.01
Mean duration*	13 (17) [1-52]	10 (15) [1-52]		0.001
Cough with bloody sputum	84 (24%)	104 (28%)	0.8 (0.6-1.1)	0.1
Mean duration*	10 (16) [1-52]	10 (16) [1-52]		0.9
Breathlessness	246 (70%)	235 (62%)	1.4 (1-1.9)	0.01
Mean duration*	12 (17) [1-52]	9 (15) [1-52]		0.04
Chest pains	314 (89%)	293 (78%)	2.3 (1.5-3.5)	0.001
Mean duration*	12 (15) [1-52]	10 (15) [1-96]		0.1
Loss of weight	315 (89%)	277 (73%)	3 (2-4.5)	0.001
Mean duration*	12 (16) [1-52]	9 (14) [1-52]		0.02
Night sweats	261 (74%)	251 (66%)	1.5 (1-2)	0.01
Mean duration*	13 (18) [1-88]	9 (15) [1-52]		0.001
Loss of appetite	244 (69%)	221 (64%)	1,6 (1.2-2)	0.002
Mean duration*	12 (17) [1.52]	9 (15) [1-52]		0.06
cervical lymph nodes	9 (3%)	14 (4%)	0.7 (0.3-1.6)	0.2
Finger clubbing	6 (2%)	2 (.5%)	3.3 (0.7-16)	0.1

Mean (SD) [Range] * duration in weeks for all symptoms

All the clinical symptoms with p value ≤ 0.20 were entered into a logistic analysis (table 9.3.4). Only chest pains was positively associated with smear-positive TB (AOR= 1.7, 95%CI, = 1.2-2.4, P= 0.002).

Table 9.3.4 Logistic regression analysis for clinical symptoms variables with p values < 0.20

Clinical symptoms	P	OR	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	AOR
Fever	0.01	1.4 (1.1-1.9)	0.58	0.58	0.59	0.64	0.65	0.67				NS	
Hemoptysis	0.1	0.8 (0.6-1.1)	0.78	0.77	0.76							NS	
Breathlessness	0.01	1.4 (1.0-1.9)	0.97									NS	
Chest pains	0.001	2.3 (1.5-3.5)	0.006	0.005	0.005	0.004	0.004	0.004	0.004	0.004	0.006	0.002	1.7 (1.2-2.4)
Weight loss	0.001	3.0 (2.0-4.5)	0.05	0.04	0.04	0.04	0.04	0.02	0.02	0.03	0.06	NS	
Night sweats	0.01	1.5 (1.0-2.0)	0.25	0.25	0.25	0.24	0.24	0.26	0.16	0.16		NS	
Anorexia	0.002	1.6 (1.2-2.0)	0.69	0.68	0.68	0.66	0.66					NS	
Enlarged lymph nodes	0.2	0.7 (0.3-1.6)	0.87	0.87								NS	
Finger clubbing	0.1	3.3 (0.7-16)	0.60	0.61	0.56	0.71						NS	
BCG	0.04		0.56	0.56	0.57	0.54	0.55	0.57	0.61			NS	

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1 to P10 = p values after running the backward logistic regression

9.3.3 Clinical signs of patients with smear-positive and smear-negative TB

Patients with smear-positive and smear-negative TB had fever and BCG scar with the same frequencies as shown in table 9.3.3, and the mean (SD) BCG scar size was not different in the two groups. Patients with smear-positive TB had significantly lower BMI (P= 0.001) and the proportion of smear-positive patients with BMI < 18.5 was higher (P= 0.001). The mean (SD) Karnofsky score was also lower in smear-positive patients, but this was was not statistically significant (table 9.3.3). However a higher proportion of smear-positive patients had Kanofsky scores < 60 (P= 0.009).

Most of the clinical signs (wheeze, bronchial breath sounds and crepitations) were more frequent in smear-positive-patients (P< 0.01 for all) as shown in table 9.3.5.

Table: 9.3.5 Clinical signs of patients with smear-positive and smear-negative TB

	ZN Smear			
Clinical Signs	**ZN positive**	**ZN negative**	**OR**	**P value**
Mean Temperature	36.9 (1) [34-40]	36.8 (1) [35-39.5]		0.2
Mean BCG size	1.1 (1.3) [0-16]	1.0 (1.1) [0-5]		0.8
Mean BMI	20 (4) [11-36]	22 (5) [12-38]		0.001
< 18.5	120 (34%)	87 (23%)		
≥ 18.5	232 (66%)	291 (77%)	1.7 (1.2-2.4)	0.001
Karnofsky score	59 (11) [20-100]	60 (13) [20-100]		0.4
< 60	238 (67%)	223 (59%)		
≥ 60	115 (33%)	155 (41 %)	1.4 (1.1-1.9)	0.009
Wheeze	87 (25%)	86 (23%)	1.1 (0.8-1.6)	0.3
Bronchial sounds	184 (53%)	142 (38%)	1.8 (1.4-2.5)	0.001
Rhonchi	140 (40%)	115 (31%)	1.5 (1.1-2.1)	0.004
Crepitations	208 (60%)	172 (46%)	1.8 (1.3-2.4)	0.001

Mean (SD) [Range]

All clinical signs among smear-positive TB with p values ≤ 0.20 were entered into logistic regression to find factors positively associated with smear-positive TB. Only rhonchi was found to be positively associated (AOR= 1.3, 95%CI = 1.0-1.7, P= 0.03).

Table 9.3.6 Logistic regression analysis for clinical signs variables with p < 0.20

Clinical signs	P	OR	P1	P2	P3	P4	P5	AOR
BMI	0.001	1.7 (1.2-2.4)	0.21	0.21	0.21		NS	
Karnofsky score	0.009	1.4 (1.1-1.9)	0.52	0.51			NS	
Bronchial sounds	0.001	1.8 (1.4-2.5)	0.77				NS	
Crepitations	0.001	1.8 (1.3-2.4)	0.17	0.16	0.19	0.20	NS	
Rhonchi	0.004	1.5 (1.1-2.1)	0.10	0.08	0.09	0.06	0.03	1.3 (1.0-1.7)

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1 to P5 = p values after running the backward logistic regression

9.3.4 Laboratory results of patients with smear-positive and smear-negative TB

Smear-positive patients had significantly lower mean (SD) haemoglobin (11 (2.2) gm/dl) compared with smear-negative patients (11.7 (2.4) gm/dl, P= 0.001). The proportion of patients with anaemia defined as an Hb < 11 gm/dl was significantly higher in smear-positive patients (148, 46% and 116, 35% respectively, P= 0.003) as shown in table 9.3.4. The mean (SD) WBC of smear-positive patients was 8.7 (3.4) x 10^9/L compared to 7.5 (4.1) x 10^9/L for smear-negative patients (P= 0.001), while the mean (SD) granulocyte percentage was 63% (13%) and 58% (13%) for smear-positive and smear-negative patients respectively (P= 0.001). In both instances, the proportion of patients with values above normal was significantly higher among smear-positive patients (P= 0.001 for both). The mean (SD) lymphocytes for smear-positive patients (37% (13%) was significantly lower than that of smear-negative patients (42% (13%),

P= 0.001). On the other hand, a higher proportion of smear-negative patients (129, 39%)) had levels of lymphocytes above normal (>45%) than smear-positive patients (75, 23%, P= 0.001).

The mean (SD) ESR was significantly higher in smear-positive than in smear-negative patients, (87 (40) mm/hr compared to 70 (47) mm/hr, P= 0.001) respectively. Similarly a higher proportion of smear-positive patients (311, 98%) had values above-normal (> 7 mm/hr) than smear-negative patients (297, 91%, P= 0.005) (table 9.3.4).

Regarding liver function parameters, the mean (SD) and the proportion of patients with abnormal alkaline phosphatase levels was significantly higher for smear-positive than for smear-negative patients, (P= 0.001). In addition, the mean serum albumin was significantly lower in smear-positive patients (3.2 (0.8) g/dl versus 3.6 (0.9) g/dl, P= 0.001), with more smear-positive patients having albumin levels below normal (P= 0.001). The differences in the mean (SD) and proportion of liver transaminases (SGOT and SGPT) above normal between the groups were not statistically significant. Finally, smear-negative patients (177, 60%) were significantly more likely to be HIV seropositive than smear-positive patients, (158, 47%, P= 0.001).

All laboratory results with p values $\leq$ 0.20 were entered into a logistic regression. Patients with smear-positive TB were more likely to have lymphocytosis (AOR = 1.3, 95%CI = 1.0-1.7, P= 0.05) and hypoalbuminuria (AOR = 1.6, 95%CI = 1.2-2.1, P= 0.001) and less likely to have raised alkaline phosphatase (AOR = 0.7, 95%CI = 0.5-1.0, P= 0.04) as shown in table 9.3.8.

Table: 9.3.7 Laboratory results in smear-positive and smear-negative TB patients

	ZN Smear		
Laboratory test	**positive**	**negative**	**P value**
Haemoglobin level	11 (2.2) [4.8-16.8]	11.7 (2.4) 5.7-16.6]	0.001
< 11 gm/dl	148 (46%)	116 (35%)	
≥ 11 gm/dl	173 (54%)	215 (65%)	0.002
White blood count	8.7 (3.4) [3.4-19.1]	7.5 (4.1) [3.3-17.2]	0.001
< 10 x 10^9	220 (69%)	271 (82%)	
≥ 10 x 10^9	100 (31%)	58 (18%)	0.001
Granulocytes	63 (13) [9-92]	58 (13) [6-92]	0.001
< 65%	167 (52%)	234 (71%)	
≥ 65%	154 (48%)	97 (29%)	0.001
Lymphocytes	37 (13) [8-91]	42 (13) [8-94]	0.001
< 45%	224 (76%)	192 (58%)	
≥ 45%	77 (24%)	139 (42%)	
ESR	87 (40) [2-160]	70 (47) [1-167]	0.001
< 7 mm/hr	6 (2%)	25 (8%)	
≥ 7 mm/hr	312 (98%)	302 (92%)	0.001
Total bilirubin	0.4 (0.4) [0-3]	0.5 (0.5) [0.1-6.3]	0.008
< 1.0 mg/dl	319 (95%)	312 (92%)	
≥1.0 mg/dl	16 (5%)	27 (8%)	0.05
Conjugated bilirubin	0.17 (0.20) [0.01-2]	0.21 (0.3) [0.01-4.5]	0.02
< 0.2 mg/dl	228 (68%)	199 (58%)	
≥ 0.2 mg/dl	108 (32%)	141 (42%)	0.006
Serum albumin	3.2 (0.8) [1.5-6.9]	3.6 (0.9) [1.2-5.9]	0.001
< 3.8 g/dl	270 (80%)	191 (56%)	
≥ 3.8 g/dl	67 (20%)	149 (44%)	0.001
SGOT	7 (6) [1-70]	7 (6) [1-58]	0.5
< 12 U/L	303 (90%)	302 (89%)	
≥ 12 U/L	33 (10%)	39 (11%)	0.3
SGPT	12 (9) [2-90]	13 (12) [2-150]	0.8
0-12 U/L	218 (65%)	226 (67%)	
> 12 U/L	118 (35%)	113 (33%)	0.3
Alkaline phosphatase	240 (153) [4-940]	202 (118) [20-937]	0.001
< 279 U/L	252 (75%)	283 (83%)	
≥ 279 U/L	84 (25%)	57 (17%)	0.005
HIV positive	158 (47%)	171 (60%)	0.001

Mean (SD) [Range]

Table 9.3.8 Logistic regression analysis for laboratory variables with p values < 0.20

Laboratory results		P	OR	P1	P2	P3	P4	P5	P6	P7	AOR
Haemoglobin	≤ 11	0.002	1.6 (0.2-2.2)	0.82	0.83	0.90				NS	
WBC	≤ 10 x 10^9	0.001	0.5 (0.3-0.7)	0.68	0.65	0.61	0.62	0.59		NS	
Granulocytes	≤ 65%	0.001	0.4 (0.3-0.6)	0.64	0.62	0.52	0.55	0.57	0.68	NS	
Lymphocytes	≤ 45%	0.001	2.3 (1.6-3.2)	0.12	0.13	0.14	0.14	0.13	0.15	0.05	1.3 (1.0-1.7)
ESR	≤ 7mm/hr	0.001	0.3 (0.2-0.8)	0.84	0.86					NS	
Total bilirubin	≤ 0.20U/L	0.05	1.7 (0.9-3.3)	0.93						NS	
Conjugated bilirubin	≤ .20U/L	0.006	1.5 (1.1-2.0)	0.58	0.53	0.62	0.63			NS	
Albumin	≤ 3.8g/dl	0.001	3.1 (2.3-4.4)	0.005	0.004	0.003	0.002	0.002	0.002	0.001	1.6 (1.2-2.1)
Alkaline phosphatase	≤ 279U/L	0.004	0.6 (0.4-0.9)	0.05	0.05	0.04	0.03	0.04	0.004	0.04	0.7 (0.5-1.0)

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1 to P7 = p values after running the backward logistic regression

9.3.5 Radiological status of smear-positive patients

For logistic reasons, only the X-rays of smear-positive patients were systematically reviewed. A large proportion of smear-negative patients underwent X-ray examinations but their X-ray films were not available to the principal investigator. The X-ray findings of patients with smear-positive TB are described here for completion.

Two hundred and seventy-six (78%) of the 353 smear positive patients enrolled had their chest X-rays reviewed for the presence of cavities. Of these 276 patients, 48 (17%) had no cavities (grade 0), 59 (21%) had cavities graded 1 (total cavity diameter < 2 cm), 114 (41%) were graded as 2 (total cavity diameter 2-4 cm) and 55 (20%) were graded as 3 (total cavity diameter > 4 cm) as shown in table 9.4.5.

Two hundred and seventy-eight smear positive patients had their X-rays reviewed for the extent of diseased lungs. Of these 4 (1%) had no diseased lungs and were graded as 0. Twenty-seven (10%) were graded as 1 (1 lung zone involved), 65 (23%) were graded as 2 (2 lung zones involved), 72 (26%) were graded as 3 (3 lung zones involved), 64 (23%) were graded 4 (4 lung zones involved), 32 (12%) were graded 5 (5 lung zones involved) and 14 (5%) were graded 6 (all lung zones involved) (table 9.4.6).

Table: 9.3.9 Radiological results of smear-positive patients showing gradings for extent of disease lungs and cavities

	Smear Positive	
grades	Extent of disease	Cavities
0	4 (1%)	48(17%)
1	27 (10%)	59 (21%)
2	65 (23%)	114 (41%)
3	72 (26%)	55 (20%)
4	64 (23%)	
5	32 (12%)	
6	14 (5%)	

9.4.1 Clinical symptoms of patients with smear-positive and smear-negative TB by HIV status

Six hundred and twenty-five (85.5%) out of the 731 patients with positive culture TB were tested for HIV I. Of these, 340 (96%) out of the 353 smear-positive patients and 285 (75%) out of the 378 smear-negative patients were tested. Smear-negative patients were more likely to be infected with HIV as 158 (47%) of the 340 smear-positive patients and 171 (60%) of the 285 smear-negative patients were HIV positive (P= 0.001).

No statistical differences were observed between the proportion of patients who were HIV positive among those who had BCG, in both smear-positive and smear-negative patients. Neither were there differences in the mean (SD) duration since having taken BCG (table 9.4.1).

The clinical symptoms on enrolment of smear-positive patients co-infected with HIV were similar to the clinical symptoms of smear-positive patients without HIV with the exception of haemoptysis and dyspnoea as shown in table 9.4.1. Among smear-positive patients, HIV-negative individuals were more likely to report haemoptysis (54, 30% versus 20, 18% for HIV-positive patients, P= 0.005) but HIV-positive patients were more likely to present with dyspnoea (118, 75% versus 117, 64% for HIV-negative patients, P= 0.02). HIV-positive individuals were also more likely to complain of anorexia (P< 0.01). The clinical presentation of patient with smear-negative TB co-infected with HIV was also similar to the presentation of patients without HIV infection with the exception of the presence of unexplained fever, a

history of weight loss, chest pains, having night sweats and anorexia. These five symptoms were more frequent in patients co-infected with HIV ($P< 0.05$) for all) as shown in table 9.4.1. A small proportion of patients had enlarged lymph nodes, and this symptom was also more frequent in patients with HIV co-infection.

Only haemoptysis was negatively associated with HIV in patients with smear-positive TB when all symptoms with p values ≤ 0.20 were entered into a logistic regression (AOR =0.6, 95%CI = 0.4-1.0, P= 0.04). While two factors were positively associated with HIV in smear-negative TB, namely weight loss (AOR = 1.6, 95%CI = 1.1-2.5, P= 0.02) and anorexia (AOR = 1.9, 95%CI = 1.3-2.6, P= 0.001) (table 9.4.3.

Table: 9.4.1 Clinical symptoms of patients with smear-positive and smear-negative TB by HIV status

	Smear positive TB			Smear negative TB		
HIV	**positive**	**negative**	**P value**	**positive**	**negative**	**P value**
N	158 (47%)	182 (53%)		171 (60%)	114 (40%)	0.001
BCG at birth or any other time	100 (63%)	119 (65%)	0.9	123(72%)	78 (68%)	0.3
Mean BCG taken (Years)	32 (9)[15-70]	32 (10)[2-82]	0.9	33 (9)[15-56]	36 (14)[14-70]	0.4
Cough	157 (99%)	182 (100%)	0.2	171 (100%)	113 (99%)	0.2
Mean duration*	14 (15)[1-80]	16 (16)[1-52]	0.3	12 (13)[2-52]	11 (12)[1-52]	0.6
Unexplained fever or sweating	115 (73%)	120 (66%)	0.09	113 (66%)	65 (57%)	0.06
Mean duration*	12 (17)[1-52]	14 (18)[1-52]	0.4	9 (14)[1-52]	8 (13)[2-52]	0.7
Cough with bloody sputum	28 (18%)	54 (30%)	0.005	51 (30%)	28 (25%)	0.2
Mean duration*	6 (10)[1-52]	11 (18)[1-52]	0.6	8 (13)[1-52]	10 (18)[1-52]	0.6
Breathlessness	118 (75%)	117 (64%)	0.02	111 (65%)	70 (61%)	0.3
Mean duration*	11 (17)[1-52]	13 (17)[1-52]	0.4	9 (15)[1-52]	7 (13)[1-52]	0.7
Chest pains	140 (89%)	162 (89%)	0.5	140 (82%)	83 (73%)	0.04
Mean duration*	11 (15)[1-52]	12 (15)[1-52]	0.7	9 (15)[1-96]	8 (12)[1-52]	0.6
Loss of weight	143 (91%)	162 (89%)	0.3	146 (85%)	73 (64%)	0.001
Mean duration*	11 (16)[2-52]	12 (16)[1-52]	0.5	8 (13)[1-52]	8 (14)[2-52]	0.9
Night sweats	118 (75%)	135 (74%)	0.5	127 (74%)	64 (56%)	0.001

Mean duration*	12 (17)[1-52]	13 (18)[1-88]	0.5	8 (14)[2-52]	8 (13)[2-52]	0.9
Loss of appetite	123 (78%)	115 (63%)	0.002	122 (71%)	53 (47%)	0.001
Mean duration*	11 (16)[1-52]	12 (17)[1-52]	0.5	8 (14)[1-52]	8 (14)[1-52]	0.9
Enlarged cervical lymph-nodes	5 (3%)	4 (2%)	0.3	10 (6%)	2 (2%)	0.05

* Values are mean (SD) [range] in weeks

Table 9.4.2 Logistic regression analysis for clinical symptoms variables with p values < 0.20 by HIV status in smear-positive patients

Clinical symptoms	P	AO	P1	P2	P3	P4	P5	AOR
Cough	0.20	Undefined	0.90	0.90			NS	
Fever	0.09	1.4 (0.9-2.2)	0.96				NS	
Haemoptysis	0.005	0.5 (0.3-0.9)	0.03	0.03	0.03	0.03	0.04	0.6 (0.4-1.0)
Dypsnoea	0.02	1.6 (1.0-2.6)	0.59	0.58	0.59	0.51	NS	
Anorexia	0.002	2.0 (1.3-3.3)	0.86	0.85	0.85		NS	

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1 to P5 = p values after running the backward logistic regression

Table 9.4.3 Logistic regression analysis for clinical symptoms variables with p values < 0.20 by HIV status in smear-negative patients

Clinical symptoms	P	OR	P1	P2	P3	P4	P5	P6	P7	AOR
Cough	0.20	Undefined	0.44	0.44					NS	
Fever	0.06	1.5 (0.9-2.4)	0.64						NS	
Haemoptysis	0.2	1.3 (0.8-2.2)	0.29	0.32	0.30	0.35			NS	
Chest pains	0.04	1.7 (1.0-3.0)	0.16	0.16	0.17	0.21	0.18		NS	
Weight loss	0.001	3.3 (1.9-5.8)	0.02	0.02	0.02	0.009	0.01	0.02	0.02	1.6 (1.1-2.5)
Night sweats	0.001	2.6 (1.4-3.7)	0.51	0.35	0.37				NS	
Anorexia	0.001	2.9 (1.7-4.7)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	1.9 (1.3-2.6)
Enlarged lymph nodes	0.05	3.5 (0.7-16)	0.12	0.12	0.12	0.13	0.14	0.15	NS	

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1 to P7 = p values after running the backward logistic regression

9.4.2 Clinical signs of patients with smear-positive and smear-negative TB by HIV status

Similar to the patterns observed for the clinical symptoms on admission, the clinical signs of patients with smear-positive TB co-infected with HIV were similar to patients not co-infected with HIV with the exception of the BMI, which was lower in co-infected individuals (P= 0.08). A higher proportion of co-infected patients had a BMI < 18.5 (62, 40% versus 53, 29% for patients without HIV, P=0.02). In addition smear-positive individuals co-infected with HIV were less likely to have wheezing than patients without HIV (32, 20% versus 51, 28% respectively, P= 0.04). Similar to the pattern observed in smear-positive patients, patients with smear-negative TB co-infected with HIV were more likely to have lower BMI and a higher proportion of them had BMI <18.5 (45, 26% for those co-infected with HIV versus 19, 17% for patients without HIV). Co-infected patients however were more likely to have wheeze than smear-negative patients without HIV and to have more bronchial breath sounds (P= 0.05 for both) as shown in table 9.4.2.

Of all the clinical signs of patients with smear-positive TB, none was positively associated with HIV infection when all clinical symptoms with p value ≤ 0.20 were entered into a logistic regression (table 9.4.5), while BMI was positively associated with a lower likelihood of HIV infection in smear-negative TB patients (AOR = 2.1, 95%CI = 1.5-3.1, P= 0.001).

Table: 9.4.4 Clinical signs of patients with smear-positive and smear-negative TB by HIV status

	Smear positive TB			Smear negative TB		
HIV	**positive**	**negative**	**P value**	**positive**	**negative**	**P value**
Mean Temperature	36.9 (1)[35-40.1]	36.9 (1)[34.4-39.9]	0.9	36.8 (1)[35-39.5]	36.8 (1)[35-39.2]	0.9
Mean BMI	19.4 (3)[11-32]	21 (4)[12-36]	0.008	21 (4)[13-37]	23 (4)[12-38]	0.001
< 18.5	62 (40%)	53 (29%)		45 (26%)	19 (17%)	
≥ 18.5	95 (60%)	129 (71%)	0.02	126 (74%)	95 (83%)	0.03
Mean Karnofsky score	59 (12)[20-100]	59 (11)[20-100]	0.9	58 (13)[20-100]	60 (11)[40-100]	0.2
< 60	103 (65%)	124 (68%)		109(64%)	67 (59%)	
≥ 60	55 (35%)	58 (32%)	0.3	62 (36%)	47 (41%)	0.2
Wheeze	32 (20%)	51 (28%)	0.04	47 (28%)	21 (18%)	0.04
Bronchial breath sounds	88 (56%)	87 (49%)	0.1	72 (43%)	36 (32%)	0.03
Rhonchi	69 (44%)	68 (38%)	0.1	52 (31%)	33 (29%)	0.4
Crepitations	93 (60%)	108 (61%)	0.4	80 (47%)	49 (43%)	0.3

Table 9.4.5 Logistic regression analysis for clinical signs with p values ≤ 0.20 by HIV status in smear-positive patients

Clinical signs	P	OR	P1	P2	P3	P4
BMI	0.02	1.6 (1.0-2.5)	0.40			NS
Bronchial sounds	0.10	1.3 (0.9-2.1)	0.09	0.08	0.10	NS
Rhonchi	0.10	1.3 (0.8-2.0)				NS
Wheeze	0.04	0.6 (0.4-1.1)	0.14	0.14		NS

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1 to P4 = p values after running the backward logistic regression

Table 9.4.6 Logistic regression analysis for clinical signs with p values ≤ 0.20 by HIV status in smear-negative patients

Clinical signs	P	OR	P1	P2	P3	P4	AOR
BMI	0.03	1.8 (1.0-2.5)	0.001	0.001	0.001	0.001	2.1 (1.5-3.1)
Karnofsky score	0.20	1.0 (0.6-1.6)	0.08	0.12		NS	
Bronchial sounds	0.03	1.6 (1.0-2.6)	0.33			NS	
Wheeze	0.04	1.7 (0.9-3.0)	0.11	0.11	0.16	NS	

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1 to P4 = p values after running the backward logistic regression

9.4.3 Laboratory results of smear-positive and smear-negative TB by HIV status

The laboratory results of patients with smear-positive and smear-negative TB are analysed by HIV status in table 9.4.3. Among smear-positive patients, patients co-infected with HIV had lower haemoglobin concentrations and were more likely to be anaemic than individuals without HIV. In addition the mean ESR was higher in HIV patients (mean (SD) of 95 (41) mm/hr) than in patients not co-infected with HIV (78 (37) mm/hr, P= 0.001). In contrast, the laboratory results of patients with smear-negative TB varied more frequently if they were or not co-infected with HIV as shown in table 9.4.3.

Haemoglobin and the prevalence of anaemia had a similar pattern to smear-positive patients, with 90 (56%) smear-negative patients co-infected with HIV being anaemic (Hb < 11 g/l) compared to 19 (17%) of those not co-infected with HIV. Similarly, the ESR was much higher in patients co-infected with HIV (mean of 87 mm/hr) than in those not co-infected (P= 0.001).

There were no significant differences in the mean (SD) total bilirubin for both HIV-positive and HIV-negative patients in both smear-positive and smear-negative patients but the proportion of patients with normal total bilirubin was higher in smear-negative, HIV-negative patients (P= 0.002). No significant differences were observed in both the mean (SD) and the proportion of patients with normal conjugated bilirubin in both HIV groups with smear-positive and smear-negative TB (table 9.4.3).

In both smear-positive and smear-negative patients, HIV negative patients had significantly higher mean (SD) albumin than HIV positive patients (P= 0.001), and the proportion of patients with hypo-albuminaemia was higher in HIV positive patients with HIV in both smear-positive and smear-negative patients (P= 0.006 and 0.001 respectively).

The mean (SD) SGOT and SGPT were higher in HIV positive patients in both smear-positive and smear-negative groups (table 9.4.9). Similarly, both patients with smear-positive and patients with smear-negative TB had higher SGOT and SGPT if they were co-infected with HIV. The mean (SD) alkaline phosphatase was higher in smear-negative patients co-infected with HIV (P= 0.001).

Table 9.4.7 Laboratory results of patients with smear-positive and smear-negative TB by HIV status

	Smear positive TB			Smear negative TB		
HIV	**positive**	**negative**	**P value**	**positive**	**negative**	**P value**
Haemoglobin level	10.3 (2)[5.6.16]	12 (2)[4.8-16.8]	0.001	10.9 (2)[5.7-16.4]	12.8 (2)[5.7-16.6]	0.001
< 11 gm/dl	95 (64%)	49 (29%)		79 (49%)	18 (16%)	
≥ 11 gm/dl	54 (36%)	119 (71%)	0.001	81 (51%)	92 (84%)	0.001
White blood count	8.6 (3)[3.4-19.1]	8.7 (3)[3.6-18.2]	0.9	7.7 (4)[3.3-31.8]	7.6 (4)[3.5-38]	0.9
$<10 \times 10^9$	99 (67%)	117 (70%)		125 (79%)	91 (83%)	
$\geq 10 \times 10^9$	49 (33%)	51 (30%)	0.3	34 (21%)	19 (17%)	0.2
Granulocytes	63 (13)[9-91]	63 (13)[18-92]	0.9	60 (14)[6-92]	57 (13)[9-88]	0.08
< 65%	76 (51%)	89 (53%)		108 (67%)	81 (74%)	
≥ 45%	73 (49%)	79 (47%)	0.4	52 (33%)	29 (26%)	0.1
Lymphocytes	37 (13)[9-91]	37 (12)[8-82]	0.87	41 (14)[8-94]	43 (13)[12-91]	0.1
< 45%	112 (75%)	128 (76%)		99 (62%)	61 (56%)	
≥ 45%	37 (25%)	40 (24%)	0.4	61 (38%)	49 (44%)	0.15
ESR	95 (41)[6-160]	78 (37)[2-160]	0.001	87 (43)[3-167]	48 (40)[1-150]	0.001
< 7 mm/hr	2 (1%)	5 (3%)		2 (1%)	17 (16%)	
≥ 7 mm/hr	148 (99%)	162 (97%)	0.08	156 (99%)	93 (84%)	0.001
Total bilirubin	.43 (.3)[0-2.5]	.45 (.4)[.1-3]	0.5	.52 (.6)[.1-6.3]	.52 (.4)[.1-3.3]	0.9
<1.0 mg/dl	149 (96%)	167 (95%)		154 (93%)	101 (92%)	
≥1.0 mg/dl	7 (4%)	9 (5%)	0.4	12 (7%)	9 (8%)	0.4
Conjugated bilirubin	.16 (.2)[.01-2]	.19 (.3)[.01-2]	0.09	.22 (.4)[.01-4.5]	.21 (.2)[.05-2.1]	0.3
0-0.2 mg/dl	113 (72%)	114 (64%)		95 (57%)	61 (55%)	
0.2 mg/dl	43 (28%)	63 (36%)	0.06	71 (43%)	50 (45%)	0.4
Serum albumin	2.9 (.8)[1.5-6.8]	3.4 (.8)[1.6-6.9]	0.001	3.3 (.9)[1.2-5.4]	4.0 (.7)[2.1-5.8]	0.001
< 3.8 g/dl	140 (90%)	126 (71%)		121 (73%)	39 (35%)	
≥ 3.8 g/dl	16 (10%)	51 (29%)	0.001	45 (27%)	72 (65%)	0.001
SGOT	7.2 (5)[1-27]	6.8 (7)[1-70]	0.1	8.3 (7)[2-58]	6.8 (6)[1-35]	0.02

	< 12 U/L	133 (85%)	158 (90%)		136 (82%)	100 (89%)	
	≥ 12 U/L	23 (15%)	18 (10%)	0.11	30 (18%)	12 (11%)	0.05
SGPT		13.2 (9)2-60]	11.3 (8)[2-90]	0.06	13.6 (15)[2-150]	11.8 (7)[2-37]	0.6
	< 12 U/L	76 (49%)	103 (59%)		81 (49%)	59 (53%)	
	≥ 12 U/L	80 (51%)	73 (41%)	0.04	85 (51%)	53 (47%)	0..3
Alkaline phosphatase		256 (177)[4-940]	225 (127)[74-825]	0.4	224 (141)[82-937]	173 (78)[33-613]	0.001
	< 279 U/L	114 (73%)	137 (78%)		127 (77%)	104 (93%)	
	≥ 279 U/L	42 (27%)	39 (22%)	0.16	38 (23%)	8 (7%)	0.001

All laboratory variables with p values ≤ 0.20 were entered into a logistic regression. Tables 9.4.7 and 9.4.8 show these analyses. One factor in patients with smear-positive TB and five factors in patients with smear-negative TB were independently associated with HIV. HIV positive patients with smear-positive TB were more likely to have anaemia (AOR = 4.6, 95%CI = 2.8-7.4, P= 0.001). HIV positive patients with smear-negative TB were more likely to have anaemia (AOR = 2.6, 95%CI = 1.8-3.9, P= 0.001) and hypoalbuminaemia (AOR = 3.1, 95%CI = 2.2-4.4, P= 0.001), were less likely to have lymphocytosis (AOR = 0.7, 95%CI = 0.3-0.9, P= 0.03) and to have lower ESR (AOR = 0.7, 95%CI = 0.1-0.6, P= 0.005) and SGOT (AOR = 0.5, 95%CI = 0.3-0.9, P= 0.02).

Table 9.4.8 Logistic regression analysis for HIV infection of laboratory variables with p values ≤ 0.20 in smear-positive patients

Laboratory results	P OR	P1	P2	P3	P4	P5	AOR
Haemoglobin		0.001	0.001	0.001	0.001	0.001	4.6 (2.8-7.4)
Conjugated bilirubin		0.87				NS	
Albumin		0.62	0.61	0.61		NS	
SGOT		0.20	0.20	0.18	0.26	NS	
SGPT		0.84	0.85			NS	

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1 to P5 = p values after running the backward logistic regression

Table 9.4.9 Logistic regression analysis for laboratory variables with p values < 0.20 by HIV status in smear-negative patients

Laboratory results	P OR	P1	P2	P3	P4	AOR
Haemoglobin		0.001	0.001	0.001	0.001	2.6 (1.8-3.9)
Lymphocytes		0.03	0.03	0.03	0.03	0.7 (0.3-0.9)
ESR		0.007	0.007	0.005	0.005	0.2 (0.1-0.6)
Total bilirubin		0.04	0.04	0.06	NS	
Albumin		0.001	0.001	0.001	0.001	3.1 (2.1-4.4)
SGOT		0.02	0.01	0.009	0.02	0.5 (0.3-0.9)
SGPT		0.52			NS	
Alkaline phosphatase		0.11	0.10		NS	

OR = Odds ratio, AOR =Adjusted odds ratio, NS = Not significant, P1 to P4 = p values after running the backward logistic regression

9.4.4 Radiological results of smear-positive patients by their HIV status

Only smear positive patients had chest X-rays read as previously described. Patients without HIV had higher cavity grades than HIV positive patients whereas more HIV positive patients had chest X-rays without abnormalities. There was no trend in the extent of disease grades for HIV positive and negative patients as shown in table 9.5.

Table 9.5 Radiology results for smear positive HIV positive and HIV negative patients scored 1-6 for extent of diseased lungs and 1-3 for cavities

	Smear positive			
	Diseased lungs		Cavities	
Scores	HIV+	HIV-	HIV+	HIV-
0	3 (2%)	0 (0)	27 (22%)	19 (13%)
1	14 (11%)	13 (9%)	27 (22%)	30 (20%)
2	29 (23%)	33 (22%)	48 (39%)	66 (45%)
3	26 (21%)	46 (31%	22 (18%)	33 (22%)
4	30 (24%)	34 (23%)		
5	18 (14%)	14 (9)		
6	5 (4%)	9 (6%)		
Total	125 (46%)	149 (54%)	124 (46%)	148 (54%)

9.5 Discussion

Clinicians are often faced with the delima of empirically treating patients with sputum-smear negative TB and these patients are often missed in the absence of culture facilities. Thus, they are more likely to rely on the same criteria used for the diagnosis of smear-positive TB, such as weight loss and chronic cough, to predict the risk of patients with negative-smear microscopy having TB. However, smear-negative patients, due to their low bacilli load, may have different clinical and radiological findings. This chapter therefore attempts to demonstrate the clinical differences between patients with smear-positive and smear-negative TB and considers the effect that HIV infection has on their clinical presentation.

Our study observed that among culture positive patients, less patients had smear-positive than smear-negative TB. This observation is comparable to other studies in high HIV prevalence areas (Alausa et al., 1977; Levy et al., 1989; Narain et al., 1971; Kim et al., 1984). Patients with smear-positive TB were more likely to be younger than patients with smear-negative TB and more likely to be male, although gender was not independently predictive after multivariate analysis. Samb et al., reported a similar age difference in smear-positive patients and that more men were diagnosed with smear-positive TB in Dakar, Senegal (Samb et al., 1999). Similar to our study, gender was not statistically significant. Tsao et al., also reported that smear-negative patients were older in a University Hospital study in Taiwan (Tsao et al., 2004).

Smear-positive patients with TB are more likely to have been in contact with patients with PTB compared to smear-negative patients. It is well established that increased

closeness of contact to a PTB patient is associated with increased risk of infection. A retrospective study of case records in Canada demonstrated that household contacts of smear-positive PTB had the highest proportion of positive TST in all age groups (Grzybowski et al., 1975). A tuberculin survey of over 3000 patients of all ages in Edinburgh, showed the level of infection was higher in known contacts of TB than in non-contacts and in particularly the household contacts (Loudon et al., 1958). There is however limited information on whether contacts of patients with smear-positive TB are also more likely to be smear-positive.

As expected, our clinical findings demonstrate that patients who are smear-negative but culture-positive are more likely to have milder symptoms and signs than patients who are smear-positive. Several studies have described the clinical presentation and risk factors of persons with AFB smear-positive sputum, who are the most infectious group of TB patients. Our findings support the results from other studies (Kobashi et al., 1995; Tsao et al., 2004) where smear-negative patients were significantly more likely to have milder symptoms of fever, dyspnoea, chest pains, loss of weight, night sweats and anorexia with shorter duration of symptoms. Our study however has shown that most of these factors are associated with each other and only one factor (chest pains) was independently associated with PTB after confounding. Clinical differences between HIV-positive and HIV-negative in patients with smear-positive and smear-negative TB were minimal as clinical features of HIV-associated PTB are frequently atypical, resembling those of primary TB (Raviglione et al., 1992). As expected, we observed patients with dual infection of TB and HIV more likely to complain of fever than patients with TB alone but this was not predictive when entered into multivariate analysis. Smear-positive patients without HIV were observed

to have a greater proportion of patients with haemoptysis than those co-infected with HIV. This might be because smear-positive patients without HIV infection are more likely to have extensive chest pathology with cavitation compared to smear-positive patients with HIV. Weight loss in smear-positive patients with and without HIV was not signifcantlly different, unlike in smear-negative TB patients where patients with HIV had significantly greater weight loss than those without HIV. As expected, anorexia was more prominent in patients dually infected with TB and HIV in the two smear groups. One of the criteria for admission into this study was cough of 3 weeks or more. This might be responsible for the absence of significant differences between smear-positive and smear-negative patients as reported in other studies (Samb et al., 1999; El-Sony et al., 2003) where the proportion of patients with cough was lower in smear-negative patients.

Our study showed an increase association between BCG and smear microscopy, as smear-negative patients were significantly more frequently vaccinated than smear-positive patients. The protective value of BCG has been questioned in areas with large burden of TB and HIV. Our study here suggest that milder clinical manifestations of smear-negative patients might be due to the protective effect of BCG. However, this association could have other explanations given the cross-sectional design of the study. To confirm this explanation, there will be need for prospective studies.

In our study, clinical signs such as bronchial breath sound, rhonchi, and crepitations occurred with greater frequency in smear-positive patients than in smear-negative-patients, though only rhonchi was independently associated with TB. Samb et al., and El-Sony et al., however did not observe any significant differences in the lung

findings (Samb et al., 1999; El-Sony et al., 2003). An interesting finding is the significant difference observed in the Karnofsky score which showed greater proportion of smear-negative patients with higher Kanofsky score of > 60 in univariate analysis compared to smear-positive patients. Even though this was expected, since smear-negative patients have milder disease, Kanofsky score had not in the past been used in many TB studies. Similarly, given the greater clinical severity of smear-positive patients, they were more malnourished compared to smear-negative TB patients. This was equally observed in other studies (Samb et al., 1999).

Not many reports have analysed biochemical differences between smear-positive and smear-negative TB. This may be because the information collected on biochemical markers gives limited value added to the diagnostic process and because routine laboratory tests are expensive. We observed that smear-positive patients were more anaemic, had lower levels of granulocytosis and higher frequencies of raised lymphocytocis and ESR. Smear-positive patients had lower levels of albumin compared to smear-negative patients, showing severity of infection in the smear-positive patients. Smear-positive patients were more likely to have higher values for total and conjugated bilirubin but lower alkaline phosphatase.

Our results support the hypothesis that smear-negative patients are more likely than smear-positive-patients to have a larger proportion of HIV seropositive cases. Our findings in Abuja associating smear-negative PTB patients with HIV more often than in smear-positive patients has been observed in previous studies in Malawi (Hargreaves et al., 2001a), Darkar, Senegal (Samb et al., 1999), Nairobi, Kenya

(Aktogu et al., 1996), Lusaka, Zambia (Elliott et al., 1993a) and South Africa (Karstaedt et al., 1998).

Our radiological findings were similar to those seen in many other studies in TB patients with and without HIV (Raviglione et al., 1992; Colebunders et al., 1989; Elliott et al., 1993d; Hira et al., 1998). HIV-positive patients compared to HIV-negative patients were observed to have fewer or no cavities on chest X-rays in TB patients, whereas there were no differences seen in the extent of diseased lungs.

Our study should provide useful information to help guide clinicians in differentiating patients with smear-positive TB from those with smear-negative TB. Our findings to some extent can guide clinical practice in centres where diagnostic facilities are inadiquate.

CHAPTER TEN

Zinc and Vitamin A as co-adjuvant for the treatment of PTB

10.1 Introduction

Malnutrition has a well-established relationship with infection (Scrimshaw 1995) and the association between infectious diseases and the host's nutritional status has generally shown increased severity and susceptibility to the infecting agent with poor host nutrition (Beck et al., 2000). Nutritional deficiencies are commonly associated with impaired immune responses, particularly cell-mediated immunity (CMI), phagocyte function, cytokine production, secretory antibody response and affinity, and the complement system (Chandra et al., 1994b; 1994a). In addition to protein and energy malnutrition, field and laboratory studies have provided evidence that micronutrient deficiencies also contribute to the morbidity and mortality of infectious diseases. Several studies examining the effect of infectious diseases on nutrition at the time of diagnosis have found micronutrient levels to be lower in infected than in non-infected patients (Karyadi et al., 2000; Guerrant et al., 2000; Onwubalili et al., 1988). Recognition of micronutrient deficiencies in infectious and non-infectious illnesses has led to large-scale supplementation, epidemiological and clinical intervention studies (Christian et al., 2003).

The link between malnutrition and TB had long been recognised. The Greeks gave the name "Phthisis", meaning literally to waste away, to TB and in the 18^{th} and 19^{th} centuries, it became known as "consumption". Epidemiological, clinical and

experimental studies have established a strong relationship between micronutrient deficiencies and TB (Onwubalili et al., 1988; Saha et al., 1989). Cross-sectional studies have shown patients with TB to suffer from micronutrient deficiencies such as vitamins A (van Lettow et al., 2004a; Evans et al., 1971; Smurova et al., 1969), B_6 (Miasnikov 1969), thiamine (Arkhipova 1975) and folate (Markkanen et al., 1967) among others. In Indonesia, patients with TB had significantly lower plasma zinc concentrations than patients without TB (Karyadi et al., 2000). In a further case control study in Miami, patients with TB were more likely to have lower plasma selenium concentrations than healthy controls (van Lettow et al., 2004a; Shor-Posner et al., 2002; Van Lettow et al., 2004).

Zinc is now recognised as an important element in a variety of metallo-enzyme systems and biochemical pathways essential for protein synthesis, the metabolism of carbohydrate, fats and proteins and many physiological pathways (Prasad et al., 1971b; Pories et al., 1967). Low plasma levels of zinc lead to impaired immune function, as such affect host defences and predispose to increases in infection and morbidity rates (Wellinghausen et al., 1997; Lesourd 1997). As zinc becomes suboptimal, its impact on the immune system is rapid and extensive, being far greater than its impact on other tissues and organs. Conversely, short periods of zinc supplementation substantially improve immune defence in individuals with infectious diseases by preventing the dismantling of the immune system (Fraker et al., 2000). Zinc pivotally influences the actions of hundreds of enzymes, stabilizes cell membranes, modulates humoral and CMI and is increasingly recognized to be responsible for the control of apoptosis and oxidative capacity (Thurnham et al., 2000). Zinc deficiency decreases the functions of T-helper 1 (Th1) cells resulting in

low production of interferon-$_\gamma$ (IFN-$_\gamma$), interleukin (IL-2) and tumour necrosis factor-α (TNF-α), (Beck et al., 1997; Prasad et al., 2000) The Th2 cell products, IL-4, IL-6 and IL-10 are unaffected (Prasad et al., 2000). This imbalance between Th1 and Th2 cell cytokines and the decreased proportion of cytotoxic T-lymphocyte cells may account for the decreased CMI, and increased susceptibility to infection. Zinc supplementation directly induce cytokine production, predominantly IL-1, IL-6, and TNF-α, by monocular cells in vitro (Driessen et al., 1994) and addition of zinc in vitro to natural killer (NK) cells appeared to enhance their activities (Ventura et al., 1986).

Zinc is one of the most closely studied micronutrients. Its deficiency was first linked to a syndrome in the Middle East in 1963, associated with severe anaemia, low iron, growth retardation and lack of secondary sexual characteristics in patients suffering from pneumonia and parasitic diseases (Prasad et al., 1963). Several studies have since shown that zinc deficiency is common in most infections, in the elderly and in children. It has been extensively studied concerning its role in wound healing, acute respiratory infections and diarrhoea in children, its effect in the immune system, cardiovascular diseases and many other medical conditions. Recent studies have demonstrated that zinc supplementation can reduce morbidity and mortality and can shorten recovery from common infectious diseases. Patients with diarrhoea, especially children, have low zinc concentrations at the time of diagnosis (Bitarakwate et al., 2003; Chaudhary et al., 1996). Low zinc concentrations in asymptomatic children are associated with a higher incidence and severity of diarrhoea (Bahl et al., 1998). Clinical trials in Asia, Africa and Latin America have consistently demonstrated that supplementation of patients with zinc reduces the incidence and severity of diarrhoea (Bhutta et al., 2000; Dutta et al., 2000; Bhandari et al., 2002a; Strand et al., 2002;

Penny et al., 1999; Alarcon et al., 2004). Albert et al., (Albert et al., 2003) reported that supplementation with zinc, improved vibrocidal antibody concentration in children given an oral cholera vaccine.

Zinc is essential for human growth and development (Walker 2004). Low zinc concentrations have been associated with dwarfism and stunted growth in growing children (Miki et al., 2002; Prasad 2001), and daily or weekly supplementation with zinc improved growth in these children and adolescents (Thu et al., 1999; Michaelsen et al., 1994; Shor-Posner et al., 2002; Castillo-Duran et al., 1994). However, dietary zinc deficiency was found unlikely to be of major overall importance for rural Gambian children's ability to thrive, and blanket zinc supplementation was found not to be justified (Bates et al., 1993). In India, children with low zinc concentrations at the time of enrolment to a prospective study had a higher incidence of acute respiratory infections (ARI) during follow up than children with normal zinc (Bahl et al., 1998). Studies to assess the effect of supplementation with zinc on ARI have shown that supplements reduced the incidence of severe ARI (Bhandari et al., 2002b; Sempertegui et al., 1996; Albert et al., 2003). Low zinc concentrations are also associated with acute malaria (Duggan et al., 2005). A randomised placebo-controlled trial in Papua New Guinea reported that zinc supplementation reduced the incidence of *Plasmodium falciparum* episodes and protected children against severe parasitaemia (Bhutta et al., 2000). A second study in Zambia reported reduced visits to the clinics with symptoms related to malaria in zinc-supplemented patients (Bates et al., 1993). Muller et al., (Muller et al., 2001) however reported that zinc supplementation had no effect on morbidity from falciparum malaria in children in rural West Africa.

As far back as 1970, Halstead and Smith (Halsted et al., 1970) observed a fall in plasma zinc concentration in patients with active PTB and this was confirmed by subsequent studies (Bogden et al., 1977; Ahmad et al., 1985). Investigators in India noted that children with TB had significantly lower plasma zinc levels than those without TB, irrespective of their nutritional status (Ray et al., 1998). Karyadi et al., (Karyadi et al., 2000) reported that zinc and retinol in plasma were lower in malnourished patients with TB than in well-nourished healthy controls. Regarding latent TB infection in children, Cuevas et al., (Cuevas et al., 2002) demonstrated that zinc supplementations increase the PPD induration size in children irrespective of their nutritional state. Investigators also observed improvement in plasma zinc status of TB patients during follow-up of anti-TB treatment (Ray et al., 1998; Pant et al., 1987; Ciftci et al., 2003). However, Taneja (Taneja 1990) did not observe any alteration in the levels of zinc with treatment with anti-TB drugs. A small supplementation of TB patients with anti-TB therapy and zinc in India resulted in more rapid improvement of the general conditions of the patients, significantly greater weight gain and more rapid sputum conversion when compared to those receiving anti-TB therapies alone (Pant et al., 1987). In addition, zinc supplementation of patients with PTB and pneumonia was shown to increase immune function (Abul et al., 1995). There is also limited information on the relationship between HIV and zinc in patients with TB. Van Lettow et al., (van Lettow et al., 2004a) did not find differences in the proportion of TB patients with zinc deficiency across categories of plasma HIV load. Co-infection with HIV and TB introduces another dimension to the pathophysiology of nutrition with increased energy expenditure, nutrient malabsorption, micronutrient malnutrition, and increased production of inflammatory cytokines with lipolytic and proteolytic activity (Niyongabo et al., 1994).

Vitamin A plays an important role in lymphocyte proliferation and maintaining the integrity and function of epithelial tissues (Chandra 1991; Thurnham et al., 2000). It also plays a major role in the immune status of patients, including expression of mucins and keratins, lymphopoiesis, apoptosis, cytokine expression, production of antibody, and the function of neutrophils, NK cells, macrophages and T and B-lymphocytes (Semba 1999). Vitamin A is a fat-soluble vitamin required for normal functioning of the visual system, growth and development, maintenance of epithelial cell integrity, immune function and reproduction (Chandra 1991). Vitamin A as retinol is chiefly found in dairy products, liver, eggs, butter and some fatty fish. In the form of carotene, it is found in yellow and red fruits and in green leafy vegetables. It is also present in palm oil and fortified margarines. Deficiency of vitamin A is associated with xerophthalmia, night blindness, Bitot's spot, corneal ulceration and blindness. Deficiency of vitamin A has also been linked with the severity of several infectious diseases especially in children. Patients with measles were observed to have low levels of vitamin A (Perry et al., 2004; van Lettow et al., 2004a; Gilbert et al., 2003) and supplementation with vitamin A reduces their morbidity and mortality (Carcillo 2005; Ray et al., 2004; Hussey 1997; Klein et al., 1990; Hussey et al., 1990). Vitamin A administered in large doses to Indonesian preschool children resulted in 34% reduction in mortality (Sommer et al., 1986). Other studies on vitamin A supplementation have shown similar results (West et al., 1991; Muhilal et al., 1988). In Tanzania, a 50% reduction in mortality was observed in HIV positive and negative patients who received vitamin A (Fawzi et al., 1999) although studies in India (Vijayaraghavan et al., 1990) and Sudan (Herrera et al., 1992) reported only modest or no effect on mortality. Also in a review of vitamin A supplementation and child mortality, vitamin A supplementation was associated with significant reduction in

mortality when given periodically to children at the community level (Fawzi et al., 1999).

Positive associations between vitamin A deficiency and the risk of respiratory and diarrhoea disease have been reported (Sommer et al., 1984). In most of the trials of vitamin A supplementation in children with measles, vitamin A supplementation resulted in a significant reduction in the occurrence and severity of respiratory and diarrhoeal complications, but no effect on non-measles respiratory infections (Brown et al., 2004; Kjolhede et al., 1995; Fawzi et al., 1998). Supplementation with vitamin A however was associated with substantial reductions in the rates of diarrhoea and pneumonia in developing countries (Bhutta et al., 1999). Several studies have suggested that vitamin A could play a role in potentiating resistance to malaria. Studies in animals and humans showed that vitamin A deficiency increased susceptibility to malaria and worsen its symptoms, which were easily reversed by vitamin A supplementation (Krishnan et al., 1976; Galan et al., 1990; Tabone et al., 1992). Also in a review of vitamin A supplementation and child mortality, supplementation was associated with significant reduction in mortality when given periodically to children at the community level (Fawzi et al., 1993). However, a trial in Ghana in preschool children did not find statistically significant effects of vitamin A supplementation on *P. falciparum* morbidity and mortality (Binka et al., 1995).

Several cross-sectional studies have suggested that patients with TB suffer from vitamin A deficiency (Evans et al., 1971; Smurova et al., 1969; Karyadi et al., 2000; Koyanagi et al., 2004) and these deficiencies are more severe in patients co-infected with HIV (Rwangabwoba et al., 1998; van Lettow et al., 2003; Mugusi et al., 2003).

Cod - liver oil, which is rich in vitamins A and D, was used as treatment of TB prior to the era of antibiotics. The low vitamin A levels observed in patients with TB however also returned to normal at the end of anti-TB treatment without the use of supplementation (Ramachandran et al., 2004). Not many studies are available on the efficacy of supplementation with vitamin A in patients with TB. Supplementation with vitamin A appears to enhance both T-lymphocyte and antibody responses to *M. tuberculosis* and in animal studies, reported an increase in survival among chicks infected with *M. tuberculosis* who received vitamin A supplements (Sklan et al., 1994). However, in South Africa, supplementation with vitamin A had no significant response on children's recovery from TB (Hanekom et al., 1997).

Zinc deficiency limits the bioavailability of vitamin A. Because zinc and vitamin A deficiencies often co-exist in malnourish and diseased patients, supplementation with the two elements may improve the vitamin A deficiencies in these patients as such their clinical status (Smith 1980). Data on the interaction between zinc and vitamin A in humans however are limited and some results of such studies are inconclusive. Zinc was observed to potentiate the effect of vitamin A in restoring night vision among night-blind pregnant Nepalese women with low initial zinc concentrations (Christian et al., 2001). Zinc supplementation was shown to improve dark adaptation among cirrhotic patients with abnormal dark adaptations that were not responsive to vitamin A and had low serum zinc concentrations (Morrison et al., 1978). In a randomised double-blind placebo-controlled intervention study, combining zinc and vitamin A supplementation, improved the bio-chemical indices of vitamin A nutriture in vitamin A-deficient children (Rahman et al., 2002). Supplementation with zinc and β-carotene of pregnant women was observed to be superior to β-carotene supplementation alone

in improving the vitamin A status of mothers and infants (Dijkhuizen et al., 2004). A double-blind, placebo-controlled study of vitamin A and zinc supplementation in persons with TB in Indonesia observed improvement in the effect of TB medication after 2 months of anti-TB therapy, resulted in the earlier sputum smear conversion and earlier resolution of X-ray lesion area, associated with significantly improved plasma retinol concentration (Karyadi et al., 2002). The study presented here duplicates the work of Karyadi et al., with some modifications, and investigates the role of weekly zinc supplementation with and without concomitant weekly zinc plus vitamin A supplementation on the efficacy of anti-TB treatment.

10.2 Materials and Methods

10.2.1 Aims and Objectives

To compare the efficacy of weekly zinc and weekly zinc plus weekly vitamin A supplementation as adjunct for the treatment of PTB.

10.2.2 Study design

The study was a double-blinded, placebo-controlled supplementation clinical trial where patients were allotted by block randomization into three treatment groups. The study was carried out in the 8 district government hospitals described in chapter 3 from September 2003 to June 2005. All patients >15 years with AFB in their smear microscopy were recruited into the study after written informed consent. The inclusion criteria for the study were: i) The patients had to be willing to take part in

the study; ii) They should have been newly diagnosed as having active pulmonary TB as per the WHO definition of smear positive TB; iii) The patients had to be ≥15 years old and iv) should not have a history of anti-TB treatment. Potential patients were excluded if they had moderate to severe surgery during the previous month; had a history of diabetes mellitus or severe cardiovascular, liver or renal diseas or previous treatment for TB. If they were taking corticosteroids or zinc supplements during the previous month or were pregnant, lactating, or taking oral contraceptives. Patients were also excluded if they could not attend follow ups regularly. The patients enrolled were asked not to take any other vitamins during the study.

A two-month pilot study (July to September) was set up to identify and address the problems involving the project. No micronutrients were used. The problems, which surfaced during this period were discussed during weekly meetings with all the research staff. These meetings continued up to the end of the follow up of all the patients.

10.2.3 Anti TB treatment

The DOTS programme of the hospitals provided anti-TB treatment free of charge to all smear positive-patients according to the Nigerian DOTS programme (NTBLCP 2004). All patients received the standard TB treatment approved by the Federal Ministry of Health of Nigeria. This treatment is divided into an initial intensive phase of ambulatory supervised daily admistration of drugs for 2 months for all new TB cases and 3 months for patients who require retreatment and a continuation phase of treatment of 6 months for new cases. Patients were seen on a monthly basis for drug

collection. The continuation phase for patients being re-treated is 5 months and should be supervised at least thrice weekly. For the Intensive phase patients were given Rifampicin, 450-600 mg daily; Isoniazid, 300 mg daily; Ethambutol 400 mg daily (15 mg/Kg); and Pyrazinamide, 1.5- 2 g daily. For the continuation phase, patients were given Isoniazid 600 mg daily and Ethambutol 750 mg daily. These treatments were pre-packed in blisters and were provided by international donors to the national control programme.

The DOTS coverage in Nigeria by the end of 2003 was estimated to be 60%. Its case detection rate for new sputum smear positive cases for the same year was 18% and the treatment success was 79% for new sputum smear positive patients (WHO 2005).

10.2.4 Micronutrient supplementation

In addition to the standard anti-TB drugs, patients were provided with micronutrient supplementations or placebo. Micronutrient supplements included zinc or zinc plus vitamin A. Patients were randomised in blocks to receive: a) anti-TB treatment alone (Group A); b) anti-TB treatment plus zinc (Group B); and c) anti-TB treatment plus zinc and vitamin A (Group C). Group A therefore received weekly placebos that were similar to the zinc tablet and vitamin A capsule. Group B received 90 mg elemental zinc weekly (as zinc sulphate, in a lactose matrix, in form of a tablet) plus a placebo that looked identical to vitamin A. Group C received 90 mg of elemental zinc weekly plus weekly 1500 retinol (this is equivalent to 5000IU of vitamin A, as retinyl acetate, in a capsular form). All capsules and tablets were prepared, coded and supplied from LSTM by Dr L. E. Cuevas. The capsules were indistinguishable to both researchers

and the patients. Because some patients that were lost to follow up very early in the study, a further 50 patients were added to the trial and divided into the groups using the pre-coded arrangements from LSTM.

10.2.5 Sample size

Sample size was calculated using as the main outcomes the proportion of patients who were positive by ZN smear microscopy at two, four and eight weeks after initiation of treatment as observed in the trial in Indonesia (Karyadi et al., 2002). Table 10.2.1 describes the proportion of adults reported to be sputum negative in this trial and the required sample size to replicate this difference.

Table: 10.2.1 Proportion of adults expected to be sputum negative

At week	Vit A + Zinc supplemented	Placebo	Sample size to attain 80%	Power attained if > 200/group
2	23	13%	200	80%
4	55	40%	69	> 90%
8	100	80%	50	> 99%

These results indicate that a sample size of 200 per study group would demonstrate differences $\geq$ 13% at week 2 with 80% power and 95% Confidence Interval and a power >90% by week 4. A sample size of 200 per study arm was attempted at the start of the study. However, for logistic reasons the sample size was reviewed downwards because of the difficulties involved in recruiting 600 smear positive patients for the trial within a decentralised DOTS programme. After consultation with my supervisors, it was decided that a sample size of 300, with 100 patients allocated to

each group was feasible. This sample size would still have a power > 90% to demonstrate differences of ≥ 20% expected at 8 weeks. A further 50 patients were included to compensate for patients lost to follow up. Of the 350 enrolled, 116 patients were allocated into group A, 117 into group B, and 117 into group C. Using the same outcome measure used in Karyardi et al., (Karyadi et al., 2002) between placebo and zinc groups at 2 weeks (Risk difference = 10%), 4 weeks (Risk difference =15%) and 8 weeks (Risk difference = 20%), the power attained by the study would be 44% at 2 weeks, 60% at 4 weeks and > 90% at 8 weeks.

10.2.6 Logistic considerations

The DOTS programme of the 8 participating hospitals was strengthened by the addition of 8 community health nurses. These nurses were responsible for the identification, follow up and supervision of the patients enrolled. Patients' compliance was assessed by examination of the empty drug foil or number of capsules remaining from the previous treatment, which the patients were asked to produce at each visit. Patients were given drugs on weekly bases for the first 2 months and drugs were distributed during clinic visits. After the initial 2 months, the patients were given the drugs on monthly bases for the remaining 6 months. At least one family member was asked to help in monitoring the patient's compliance. Patients were asked to swallow the micronutrients or placebo in the presence of the nurse during the first 2 months of follow up and were given the supplements together with their anti-TB drugs in subsequent months. Patients who did not come to the clinic at the time of their appointment were visited and treated at home by the nurses and requested sputum and blood samples. Patients who defaulted from taking their drugs for more than a week

were dropped from the per protocol analysis but were included in the intention to treat analysis.

10.2.7 Procedures

Patients who fulfilled the enrolment criteria were interviewed with a structured questionnaire to obtain information on their social and medical background, contact and clinical data. On each follow up, patients were asked structured questions related to the state of their health and symptoms of TB. The senior medical officer in charge of the DOTS programme, in each participating hospital, conducted clinical examinations at each visit. The initial screening consisted of 3 sputum smear examinations, collected in two days. The smears were graded according to the IUATLD, as described in chapter 3. During the follow up, sputum examinations were done on weekly bases for the first 2 months and then at 6 months. Patients' sputum samples were cultured at enrolment, 2^{nd} and at 6^{th} month on the BACTEC 960 as described in chapter 3. Ten millilitres of blood were collected from each patient at enrolment and at 1^{st}, 2^{nd}, 3^{rd} and 6^{th} month. Blood samples were examined in the Zankli Medical Centre laboratory. Blood tests included haemoglobin, white blood count, granulocytes, lymphocytes, erythrocyte sedimentation rate, total and conjugated bilirubin, serum albumin, alkaline phosphatase and liver enzymes at enrolment, 2^{nd} and 6^{th} month. Patients were given pre- and post-test counselling before blood was taken for HIV testing at enrolment and before the results of the test were explained. Part of each blood sample was centrifuged and the plasma was stored at -20°C in safelock tubes for future transportation to Liverpool for micronutrient assays. Blood for the measurement of micronutrient concentrations were taken at

enrolment, 1st, 2nd, 3rd and 6th month. Thirteen patients were randomly chosen from each group and their blood samples at enrolment, 2nd and 6th month were tested for C-reactive protein.

10.2.8 Chest X-rays

Chest X-rays were taken at enrolment and at the 2nd and 6th month. A consultant radiologist (Dr. Olatunji) from the National Hospital, in Abuja, Nigeria and a consultant chest physician (Prof. Davies) from the Cardio-thoracic Centre, of the University of Liverpool, read each film independently. The two readers met at the start of the trial and agreed on the criteria for grading the X-rays as described in chapter 9.

Patients were followed in the trial period for the first six months of therapy. Treatments with anti-TB drugs without the supplements were continued for the remaining 2 months, to complete the 8 months of the standard anti-TB drugs. After 6 months, patients were declared as being "cured", if the sputa collected at the 6th month were negative for tubercle bacilli by microscopy and treatment failure if one or more sputum samples were positive. This is the TB outcome classification used by the National TB Control Programme of Nigeria. Patients declared as treatment failure were referred to the DOTS programme for the initiation of re-treatment chemotherapy for relapses or failures (RAD) which involves the addition of 1-gram of streptomycin daily for 2 months to the initial intensive phase, which is extended to 3 months. The continuation phase involves patients being given a four drug in fixed dose combination (Rifampicin, Isoniazid, Pyrazinamide and Ethanbuthol) for 5 months.

Patients co-infected with HIV did not receive ART during the first 6 months of anti-TB treatment following the National recommendations of the HIV Control Programme, as the anti-TB treatment is based on Rifampicin. At the end of treatment, HIV positive patients were referred to the GEDE foundation where they were able to received subsidised antiretroviral drugs (http://www.gedefoundation.org). Doctors in the participating hospitals were notified of patients who at 6 months of therapy were still positive for AFB in sputum.

10.2.9 Statistical analysis

Primary outcome measures included the time to sputum clearance (proportion of bacilli cleared at 1st, 2nd, 4th, 6th weeks, 2nd and 6th months) and resolution of the lesion areas in the chest X-ray. Secondary outcome included, clinical and laboratory differences between the 3 groups.

Data on the patients' age and sex distributions, educational and occupational status, nutritional status, blood chemistry, clinical signs and symptoms and results of radiological signs at enrolment and at subsequent visits were summarised and used to assess the comparability of the patients randomly assigned to the three treatment groups. Missing values were considered as negative for the purpose of the analysis and were not replaced.

The data on efficacy was analysed as Intention-to-treat analysis. Pearson's chi-square test was used to compare categorical variables between the groups, examining each characteristic using univariate analysis. Descriptive statistics included analysis of

variance (ANOVA) to compare the means of continuous variables, where Barlett's chi-square was small (> 0.05). For continuous variables with not normally distributed, non-parametric tests were used instead. A P-value of > 0.05 was considered statistically significant.

10.3.1 Characteristics of participants in the three study groups

Three hundred and fifty patients were enrolled into the trial. One hundred and sixteen patients were allocated into the placebo group (group A), 117 into the group receiving zinc (group B) and 117 into the group receiving zinc and vitamin A (group C).

The mean (SD) age was 29 (8), 31 (9) and C, 34 (12) years for patients in groups A, B and C respectively (> 0.1). There were 73 (63%) male patients in group A, 76 (65%) in group B and 83 (71%) in group C (table 10.3.1). The employment characteristics of the patients are discribed in table 10.3.1. Similarly, there were no differences in the educational characteristics across the groups.

The mean (SD) number of people who shared a room with each patient was three in all the groups and almost a third (32% to 34%) of the participants from each group smoked cigarettes. The mean (SD) number of cigarettes smoked per day was similar in the three groups but patients in group C had smoked for a longer period (table 10.3.1).

Nineteen (36%) patients in group A, 16 (30%) in group B and 18 (34%) in group C had been in contact with patients with cough in the previous 3 weeks. The duration of

such contact was longest in group C but this was not statistically significant (table 10.3.1). Ten (35%) patients in group A, 5 (17%) in group B and 14 (48%) in group C had been in contact with patients with fever while 30 (43%) patients from group A, 20 (29%) from group B and 20 (29%) from group C had been in contact with PTB patients in the past 2 years.

Table 10.3.1 General characteristics of participants of the study groups

		Group A	Group B	Group C	P value
N=350		116	117	117	
Age (years) Mean (SD)					
	All	29 (8) [15-58]	31 (9) [15-70]	34 (12) [18-92]	0.006
	Male	30 (8) [20-58]	32 (9) [19-65	35 (13) [19-92]	0.02
	Female	27 (8) [15-50]	29 (11) [15-70]	30 (9) [18-16]	0.5
Sex: M: F (%male)		73:43 (63%)	76:41 (65%)	83:34 (71%)	0.4
Activitie	Working	66 (29%)	78 (34%)	83 (37%)	
	Not working	10 (32%)	11 (36%)	10 (32%)	
	Student	31 (51%)	15 (25%)	15 (25%)	
	Housewife	9 (29%)	13 (42%)	9 (29%)	0.4
Education	None	10 (29%)	9 (26%)	16 (46%)	
	Primary	24 (32 %)	29 (38%)	23 (30%)	
	Secondary	54 (32 %)	57 (34%)	58 (34%)	
	Tertiary	28 (40%)	22 (31%)	20 (29%)	0.6
Person/ room*		3 (2) [1-10]	3 (2) [1-19]	3 (2) [1-10]	0.8
Number of smokers		30 (33%)	31 (34%)	29 (32 %)	0.9
Time smoked (years)*		9 (11) [1-46]	10 (7) [1-27]	12 (12) [1-43]	0.3
Cigarettes per day*		8 (6) [2-30]	8 (8) [1-40]	8 (8) [1-30]	0.9
Contact with cough		19 (36%)	16 (30%)	18 (34%)	0.8
Duration (weeks)*		5 (5) [1-20]	3 (5) [1-20]	7 (11) [1-44]	0.6
Contact with fever		10 (35%)	5 (17%)	14 (48%)	0.1
Contact with PTB		30 (43%)	20 (29%)	20 (29%)	0.4
Duration (years)*		6 (6) [1-25]	4 (3) [1-16]	4 (3) [1-12]	0.4

*Mean (SD) [range]

10.3.2 Number of patients at enrolment and follow-up by treatment group

Table 10.3.2 and figure 10.3.1 show the number of patients at enrolment and the number followed up in the three treatment groups, the number of patients who were lost or died. Figure 10.3.1 describes their HIV status at 6 months. Patients lost to follow up include those who stopped without any obvious reason, those who relocated from Abuja and those who became pregnant or had major surgery and withdrew from the trial.

Of the 116 patients enrolled in group A, 117 in group B and 117 in group C, 110 (95%) in group A, 94 (80%) in group B and 91 (78%) in group C were followed at the 2^{nd} month. Fourteen (12%) and 1 (1%) of the patients in group A, 15 (13%) and 8 (7%) in group B and 21 (18%) and 5 (4%) in group C respectively were lost to follow up or had died by the 2^{nd} month.

At 6 months, 91 (78%) patients in group A, 89 (76%) in group B and 81 (69%) in group C were left at the end of the trial. Twenty-four (21%) patients in group A, 19 (16%) in group B and 27 (23%) in group C were lost to follow up. One (1%) patient in group A, 9 (8%) in each of groups B and C had died by the 6^{th} month of follow up period (P= 0.01).

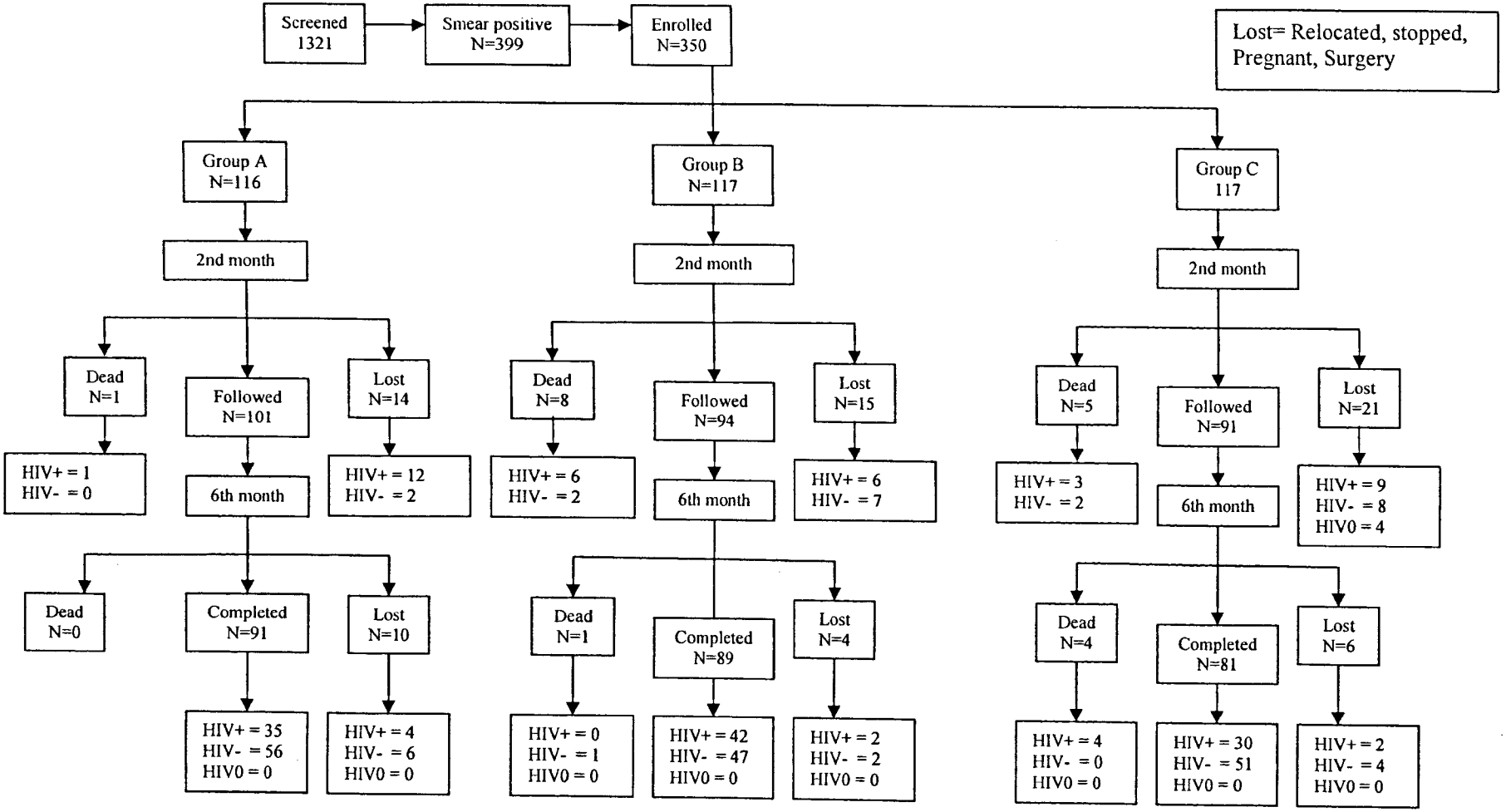
The HIV status of patients left in the study, those lost to follow up and those who had died at 2^{nd} and 6^{th} months in the three groups are shown in figure 10.3.1.

Two hundred and fourteen (61%) of the 350 patients enrolled attended the required 8 follow up visits, 53 (15%) attended 7, 25 (7%) attended 6, 16 (5%) attended 5, 9 (3%) attended 4 and 33 (9%) attended less than 4 visits.

Table 10.3.2 Number of patients at enrolment and follow-ups

Follow up visits	Micronutrient groups	Number per follow up	Number lost	Number dead	Total
Enrolment	A	116 (100%)			116
	B	117 (100%)			117
	C	117 (100%)			117
1st week	A	110 (95%)	6 (5%)	0 (0)	116
	B	111 (95%)	1 (1%)	5 (4%)	117
	C	114 (97%)	2 (2%)	1 (1%)	117
2nd week	A	109 (94%)	7 (6%)	0 (0)	116
	B	106 (91%)	5 (4%)	6 (5%)	117
	C	110 (94%)	6 (5%)	1 (1%)	117
4th week	A	98 (84%)	17 (15%)	1 (1%)	116
	B	101 (86%)	8 (7%)	8 (7%)	117
	C	105 (90%)	10 (8%)	2 (2%)	117
6th week	A	94 (81%)	21 (18%)	1 (1%)	116
	B	93 (79%)	16 (14%)	8 (7%)	117
	C	97 (83%)	17 (26%)	3 (3%)	117
2nd month	A	101 (87%)	14 (12%)	1 (1%)	116
	B	94 (80%)	15 (13%)	8 (7%)	117
	C	91 (78%)	21 (18%)	5 (4%)	117
6th month	A	91 (78%)	24 (21%)	1 (1%)	116
	B	89 (76%)	19 (16%)	9 (8%)	117
	C	81 (69%)	27 (23%)	9 (8%)	117

Figure 10.3.1 Flow chart at enrolment and follow up at 2nd and 6th month of patients



10.4 Clinical presentation of patients in the three arms by treatment duration

The changes in the clinical symptoms by duration of treatment of the three treatment groups are shown in table 10.4.1. Selected clinical symptoms are illustrated in figures 10.4.1. A general improvement was noticed for all the symptoms in all the three groups of patients as treatment progressed and by the 6^{th} month, the majority of the patients no longer complained of any symptoms. Even though the proportion of patients with cough decreased with treatment in all the groups, improvement of cough was best noticed in groups C and B from the 2^{nd} to the 6^{th} month compared to patients in group A.

There was no difference noticed in the pattern of improvement of fever with treatment within the groups, neither was there in the symptoms of night sweats and haemoptysis, though patients in group C had slightly better improvement of night sweats in the last few months of treatment compared to the other two groups. No difference was noticed in the pattern of improvement of patients regarding the presence of headache in the three groups even though patients in group C seem to have done better in the first few weeks of treatment.

Symptoms of dyspnoea and chest pains improved better in the group C compared to groups A and B from the first few weeks of treatment while no difference was observed with loss of appetite within the three groups. None of these improvements in clinical symptoms however, reached statistical significance.

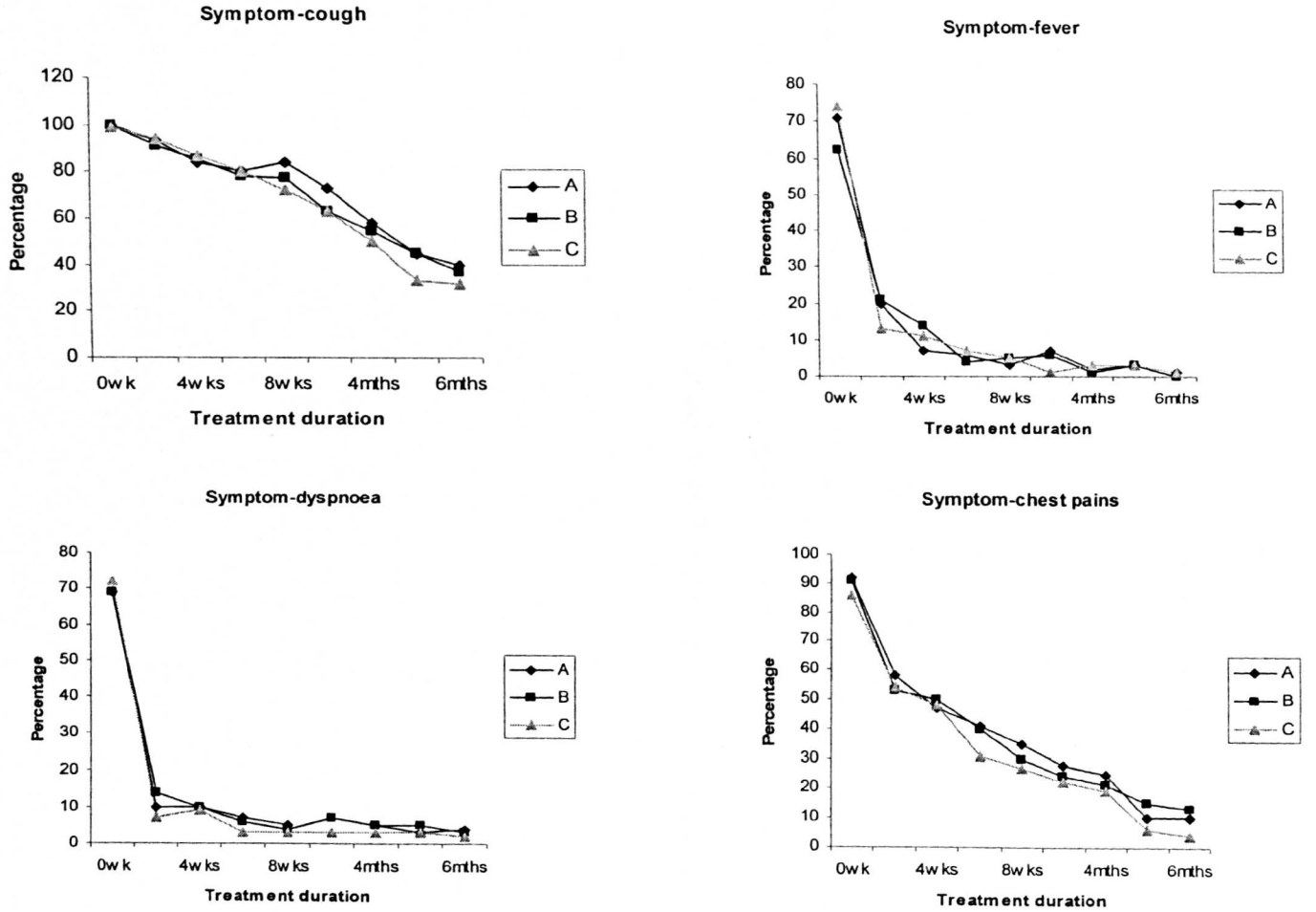
Table 10.4.1 Clinical symptoms of patients in the three arms by treatment duration

Symptoms	Treatment groups	Duration of treatment								
		0 wk	2wks	4wks	6wks	8wks	3mths	4mths	5mths	6mths
Cough	**A**	116 (100)	108 (93)	97 (84)	93 (80)	97 (84)	85 (73)	67 (58)	52 (45)	46 (40)
	B	117 (100)	106 (91)	100 (85)	91 (78)	90 (77)	74 (63)	63 (54)	53 (45)	43 (37)
	C	116 (99)	110 (94)	102 (87)	94 (80)	84 (72)	74 (63)	58 (50)	39 (33)	37 (32)
Fever	**A**	82 (71)	25 (20)	8 (7)	7 (6)	3 (3)	8 (7)	2 (2)	3 (3)	1 (1)
	B	72 (62)	24 (21)	16 (14)	5 (4)	6 (5)	7 (6)	1 (1)	3 (3)	0 (0)
	C	87 (74)	15 (13)	13 (11)	8 (7)	6 (5)	1 (1)	3 (3)	4 (3)	1 (1)
Night sweats	**A**	86 (74)	35 (30)	19 (16)	14 (12)	9 (8)	6 (5)	3 (3)	1 (1)	0 (0)
	B	83 (71)	37 (32)	23 (20)	15 (13)	10 (9)	10 (9)	2 (2)	2 (2)	1 (1)
	C	91 (78)	36 (31)	19 (16)	10 (19)	3 (3)	2 (2)	1 (1)	1 (1)	1 (1)
Haemoptysis	**A**	28 (24)	7 (6)	8 (7)	4 (3)	4 (3)	2 (2)	3 (3)	0 (0)	0 (0)
	B	34 (29)	6 (5)	9 (8)	7 (6)	4 (3)	1 (1)	0 (0)	1 (1)	1 (1)
	C	28 (31)	4 (3)	6 (5)	6 (5)	2 (2)	3 (3)	1 (1)	0 (0)	0 (0)
Headache	**A**	20 (18)	26 (21)	16 (14)	14 (12)	10 (9)	9 (8)	4 (3)	4 (3)	2 (2)
	B	25 (23)	20 (17)	17 (15)	14 (12)	7 (6)	5 (4)	4 (3)	4 (3)	3 (3)
	C	29 (25)	18 (15)	15 (13)	9 (8)	7 (6)	9 (8)	2 (2)	5 (4)	3 (3)
Dyspnoea	**A**	80 (69)	12 (10)	12 (10)	8 (7)	8 (7)	6 (5)	6 (5)	4 (3)	5 (4)

	B	81 (69)	16 (14)	12 (10)	7 (6)	5 (4)	8 (7)	6 (5)	6 (5)	3 (3)
	C	84 (72)	8 (7)	10 (9)	4 (3)	4 (3)	3 (3)	4 (3)	4 (3)	2 (2)
Chest pains	**A**	107 (92)	67 (58)	54 (47)	48 (41)	41 (35)	32 (28)	29 (25)	12 (10)	12 (10)
	B	106 (91)	63 (53)	58 (50)	47 (40)	35 (30)	28 (24)	25 (21)	18 (15)	15 (13)
	C	100 (86)	64 (54)	56 (48)	36 (31)	32 (27)	26 (22)	22 (19)	7 (6)	5 (4)
Lost of appetite	**A**	83 (72)	13 (11)	7 (6)	7 (6)	6 (5)	3 (3)	1 (1)	2 (2)	1 (1)
	B	75 (64)	16 (14)	7 (6)	10 (9)	6 (5)	6 (5)	0 (0)	4 (3)	1 (1)
	C	81 (69)	13 (11)	8 (7)	4 (3)	3 (3)	3 (3)	2 (2)	2 (2)	3 (3)

* Mean (SD); Underlined P $\leq$0.20; Bolded P $\leq$0.05

Figure 10.4.1 Selected changes in clinical symptoms of the three treatment groups



Changes in the clinical signs of patients in the three treatment groups are shown in table 10.4.2 and figures 10.4.9 to 10.4.11. A general improvement of all clinical signs was observed in all the three groups of patients with treatment.

A better improvement in chest abnormalities was observed in group C than in groups A and B. In general, patients in groups B and C were observed to have better Karnofsky scores at 2 months of treatment compared to patients in group A.

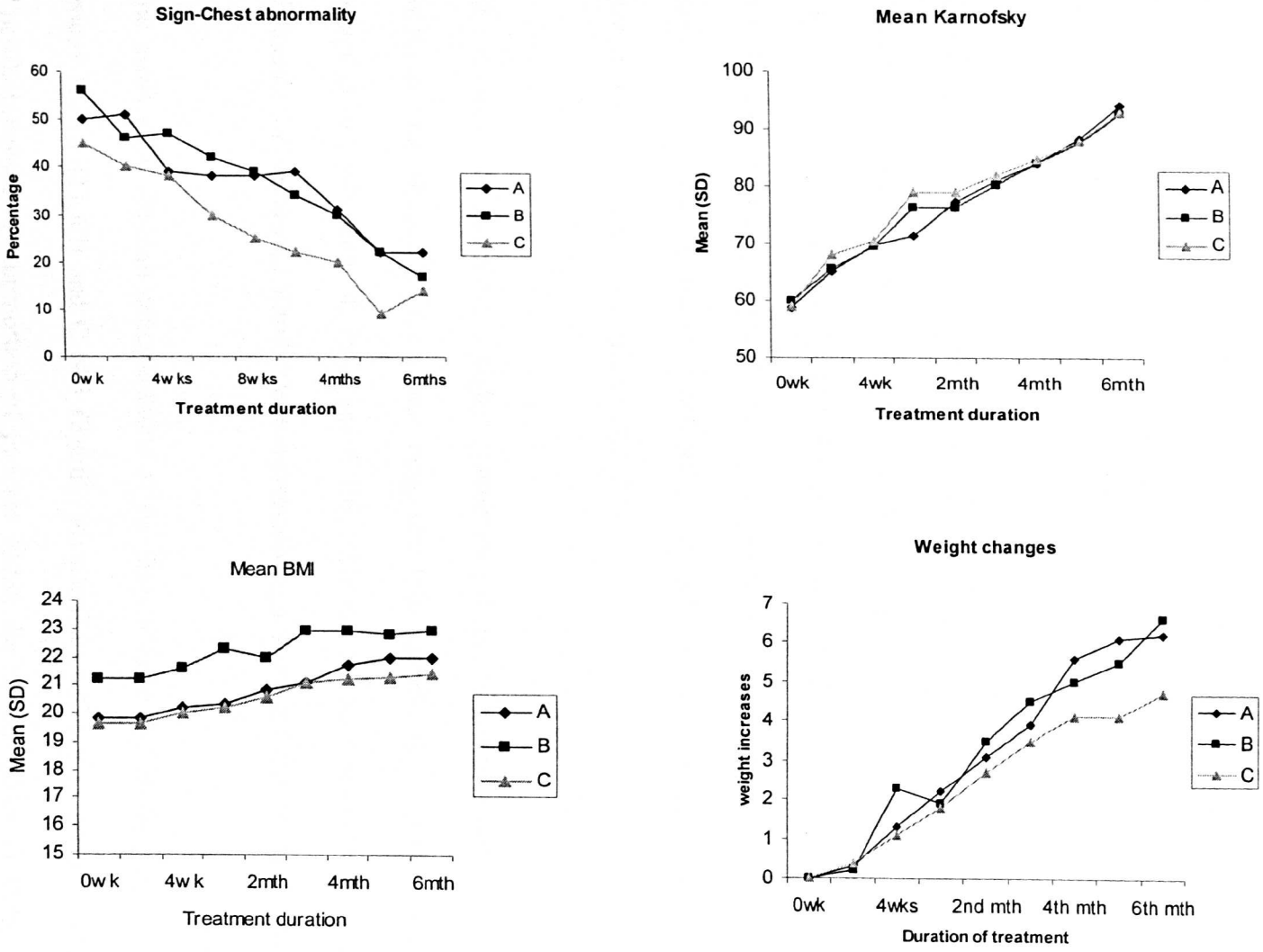
Weight increases, as shown in figure 10.4.12 were significantly higher in group B than in groups A and C. There were improvements in the BMI in the three groups with duration of treatment, though patients in group B had significantly better BMI especially in the first few weeks than patients in groups A and C. The difference in the BMI and weight were statistically significant between 2 weeks and 3 months. Differences in the proportion of patients with chest anomalies were significant after 8 weeks and at 5 months.

Table 10.4.2 Clinical signs of patients in groups and treatment duration

Signs	Treatment groups	Duration of treatment								
		0wk	2wks	4wks	6wks	8wks	3mths	4mths	5mths	6mths
Chest exam(normal /abnormal)	**A**	58 (50)	59 (51)	45 (39)	44 (38)	**44 (38)**	45 (39)	36 (31)	**25 (22)**	25 (22)
	B	65 (56)	54 (46)	55 (47)	49 (42)	**46 (39)**	40 (34)	35 (30)	**26 (22)**	20 (17)
	C	53 (45)	47 (40)	44 (38)	35 (30)	**29 (25)**	26 (22)	23 (20)	**11 (9)**	16 (14)
Karnofsky score	***A**	59(10)	65.3(10)	69.8(11)	71.4(10)	77.3(10)	80.9(10)	84(9)	88.4(7)	94.1 (8)
	***B**	60 (12)	65.7(12)	69.6(10)	71.3(10)	76.5(10)	80.3(10)	84.1(8)	87.6(8)	92.6 (9)
	***C**	59 (11)	68.2(10)	70.4(12)	74.1(11)	79.1(10)	81.8(11)	84.7(10)	87.8(10)	92.8 (9)
BMI	***A**	19.8 (3)	**19.8 (4)**	**20.2 (4)**	**20.3 (4)**	20.8 (4)	**21.1 (4)**	**21.7 (4)**	22 (5)	22 (4)
	***B**	21.2 (5)	**21.2 (5)**	**21.6 (5)**	**22.3 (7)**	22 (5)	**22.9 (5)**	**22.9 (5)**	22.8 (5)	22.9 (5)
	***C**	19.6 (3)	**19.6 (3)**	**20 (3)**	**20.2 (3**	20.6 (4)	**21.1 (5)**	**21.2 (4)**	21.3 (4)	21.4 (4)
Mean (SD) weight (kg)	***A**	51.5(9)	51.8 (9)	**52.8 (9)**	53.7 (9)	**54.6(9)**	**55.4 (9)**	57.1 (9)	57.6 (9)	**57.7 (9)**
	***B**	54.2 (9)	54.4 (10)	**56.5 (9)**	56.1 (9)	**57.7 (10**	**58.7 (10**	59.2 (10	59.7 (10	**60.8 (10**
	***C**	53.1 (8)	53.5 (8)	**54.2 (8)**	54.9 (9)	**55.8 (8)**	**56.6 (8)**	57.2 (8)	57.2 (8)	**57.8 (8)**

* Mean (SD); Bolded= P ≤0.05

Figure 10.4.2 Changes in clinical signs of the three treatment groups



10.5 Laboratory results during treatment by study group

There was a general improvement in the laboratory results as the treatment progressed in the three participating groups as shown in table 10.5.1. The mean (SD) Hb concentrations and the proportion of patients with an increase in Hb level were higher in group B than in groups A and C. There were no differences with the WBC counts, granulocyte or lymphocyte percentages by study groups, although slightly more patients had values within the normal ranges in patients in groups B and C (not significant).

The mean (SD) ESR at 2 months was lower for patients in group C (51 (34)) compared to groups A (59 (38) and B (59 (36) and at 6 months, patients in group B had lower ESR values (31 (29)), followed by patients in group C (38 (35)) and group A (49 (42)). Likewise, the proportion of patients whose ESR was within the normal range at 6 months was higher in patients in group B (14, 19%) than in patients in groups A (14%) and C (11%) (figure 10.5.2)

At six months of treatment, a higher number of patients in group A had total and conjugated bilirubin within the normal range (69, 95% and 68, 92%) than patients in groups B (67, 94% and 54, 76%) and C (69, 93% and 64, 89% respectively). The difference for conjugated bilirubin was statistically significant at 6 months ($p< 0.02$).

The mean (SD) albumin concentrations at six months were higher for patients in groups B (4.1 (1)g/dl) and C (4.3 (3)g/dl) compared to patients in group A (3.1 (1)g/dl). Likewise, the proportion of patients within the normal range of albumin

levels was higher in groups B (51 (73%)) and C (53 (74%)) compared to group A (46 (65%)). The change in albumin concentration between enrolment and 6 months of treatment was larger in patients in group C compared to groups B and C (figure 10.5.4) but this was not statistically significant.

At six months, a higher proportion of patients in group B had SGOT and SGPT within the normal range (67, 94% and 58, 82% respectively) than for patients in group A (69, 93% and 58, 78%) and group C (68, 90% and 61, 80% respectively). The proportion of patients whose alkaline phosphatase level fell within normal range at six months was higher in patients in group C (66, 94%) compared to 62 (86%) for patients in group A and 59 (88%) for patients in group B (figure 10.5.5).

The mean CRP was lower for patients in group A compared to patients in groups B and C. The mean CRP for patients in group C was lower at the end of treatment than patients in group B (figure 10.5.6). This was not statistically significant.

Table 10.5.1 Laboratory results of patients in groups A, B, and C at the time of enrolment, 2nd, and 6th months

		Treatment duration		
		0 month	**2nd month**	**6th month**
Hb	***A**	11.1 (2) [6-17]	12.5 (2) [8-17]	12.8 (2) [7-16]
	<11gm/dl	53 (46%)	15 (13%)	15 (13%)
	≥11gm/dl	58 (50%)	71 (61%)	61 (53%)
	Missing	5 (4%)	30 (26%)	40 (34%)
	***B**	11.3 (2) [6-16]	12.8 (2) [8-16]	13.2 (2) [9-18]
	<11gm/dl	50 (43%)	12 (10%)	9 (12%)
	≥11gm/dl	62 (53%)	72 (62%)	64 (88%)
	Missing	5 (4%)	33 (28%)	44 (38%)
	***C**	11 (2) [5-16]	12.6 (2) [7-16]	12.5 (2) [5-17]
	<11gm/dl	42 (41%)	14 (12%)	14 (19%)
	≥11gm/dl	61 (59%)	73 (62%)	61 (81%)
	Missing	14 (12%)	30 (26%)	42 (36%)
WBC	***A**	8.9 (4) [4-19]	6.7 (2) [4-14]	5.7 (2) [4-17]
	Low	4 (3%)	2 (2%)	6 (5%)
	Normal	66 (57%)	78 (67%)	67 (58%)
	High	40 (34%)	6 (5%)	3 (3%)
	Missing	6 (5%)	30 (26%)	40 (34%)
	***B**	8.7 (3) [4-17]	6.8 (3) [3-15]	5.6 (2) [3-11]
	Low	0 (0)	4 (3%)	9 (8%)
	Normal	78 (67%)	68 (58%)	62 (53%)
	High	34 (29%)	12 (10%)	2 (2%)
	Missing	5 (4%)	33 (28%)	44 (38%)
	***C**	8.3 (3) 3-18]	6.7 (3) [4-16]	5.9 (2) [3-18]
	Low	4 (3%)	3 (3%)	4 (3%)
	Normal	73 (62%)	76 (65%)	68 (58%)
	High	26 (22%)	8 (7%)	3 (3%)
	Missing	14 (12%)	30 (26%)	42 (36%)
Granulocytes	***A**	63 (13) [20-92]	59 (11) [26-94]	53 (10) [26-74]
	Low	8 (5%)	6 (5%)	15 (13%)
	Normal	50 (43%)	56 (48%)	55 (47%)
	High	53 (45%)	24 (21%)	6 (5%)
	Missing	5 (4%)	30 (26%)	40 (34%)
	***B**	62 (13) [9-90]	58 (9) [35-78]	55 (9) [28-70]
	Low	6 (5%)	6 (5%)	8 (7%)
	Normal	58 (50%)	58 (50%)	54 (46%)
	High	48 (41%)	20 (21%)	11 (9%)
	Missing	5 (4%)	33 (28%)	44 (38%)
	***C**	63 (12) [18-86]	58 (9) [40-79]	55 (9) [26-74]
	Low	7 (6%)	5 (4%)	7 (9%)
	Normal	48 (41%)	64 (55%)	55 (73%)
	High	48 (41%)	18 (15%)	13 (17%)
	Missing	14 (12%)	30 (26%)	42 (36%)

*Mean (SD)[Range]; Underlined P ≤0.20; Bolded P ≤0.05; Normal values for laboratory test are defined in table 8.5.5, page 125

Table 10.5.1 Laboratory results of patients in groups A, B, and C at the time of enrolment, 2nd, and 6th months (cont)

		Treatment duration		
		0 month	**2nd month**	**6th month**
ESR	***A**	86 (39) [7-160]	59 (38) [4-160]	49 (42) [1-153]
	Low	0 (0)	0 (0)	2 (2%)
	Normal	1 (1%)	5 (4%)	10 (9%)
	High	107 (92%)	78 (67%)	61 (53%)
	Missing	8 (7%)	33 (28%)	43 (37%)
	***B**	82 (41) [2-150]	59 (36) [2-140]	31 (29) [1-123]
	Low	1 (1%)	1 (1%)	5 (4%)
	Normal	3 (3%)	3 (3%)	14 (12%)
	High	107 (91%)	80 (68%)	53 (45%)
	Missing	6 (5%)	33 (28%)	45 (38%)
	***C**	88 (37) [5-152]	51 (34) [2-141]	38 (35) [2-124]
	Low	0 (0)	1 (1%)	3 (3%)
	Normal	1 (1%)	9 (8%)	8 (7%)
	High	100 (85%)	77 (66%)	63 (54%)
	Missing	16 (14%)	31 (26%)	43 (38%)
Bilirubin T*	**A**	.45 (.4) [0-3]	.38 (.3) [0-1.6]	.47 (.5) [0.1-3]
	Low	2 (2%)	1 (1%)	2 (2%)
	Normal	105 (90%)	85 (73%)	69 (59%)
	High	5 (4%)	2 (2%)	2 (2%)
	Missing	4 (3%)	28 (24%)	43 (38%)
	***B**	.49 (.5) [.1-3.8]	.43 (.5) [0-4.9]	.55 (.6) [.05-4]
	Low	3 (3%)	0 (0)	1 (1%)
	Normal	105 (90%)	85 (73%)	67 (94%)
	High	5 (4%)	1 (1%)	3 (4%)
	Missing	4 (3%)	31 (26%)	46 (39%)
	***C**	.47 (.4) [.1-3]	.41 (.7) [0.1-6]	.47 (.6) [0.1-3.9]
	Low	4 (4%)	1 (1%)	1 (1%)
	Normal	104 (93%)	85 (73%)	69 (59%)
	High	4 (4%)	2 (2%)	4 (3%)
	Missing	5 (4%)	29 (25%)	43 (36%)
Bilirubin C	***A**	.18 ((.3) [.01-2]	.14 (.2) [.01-.8]	.2 (.4) [.01-3]
	Normal	88 (76%)	74 (64%)	**68 (58%)**
	High	26 (22%)	15 (13%)	**6 (5%)**
	Missing	2 (2%)	27 (23%)	**43 (37%)**
	***B**	.17 (.2) [.01-1.3]	.17 (.2) [.01-2]	.19 (.2) .01-1]
	Normal	73 (62%)	73 (62%)	**54 (46%)**
	High	41 (35%)	13 (11%)	**17 (15%)**
	Missing	3 (3%)	31 (26%)	**46 (39%)**
	***C**	.2 (.3) [.01-2]	.14 (.1) [.01-.8]	.15 (.1) [.01-1]
	Normal	73 (62%)	80 (68%)	**64 (55%)**
	High	39 (33%)	8 (7%)	**8 (7%)**
	Missing	5 (4%)	29 (25%)	**45 (38%)**

*Mean (SD)[Range]; Underlined P ≤0.20; Bolded P ≤0.05; Normal values for laboratory test are defined in table 8.5.5, page 125

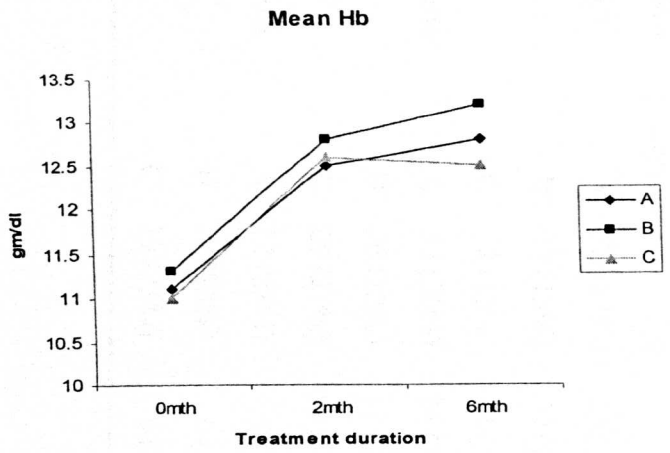
Table 10.5.1 Laboratory results of patients in groups A, B, and C at the time of enrolment, 2nd, and 6th months (cont)

		Treatment duration		
		0 month	**2nd month**	**6th month**
SGOT	***A**	7.6 (8) [1-70]	5.6 (5) [2-35]	6.3 (4) [1-23]
	Normal	101 (86%)	86 (74%)	69 (93%)
	High	13 (11%)	4 (3%)	5 (7%)
	Missing	2 (2%)	27 (23%)	43 (37%)
	***B**	6.3 (5) [1-26]	5.6 (4) [1-22]	6.4 (5) [2-29]
	Normal	106 (92%)	84 (72%)	67 (57%)
	High	8 (8%)	4 (3%)	4 (3%)
	Missing	3 (%)	29 (25%)	46 (39%)
	***C**	6.6 (5) [1-49]	5.6 (3) [1-24]	7.4 (7) [2-47]
	Normal	103 (88%)	85 (73%)	68 (58%)
	High	8 (7%)	2 (2%)	8 (7%)
	Missing	6 (5%)	30 (27%)	41 (35%)
SGPT	***A**	12.7 (9) [2-60]	12.1 (13) [2-90]	11.6 (9) [2-53]
	Normal	75 (65%)	71 (61%)	58 (78%)
	High	37 (32%)	18 (15%)	16 (22%)
	Missing	4 (3%)	28 (24%)	43 (37%)
	***B**	11.6 (8) [2-52]	11 (11) [2-90]	11.9 (13) [2-90]
	Normal	76 (65%)	71 (61%)	58 (50%)
	High	38 (32%)	17 (15%)	13 (11%)
	Missing	3 (2%)	29 (25%)	46 (39%)
	***C**	12.2 (10) [2-90]	11.4 (8) [2-52]	11.2 (11) [2-76]
	Normal	70 (60%)	67 (57%)	61 (80%)
	High	40 (34%)	20 (17%)	15 (20%)
	Missing	7 (6%)	30 (26%)	41 (35%)
Alk phosphatase	***A**	247 (173) [74-911]	202 (125) [68-958]	175 (78) [76-433]
	Low	3 (3%)	2 (2%)	4 (3%)
	Normal	82 (70%)	72 (62%)	62 (53%)
	High	29 (25%)	8 (7%)	6 (5%)
	Missing	2 (2%)	34 (29%)	44 (38%)
	***B**	238 (123) [92-675]	189 (71) [111-438]	171 (88) [88-541]
	Low	1 (1%)	0 (0)	5 (8%)
	Normal	82 (70%)	73 (90%)	59 (88%)
	High	31 (26%)	8 (10%)	3 (5%)
	Missing	3 (2%)	36 (31%)	50 (43%)
	***C**	261 (168) [44-940]	183 (78) [75-512]	172 (51) [119-326]
	Low	1 (1%)	5 (4%)	2 (3%)
	Normal	75 (64%)	72 (62%)	66 (94%)
	High	32 (27%)	7 (6%)	2 (3%)
	Missing	9 (8%)	33 (28%)	47 (40%)
C-reactive protein	***A**	82.25	27.60	17.8
	***B**	59.08	42.02	32.28
	***C**	72.47	21.08	17.68

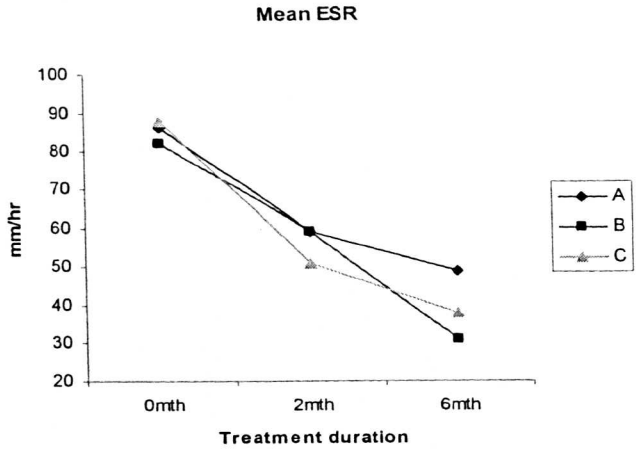
*Mean (SD)[Range]; Underlined P ≤0.20; Bolded P ≤0.05; Normal values for laboratory test are defined in table 8.5.5, page 125

Figure 10.5.1 Mean haemoglobin (a), ESR (b), CRP (c) and proportion of patients with normal bilirubin (d) at the time of enrolment, 2nd and 6th month follow up by study group

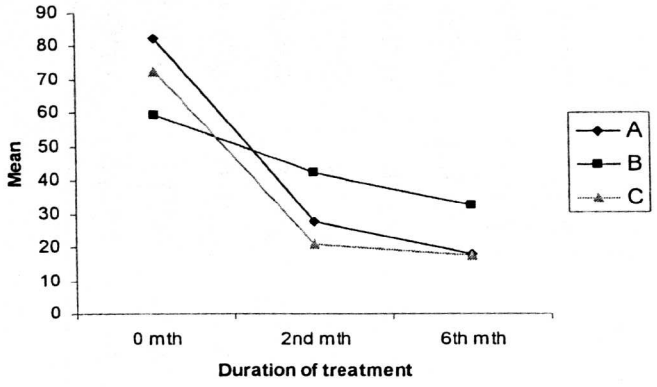
a



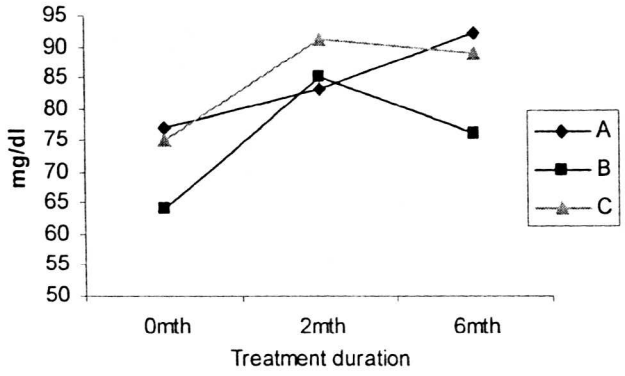
b



c



d



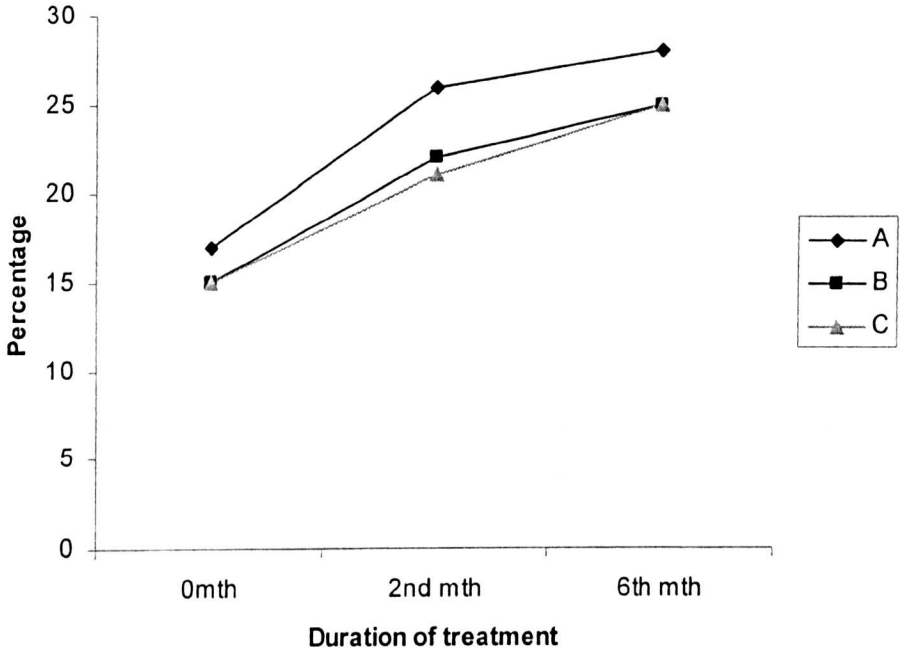
10.6 Radiological presentation of patients by study group

Radiological results by treatment groups are shown in table 10.6.1 and illustrated in figure 10.6.1. There was general improvement in the radiological status of patients in the three groups with treatment. At enrolment only 20 (17%) patients in group A (placebo), 17 (15%) in group B (Zinc) and 18 (15%) in group C (Zinc plus vitamin A) did not have chest cavities. By the 2nd month, 30 (26%) of the patients in group A, 26 (21%) of those in group B and 25 (21%) patients in group C did not have chest cavities. At 6 months, the number of patients without chest cavities had increased, with the largest proportion of patients without cavities in group A (32, 28%), followed by 29 (24%) in group B and 29 (24%) in group C. The differences however, were not statistically significant ($p > 0.5$).

Table 10.6.1 Radiological results of the three treatment groups and clearance of cavities

Treatment	Patients	Cavity grading					
Duration	groups	0	1	2	3	Missing	Total
0 month	A	20 (17%)	23 (20%)	45 (39%)	17 (15%)	11(9%)	116
	B	17 (15%)	20 (17%)	42 (35%)	19 (16%)	19 (16%)	117
	C	18 (15%)	24 (20%)	38 (32%)	24 (20%)	13 (11%)	117
2nd month	A	30 (26%)	24 (21%)	13 (11%)	4 (3%)	45 (39%)	116
	B	26 (22%)	17 (15%)	13 (11%)	4 (3%)	58 (50%)	117
	C	25 (21%)	16 (14%)	16 (14%)	3 (3%)	57 (49%)	117
6th month	A	32 (28%)	9 (8%)	5 (4%)	0 (0%)	70 (60%)	116
	B	29 (25%)	6 (5%)	0 (0%)	1 (1%)	81 (69%)	117
	C	29 (25%)	9 (8%)	3 (3%)	0 (0%)	76 (65%)	117

Figure 10.6.1 Proportion of patients without lung cavities on X-rays by study group



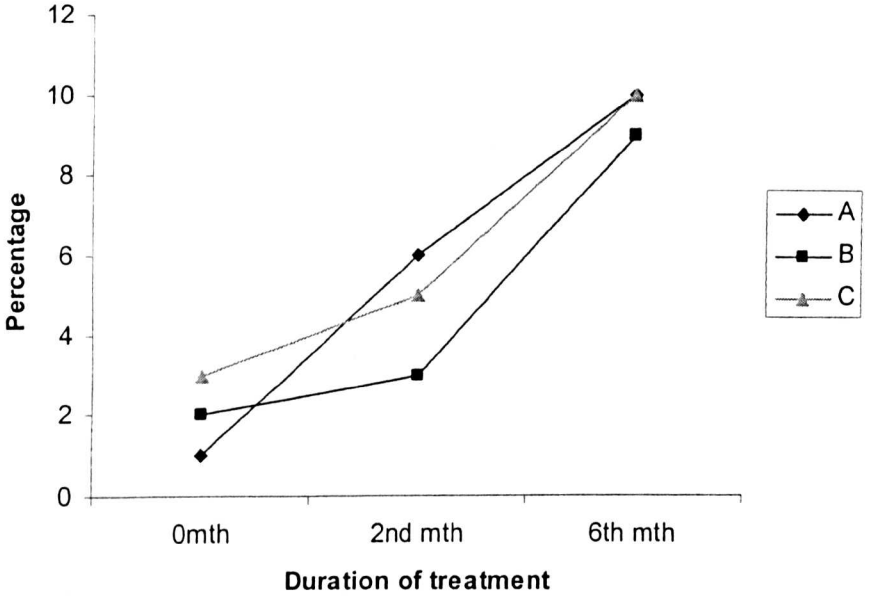
The number of patients without diseased lungs at enrolment was 1 (1%) in group A, 2 (2%) in group B and 4 (3%) in group C. By the 2nd month, the number of patients who were declared to have no lung involvement in their lungs (a score of 0 for extent of disease) were 7 (6%) in group A, 4 (3%) in group B and 6 (5%) in group C as shown in table 10.6.2 and illustrated in figure 10.6.2. By 6 months, 12 (10%) patients in group A, 11 (9%) in group B and 12 (10%) in group C had no signs of significant disease in their lungs X-rays. These differences however, were not statistically significant.

Table 10.6.2 Radiological findings describing the extent of disease by study group on enrolment, 2nd and 6th month follow up

Treatment Duration	Patients groups	Extent of disease (lung grading)								
		0	1	2	3	4	5	6	Missing	Total
0 month	A	1 (1%)	13 (11%)	24 (21%)	26 (22%)	18 (17%)	15 (16%)	8 (7%)	11 (9%)	116
	B	2 (2%)	13 (11%)	17 (15%)	28 (24%)	26 (22%)	11 (9%)	3 (3%)	17 (15%)	117
	C	4 (3%)	7 (6%)	30 (26%)	29 (25%)	21 (18%)	9 (8%)	4 (3%)	13 (11%)	117
2nd month	A	7 (6%)	21 (18%)	16 (14%)	17 (15%)	10 (9%)	2 (2%)	3 (3%)	40 (34%)	116
	B	4 (3%)	14 (12%)	23 (20%)	13 (11%)	12 (10%)	3 (3%)	1 (1%)	47 (40%)	117
	C	6 (5%)	20 (17%)	13 (11%)	14 (12%)	10 (9%)	3 (3%)	0 (0)	51 (44%)	117
6th month	A	12 (10%)	16 (14%)	12 (10%)	8 (7%)	1 (1%)	0 (0)	0 (0)	67 (58%)	116
	B	11 (9%)	18 (15%)	8 (7%)	1 (1%)	1 (1%)	0 (0)	0 (0)	78 (67%)	117
	C	12 (10%)	18 (15%)	6 (5%)	5 (4%)	1 (1%)	1 (1%)	0 (0)	74 (63%)	117

* The numbers indicate the numbers of lobes involved. 0 = none of the 6 lung lobes had significant abnormal findings in the chest X-rays

Figure 10.6.2 Proportion of patients who had a score of '0' (no lung lobes affected) on X-rays by study group



10.7 Clinical presentation of patients by HIV status and treatment group

10.7.1 Clinical symptoms of patients by HIV status and treatment group

The clinical symptoms of patients in the three treatment groups by HIV status are shown in table 10.7.1. The proportion of patients with improved symptoms as treatment progressed was higher among HIV negative patients compared to HIV positive patients irrespective of the treatment group.

By the 2nd, 4th and 6th month of treatment, the proportion of HIV-positive and HIV-negative patients with cough had reduced in groups B and C compared to group A (figures 10.7.1 and 10.7.2).

No difference in the improvement of fever was observed in the three treatment groups irrespective of their HIV status. At 6 weeks, patients in group C were less likely to have symptoms of night sweats, haemoptysis, headache, dyspnoea, chest pains or anorexia compared to patients in groups B and A in both HIV groups and by the 2nd month of treatment most of the clinical symptoms had abated as shown in figure 10.7.1.

Table 10.7.1 Clinical symptoms of HIV positive and HIV negative patients in the three arms by treatment duration

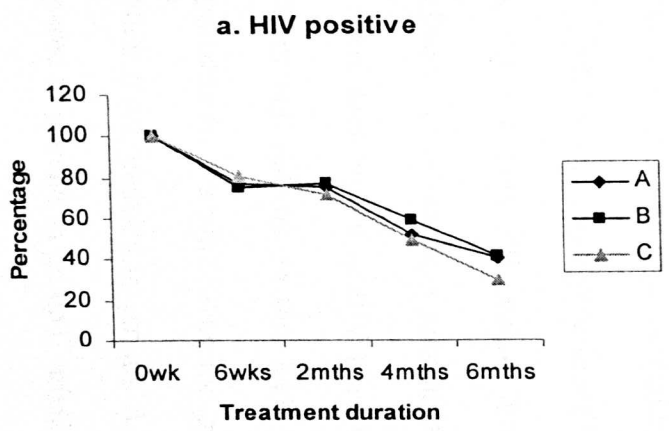
		Duration of treatment									
		HIV status									
Groups		Positive					Negative				
Symptoms		0wk	6wk	2mth	4mth	6mth	0wk	6wk	2mth	4mth	6mth
Cough	A	52 (100)	40 (77)	39 (75)	27 (52)	21 (40)	64 (100)	53 (83)	58 (91)	40 (63)	25 (39)
	B	56 (100)	42 (75)	43 (77)	33 (59)	23 (41)	59 (100)	49 (83)	47 (80)	30 (51)	20 (34)
	C	47 (100)	38 (81)	34 (72)	23 (49)	14 (30)	66 (100)	54 (82)	49 (74)	35 (50)	23 (35)
Fever	A	37 (71)	6 (12)	2 (4)	1 (2)	0 (0)	45 (70)	1 (2)	1 (2)	1 (2)	1 (2)
	B	41 (73)	4 (7)	3 (5)	1 (2)	0 (0)	30 (54)	1 (2)	3 (5)	0 (0)	0 (0)
	C	37 (79)	4 (9)	4 (9)	1 (2)	0 (0)	49 (74)	4 (6)	1 (2)	2 (3)	1 (2)
Night sweats	A	37 (71)	8 (15)	4 (8)	2 (4)	0 (0)	49 (77)	6 (9)	5 (8)	1 (2)	0 (0)
	B	40 (71)	7 (17)	4 (9)	2 (5)	0 (0)	42 (71)	8 (14)	6 (10)	0 (0)	1 (2)
	C	39 (83)	6 (13)	1 (2)	1 (2)	0 (0)	48 (73)	4 (6)	2 (3)	0 (0)	0 (0)
Haemoptysis	A	8 (15)	3 (6)	1 (2)	2 (4)	0 (0)	20 (31)	1 (2)	3 (5)	1 (2)	0 (0)
	B	13 (23)	5 (9)	2 (5)	0 (0)	1 (2)	21 (36)	2 (3)	2 (3)	0 (0)	0 (0)
	C	9 (19)	1 (2)	0 (0)	0 (0)	0 (0)	18 (27)	5 (11)	2 (3)	1 (2)	0 (0)

Table 10.7.1 Clinical symptoms of HIV positive and HIV negative patients in the three arms by treatment duration (cont)

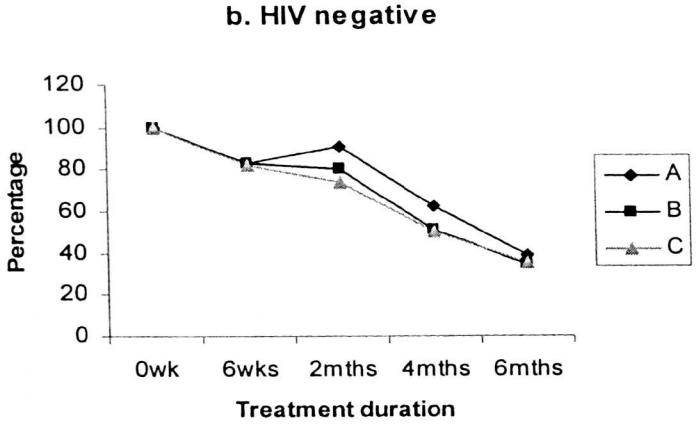
		Duration of treatment									
		HIV status									
Groups		Positive					Negative				
Headache	**A**	10 (19)	7 (13)	7 (13)	1 (2)	0 (0)	10 (16)	7 (11)	3 (5)	3 (5)	2 (3)
	B	12 (21)	7 (13)	4 (7)	3 (5)	2 (4)	12 (21)	7 (12)	3 (5)	1 (2)	1 (2)
	C	9 (19)	6 (13)	3 (6)	1 (2)	1 (2)	18 (29)	3 (5)	3 (5)	1 (2)	2 (4)
Dyspnoea	**A**	38 (73)	3 (5)	3 (5)	2 (4)	3 (5)	42 (66)	5 (8)	5 (8)	4 (6)	2 (3)
	B	41 (73)	2 (4)	2 (4)	5 (9)	1 (2)	38 (64)	5 (8)	3 (5)	1 (2)	2 (3)
	C	38 (81)	2 (4)	1 (2)	1 (2)	0 (0)	43 (65)	1 (2)	2 (3)	3 (5)	2 (3)
Chest pains	**A**	47 (90)	24 (46)	18 (35)	13 (25)	6 (12)	60 (94)	24 (38)	23 (36)	16 (25)	6 (9)
	B	53 (95)	25 (45)	17 (30)	13 (23)	9 (16)	52 (88)	22 (37)	18 (31)	12 (20)	6 (10)
	C	42 (89)	15 (32)	10 (21)	9 (19)	4 (9)	54 (82)	19 (29)	21 (31)	13 (19)	2 (3)
Lost of appetite	**A**	43 (83)	6 (12)	3 (5)	1 (2)	1 (2)	40 (63)	1 (2)	3 (5)	0 (0)	0 (0)
	B	42 (75)	5 (8)	3 (5)	0 (0)	0 (0)	32 (54)	4 (7)	3 (5)	0 (0)	1 (2)
	C	35 (75)	2 (4)	1 (2)	1 (2)	2 (4)	44 (67)	1 (2)	2 (3)	1 (2)	1 (2)

( ) percentage

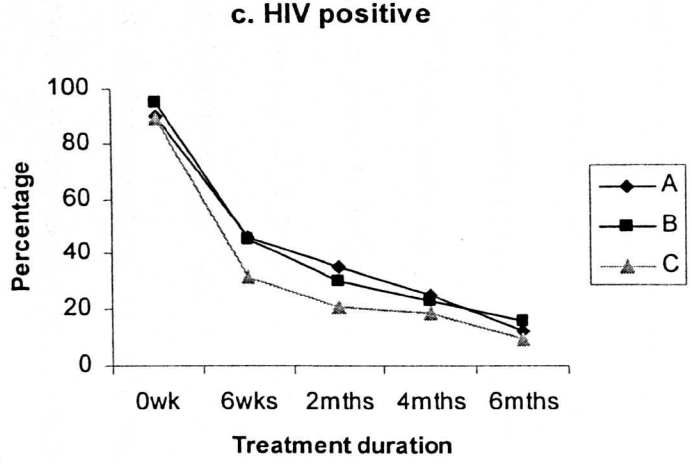
Figure 10.7.1 Proportion of patients who reported cough (a and b) or chest pains (c and d) by study group and HIV status



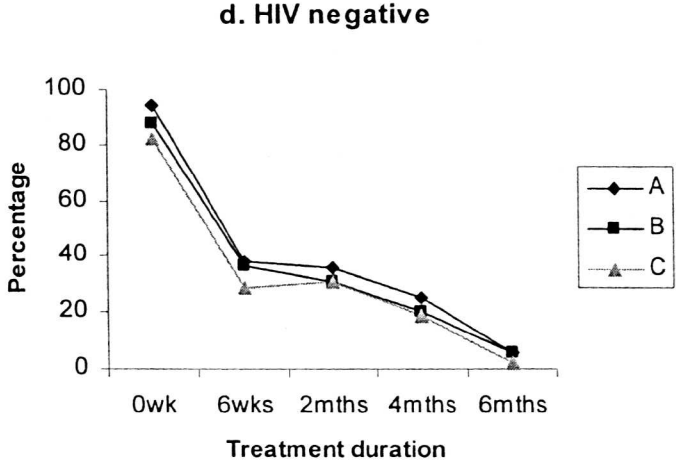
a)



b)



c)



d)

10.7.2 Clinical signs of patients by HIV status and treatment group

The clinical signs of the patients by treatment group and HIV status are described in table 10.7.2. Similarly to the pattern observed for the clinical symptoms, the clinical signs in the three groups improved with treatment. There was no difference observed in the temperature pattern between the treatment groups in patients with and without HIV. The chest pathology in patients in groups C improved better than the other groups in both HIV positive and HIV negative patients. At 6 months, patients in group B had improved more than patients in A. The differences however were not statistically significant.

The mean Karnofsky score in patients in group C and B improved at a faster rate than those in group A especially in HIV-positive patients and these differences were statistically significant at the 6^{th} week follow up (figure 10.7.2). The Kanofsky scores of HIV-negative patients also improved with time, but the three groups had a similar pattern independently of the supplements received. Improvement in the mean (SD) BMI changes was more prominent in patients in group B than in groups A and C irrespective of their HIV status. However, these differences were only statistically significant in HIV-negative patients during the 6^{th} week follow up. Increase in weight was more pronounced in HIV positive patients in group B during the 6 months of treatment and patients in this had gained an average of 7 kg by the 6^{th} month, compared to an average of 6 kg for group A and 4 kg for group C (figure 10.7.3). On the other hand, patients in groups A and B had more pronounced weight increases compared to patients in group C among HIV negative patients (figure 10.7.3). Weight gain increases however were not statistically significant across the groups

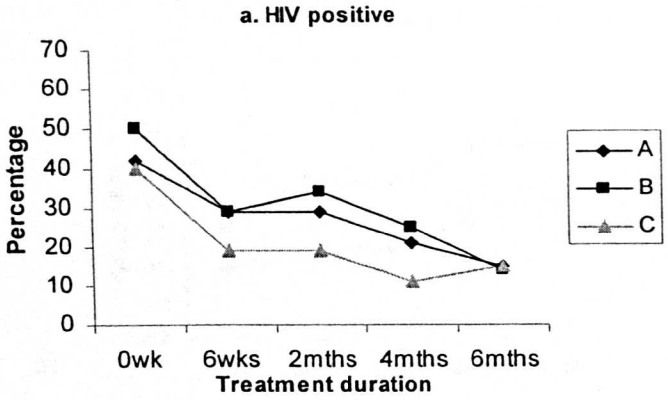
Table 10.7.2 Clinical signs of HIV-positive and HIV-negative patients by treatment group

		Duration of treatment									
		HIV status									
		Positive					Negative				
		0wk	6wk	2mth	4mth	6mth	0wk	6wk	2mth	4mth	6mth
Chest exam(normal /abnormal)	**A**	22 (42)	15 (29)	15 (29)	11 (21)	8 (15)	36 (56)	13 (20)	29 (45)	25 (39)	17 (27)
	B	28 (50)	16 (29)	19 (34)	14 (25)	8 (14)	35 (59)	17 (29)	26 (44)	21 (35)	12 (20)
	C	19 (40)	9 (19)	9 (19)	5 (11)	7 (15)	32 (48)	19 (29)	19 (29)	18 (27)	9 (14)
Temperature*	**A**	36.9 (1)	36.4 (1)	36.5 (1)	36.1 (1)	36.5 (1)	36.8 (1)	36.4 (1)	36.4 (1)	36.5 (1)	36.7 (1)
	B	36.7 (1)	36.4 (1)	36.5 (1)	36.5 (1)	36.7 (1)	36.9 (1)	36.5 (1)	36.3 (1)	36.6 (1)	36.2 (1)
	C	36.9 (1)	36.4 (1)	36.6 (1)	36.4 (1)	36.5 (1)	37.1 (1)	36.6 (1)	36.5 (1)	36.8 (1)	36.5 (1)
BMI*	**A**	19 (3)	19.4 (3)	19.6 (4)	20.4 (4)	21.7 (5)	**20.5 (3)**	**21 (4)**	21.6 (4)	22.5 (4)	22.3 (4)
	B	20.2 (4)	21.6 (7)	21.4 (5)	22.5 (5)	22.6 (4)	**22 (5)**	**22.9 (7)**	22.5 (5)	23.2 (5)	23.2 (5)
	C	19.4 (3)	20.3 (3)	20.6 (3)	21.3 (3)	20.9 (3)	**19.6 (4)**	**20.3 (4)**	20.6 (4)	21.2 (4)	21.7 (5)
Karnofsky score*	**A**	58.2 (13)	**69.6 (10)**	76.0 (11)	83.1 (11)	92.3 (9)	52.3 (9)	72.7 (11)	78.2 (9)	84.6 (8)	95.3 (7)
	B	60.2 (10)	**72.7 (10)**	77.0 (9)	83.8 (8)	90.6 (9)	60.7 (14)	70 (9)	76.1 (10)	84.3 (8)	94.4 (9)
	C	59.6 (12)	**75.4 (10)**	80.8 (8)	87.6 (10)	94.6 (6)	58.8 (11)	75.1 (16)	78.3 (11)	82.9 (9)	91.9 (10)
Mean weight (kg)	**A**	48.5 (9)	49.9 (8)	50.5 (8)	53.3 (9)	54.6 (9)	53.9 (8)	56.5 (8)	57.1 (8)	59.4 (8)	59.8 (8)
	B	53.5(9)	55.1 (11)	56.8 (10)	58.9 (11)	60.5 (11)	55.1 (10)	57.3 (9)	58.4 (9)	59.5 (9)	61.0 (9)
	C	53.1 (8)	55.2 (8)	56.1 (7)	57.9 (7)	56.9 (8)	52.9 (8)	55.2 (8)	55.7 (8)	56.8 (8)	58.3 (9)

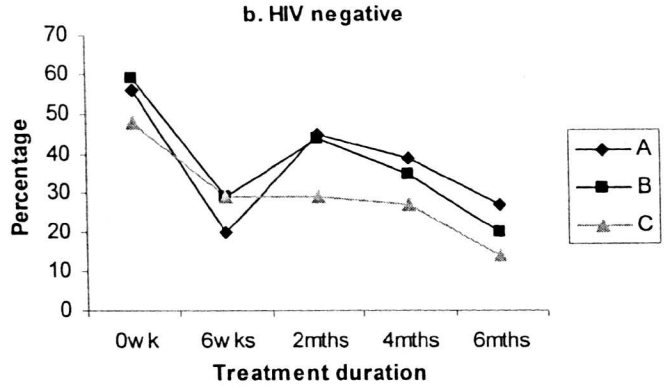
* Mean (SD);

Figure 10.7.2 Proportion of patients with chest anomalies (a and b) and mean Karnofsky scores (c and d) by treatment group and HIV status

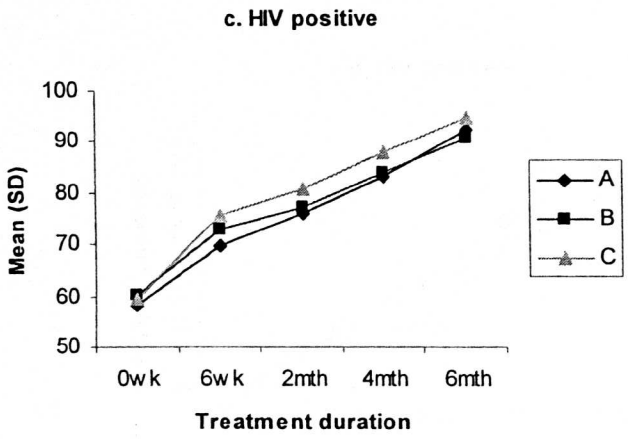
a)



b)



c)



d)

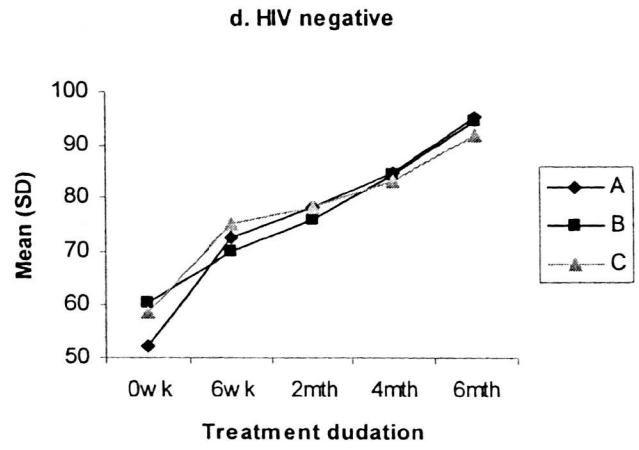
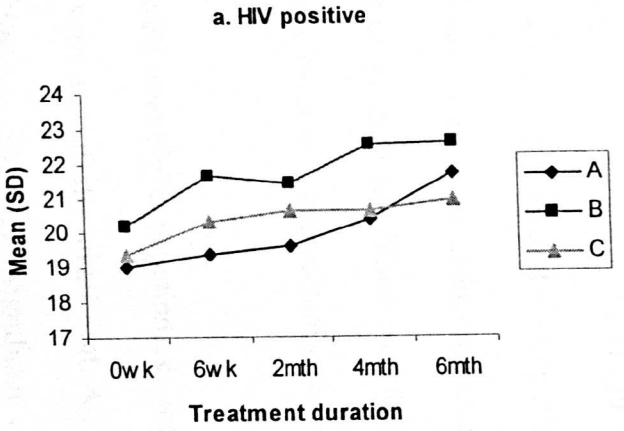
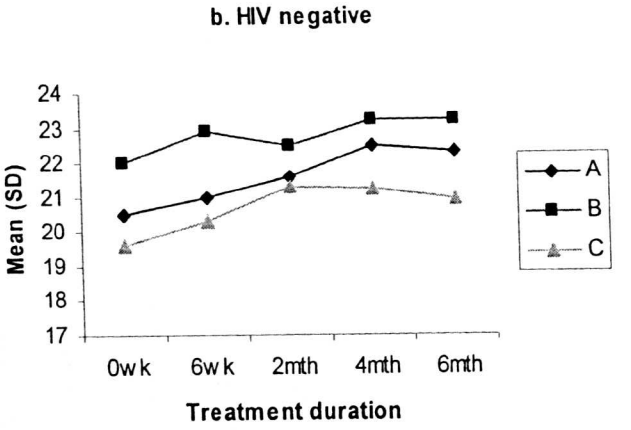


Figure 10.7.3 BMI (a and b) and weight changes (c and d) of patients by HIV status and treatment group

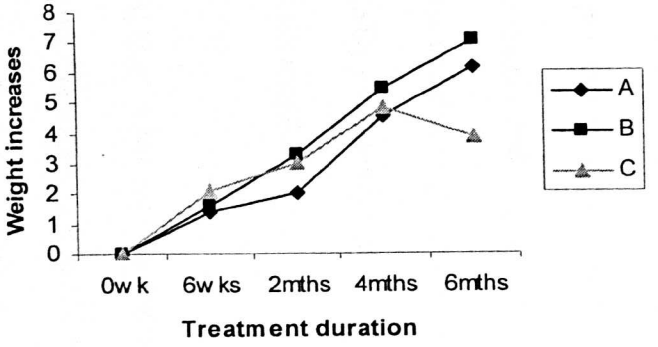
a)



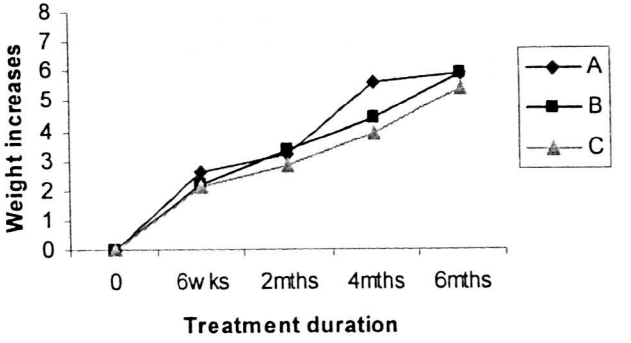
b)



c)



d)



10.8 Laboratory results of patients by HIV status and treatment group

The laboratory results of patients with and without HIV by treatment group by HIV status are shown in table 10.8.1. There was general improvement in the laboratory results in patients with and without HIV in the three groups as treatment progressed.

In HIV positive patients, the mean (SD) Hb and the proportion of patients who had normal Hb (> 11 g/L was higher in patients in group B (mean of 12.9 (2) g/L and 34, 87%) respectively) at the end of 6 months treatment than in patients in groups A (mean of 12 (2) g/L and 25, 74%) respectively) and C (mean of 12 (2) g/L and 19, 70%) respectively). In HIV negative patients, there was no difference in the proportion of patients with normal Hb at six months by treatment group nor differences in the mean (SD) Hb of patients.

There was no difference in the mean (SD) WBC counts or the proportion of patients with normal WBC counts by treatment group in both patients with or without HIV. Among patients with HIV, the mean (SD) granulocyte counts and the proportion of patients with granulocyte counts within normal range were higher in groups A and B. Patients in groups B and C however, had higher mean (SD) granulocytes and a higher proportion of patients had normal granulocyte counts at six months.

ESR levels were higher in patients co-infected with HIV than in patients without HIV. These were statistically significant. Among HIV patients the difference in the mean (SD) ESR was statistically significant in patients in group C at 2 months (58.5 (36) mm/hr and in patients in groups B and C at 6 months (39.5 (33) mm/hr and 54.1 (42)

mm/hr, respectively (P= 0.03 at 2 months and 0.02 at 6 months). Among HIV negative patients, the ESR were lower at 6 months in patients in groups B and C, but these differences were not statistically significant.

The proportion of patients with normal serum concentration of conjugated bilirubin, was statistically significant in group A at 6 months of treatment in HIV positive patients, compared to patients in groups B and C (P= 0.02). The mean (SD) total bilirubin and the proportion of patient with bilirubin concentrations within normal range had improved at two months but this was no longer obvious by the 6^{th} month in the three groups irrespective of HIV status.

A similar trend was observed for SGOT and to some extent, for SGPT in the three groups. The mean (SD) alkaline phosphatase improved more with treatment in patients in group B at 2 and 6 months in HIV positive patients but in HIV negative patients the mean (SD) and the proportion of patients with normal alkaline phosphatase was higher in patients in group C. These differences however were not statistically significant.

Table 10.8.1 Laboratory results by HIV status and treatment group

Laboratory Test		Duration of treatment HIV positive			HIV negative		
		0 week	2nd mth	6th mth	0 week	2nd mth	6th mth
Hb	***A**	10.1 (2)	11.7 (2)	12 (2)	11.9 (2)	13 (2)	13.3 (2)
	<11gm/dl	35 (67%)	11 (21%)	9 (17%)	18 (28%)	4 (13%)	6 (9%)
	≥11gm/dl	16 (31%)	23 (44%)	25 (48%)	42 (66%)	48 (75%)	36 (56%)
	Missing	1 (2%)	18 (35%)	18 (35%)	4 (6%)	12 (19%)	22 (34%)
	***B**	10.6 (2)	12.6 (2)	12.9 (2)	12 (2)	13 (2)	13.3 (2)
	<11gm/dl	34 (60%)	7 (13%)	4 (7%)	14 (24%)	5 (8%)	5 (13%)
	≥11gm/dl	21 (38%)	34 (61%)	32 (57%)	41 (69%)	38 (64%)	32 (87%)
	Missing	1 (2%)	15 (27%)	20 (36%)	4 (8%)	16 (27%)	22 (37%)
	***C**	10.4 (2)	12 (2)	12 (2)	11.6 (2)	13 (2)	12.7 (2)
	<11gm/dl	23 (58%)	10 (21%)	8 (17%)	17 (26%)	4 (6%)	6 (9%)
	≥11gm/dl	17 (42%)	23 (49%)	19 (40%)	44 (67%)	50 (76%)	42 (64%)
	Missing	7 (15%)	14 (30%)	20 (43%)	5 (8%)	12 (18%)	18 (27%)
WBC	***A**	8.7 (3)	6.9 (2)	5.4 (2)	9.1 (4)	6.6 (2)	5.8 (2)
	Low	2 (4%)	1 (2%)	3 (6%)	2 (3%)	1 (2%)	3 (7%)
	Normal	29 (56%)	30 (58%)	30 (58%)	37 (58%)	48 (75%)	37 (88%)
	High	19 (37%)	3 (6%)	1 (2%)	21 (33%)	3 (5%)	2 (5%)
	Missing	2 (4%)	18 (35%)	18 (35%)	6 (9%)	12 (19%)	22 (34%)
	***B**	9.2 (3)	7.3 (3)	5.7 (2)	8.2 (3)	6.4 (2)	5.7 (2)
	Low	0 (0)	0 (0)	5 (9%)	0 (0)	4 (7%)	4 (7%)
	Normal	34 (62%)	33 (59%)	30 (54%)	42 (71%)	35 (59%)	32 (54%)
	High	21 (38%)	8 (14%)	1 (2%)	13 (22%)	4 (7%)	1 (2%)
	Missing	1 (2%)	15 (27%)	20 (36%)	4 (7%)	16 (27%)	22 (37%)
	***C**	8.1 (3)	6.9 (3)	5.8 (2)	8.5 (2)	6.6 (2)	5.9 (3)
	Low	2 (4%)	1 (2%)	2 (4%)	2 (3%)	2 (3%)	2 (3%)
	Normal	28 (60%)	28 (60%)	25 (53%)	43 (65%)	48 (73%)	43 (65%)
	High	10 (21%)	4 (9%)	0 (0)	16 (24%)	4 (6%)	3 (5%)
	Missing	7 (15%)	14 (30%)	20 (42%)	5 (8%)	12 (18%)	18 (27%)
Granulocytes *A		63.3 (14)	59.4 (10)	51.5 (10)	62.7 (13)	59.5 (11)	53.8 (10)
	Low	5 (10%)	3 (6%)	7 (13%)	3 (5%)	3 (5%)	8 (13%)
	Normal	20 (38%)	21 (40%)	25 (48%)	30 (47%)	35 (55%)	30 (47%)
	High	26 (50%)	10 (19%)	2 (4%)	27 (42%)	14 (22%)	4 (6%)
	Missing	1 (2%)	18 (35%)	18 (35%)	4 (6%)	12 (19%)	22 (34%)
	***B**	61.4 (15)	58 (9)	54.6 (10)	63.7 (10)	57.7 (10)	55.1 (8)
	Low	5 (9%)	2 (4%)	4 (7%)	1 (2%)	4 (7%)	4 (6%)
	Normal	26 (46%)	30 (54%)	25 (45%)	30 (51%)	28 (47%)	29 (49%)
	High	24 (43%)	9 (16%)	7 (13%)	24 (41%)	11 (19%)	4 (6%)
	Missing	1 (2%)	15 (27%)	20 (36%)	4 (6%)	16 (27%)	22 (37%)
	***C**	64.9 (10)	57.3 (9)	57.1 (10)	61.7 (13)	58.1 (9)	54 (9)
	Low	1 (3%)	2 (4%)	2 (4%)	6 (19%)	3 (5%)	5 (8%)
	Normal	20 (50%)	23 (49%)	17 (36%)	28 (42%)	41 (62%)	38 (58%)
	High	19 (47%)	8 (17%)	8 (17%)	27 (41%)	10 (15%)	5 (8%)
	Missing	7 (15%)	14 (30%)	20 (43%)	5 (8%)	12 (18%)	18 (27%)

Table 10.8.1 Laboratory results by HIV status and treatment group (cont)

Laboratory Test		Duration of treatment					
		HIV positive			HIV negative		
		0 week	2nd mth	6th mth	0 week	2nd mth	6th mth
ESR	***A**	95.9 (41)	**80.6 (39)**	**66.7 (42)**	78 (35)	44.7 (30)	30.6 (35)
	Low	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (3%)
	Normal	1 (2%)	0 (0)	2 (6%)	0 (0)	5 (8%)	8 (13%)
	High	48 (98%)	33 (63%)	30 (94%)	59 (92%)	45 (70%)	31 (48%)
	Missing	3 (6%)	19 (37%)	20 (38%)	5 (8%)	14 (22%)	23 (36%)
	***B**	92 (42)	**61 (35)**	**39.5 (33)**	70.8 (37)	56.5 (37)	23.7 (21)
	Low	0 (0)	0 (0)	2 (4%)	1 (2%)	1 (2%)	3 (5%)
	Normal	1 (2%)	1 (2%)	6 (11%)	2 (4%)	2 (4%)	8 (14%)
	High	54 (98%)	40 (71%)	27 (48%)	52 (88%)	40 (68%)	26 (44%)
	Missing	1 (2%)	15 (27%)	21 (38%)	4 (7%)	16 (27%)	22 (37%)
	***C**	94.9 (38)	**58.5 (36)**	**54.1 (42)**	81.6 (36)	47.2 (33)	25.7 (23)
	Low	0 (0)	1 (2%)	1 (2%)	0 (0)	0 (0)	2 (3%)
	Normal	0 (0)	1 (2%)	0 (0)	1 (2%)	8 (12%)	8 (12%)
	High	40 (100%)	31 (66%)	26 (55%)	59 (89%)	46 (70%)	37 (56%)
	Missing	7 (15%)	14 (30%)	20 (43%)	6 (9%)	12 (18%)	19 (29%)
Bilirubin T	***A**	.44 (.38)	.32 (.16)	.45 (.49)	.46 (.41)	.43 (.30)	.45 (.44)
	Low	1 (2%)	0 (0)	0 (0)	1 (2%)	1 (2%)	2 (3%)
	Normal	47 (90%)	36 (69%)	30 (58%)	58 (91%)	49 (77%)	39 (61%)
	High	2 (4%)	0 (0)	1 (2%)	3 (5%)	2 (3%)	1 (2%)
	Missing	2 (4%)	16 (31%)	21 (40%)	2 (3%)	12 (19%)	22 (34%)
	***B**	.52 (.63)	.36 (.16)	.48 (.49)	.45 (.23)	.49 (.69)	.58 (.63)
	Low	2 (4%)	0 (0)	0 (0)	1 (2%)	0 (0)	1 (2%)
	Normal	50 (89%)	42 (75%)	34 (61%)	54 (92%)	43 (73%)	33 (56%)
	High	4 (7%)	0 (0)	2 (4%)	1 (2%)	1 (2%)	1 (2%)
	Missing	0 (0)	14 (25%)	20 (36%)	3 (5%)	15 (25%)	24 (41%)
	***C**	.43 (.25)	.29 (.08)	.48 (.69)	.49 (.45)	.49 (.82)	.51 (.65)
	Low	2 (4%)	0 (0)	0 (0)	2 (3%)	1 (2%)	1 (2%)
	Normal	43 (91%)	33 (70%)	26 (55%)	59 (89%)	52 (95%)	43 (65%)
	High	1 (2%)	0 (0)	1 (2%)	3 (5%)	2 (4%)	3 (5%)
	Missing	1 (2%)	14 (30%)	20 (42%)	2 (3%)	11 (17%)	19 (29%)
Bilirubin C	***A**	.14 (.15)	.11 (.08)	.13 (.08)	.21 (.31)	.17 (.18)	.23 (.47)
	Normal	**44 (85%)**	33 (63%)	**31 (60%)**	44 (69%)	41 (64%)	**37 (58%)**
	High	**7 (13%)**	4 (8%)	**1 (2%)**	19 (30%)	11 (17%)	**5 (8%)**
	Missing	**1 (2%)**	15 (29%)	**20 (38%)**	1 (2%)	12 (19%)	**22 (34%)**
	***B**	.18 (.23)	.14 (.11)	.18 (.19)	.16 (.10)	.20 (.30)	.18 (.17)
	Normal	**39 (70%)**	37 (66%)	**27 (48%)**	34 (58%)	36 (61%)	**27 (46%)**
	High	**17 (30%)**	5 (9%)	**9 (16%)**	23 (39%)	8 (14%)	**8 (14%)**
	Missing	**0 (0)**	14 (25%)	**20 (36%)**	2 (3%)	15 (25%)	**24 (41%)**
	***C**	.19 (.31)	.11 (.06)	.12 (.09)	.21 (.30)	.16 (.15)	.16 (.04)
	Normal	**30 (64%)**	32 (68%)	**24 (51%)**	42 (64%)	48 (73%)	**40 (87%)**
	High	**16 (34%)**	1 (2%)	**2 (4%)**	22 (33%)	7 (11%)	**6 (13%)**
	Missing	**1(2%)**	14 (30%)	**21 (45%)**	2 (3%)	11 (17%)	**20 (30%)**

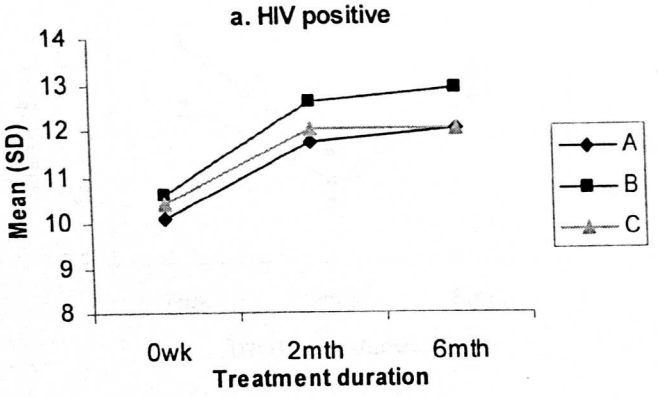
Table 10.8.1 Laboratory results by HIV status and treatment group (cont)

Laboratory Test		Duration of treatment					
		HIV positive			HIV negative		
		0 week	2nd mth	6th mth	0 week	2nd mth	6th mth
SGOT	***A**	7.6 (6)	6.6 (5)	6.5 (5)	7.6 (9)	4.8 (5)	5.9 (4)
	Normal	43 (83%)	34 (65%)	28 (54%)	58 (91%)	52 (81%)	41 (64%)
	High	8 (15%)	3 (6%)	3 (6%)	5 (8%)	1 (2%)	2 (3%)
	Missing	1 (2%	15 (29%)	21 (40%)	1 (2%)	11 (17%)	21 (33%)
	***B**	7.1 (6)	5.0 (3)	6.6 (6)	5.6 (4)	6.2 (4)	5.4 (4)
	Normal	49 (88%)	41 (73%)	33 (59%)	55 (93%)	43 (73%)	34 (58%)
	High	7 (12%)	1 (2%)	3 (5%)	2 (3%)	3 (5%)	1 (2%)
	Missing	0 (0)	14 (25%)	20 (36%)	2 (3%)	13 (22%)	24 (41%)
	***C**	6.4 (3)	5.6 (3)	9.5 (10)	6.6 (7)	5.7 (4)	6.3 (4)
	Normal	43 (94%)	32 (68%)	22 (82%)	59 (89%)	53 (80%)	46 (94%)
	High	3 (6%)	1 (2%)	5 (18%)	4 (6%)	1 (2%)	3 (6%)
	Missing	1 (2%)	14 (30%)	20 (43%)	3 (5%)	12 (18%)	17 (26%)
SGPT	***A**	14.9 (12)	14.1 (17)	13.5 (11)	10.6 (5)	10.8 (9)	9.4 (6)
	Normal	28 (57%)	27 (52%)	21 (40%)	47 (73%)	44 (69%)	37 (58%)
	High	21 (43%)	9 (17%)	10 (19%)	16 (25%)	9 (14%)	6 (9%)
	Missing	3 (6%)	16 (31%)	21 (40%)	1 (2%)	11 (17%)	21 (33%)
	***B**	12.3 (9)	10.3 (7)	13.8 (15)	10.9 (8)	11.6 (14)	9.3 (5)
	Normal	37 (66%)	33 (59%)	26 (46%)	37 (63%)	38 (64%)	32 (91%)
	High	19 (34%)	9 (16%)	10 (18%)	19 (32%)	8 (14%)	3 (9%)
	Missing	0 (0)	14 (25%)	20 (36%)	3 (5%)	13 (22%)	24 (41%)
	***C**	12.3 (7)	12.3 (8)	15.0 (16)	11.6 (11)	10.9 (8)	10.4 (7)
	Normal	29 (62%)	23 (49%)	20 (43%)	41 (62%)	44 (67%)	41 (62%)
	High	17 (36%)	10 (21%)	7 (15%)	21 (32%)	10 (15%)	8 (12%)
	Missing	1 (2%)	14 (30%)	20 (42%)	4 (6%)	12 (18%)	17 (26%)
Alk phosph.	***A**	283 (203)	214 (165)	178 (88)	214 (134)	185 (80)	161 (47)
	Low	1 (2%)	2 (4%)	4 (8%)	2 (3%)	0 (0)	0 (0)
	Normal	33 (63%)	26 (50%)	24 (46%)	49 (76%)	46 (72%)	38 (59%)
	High	17 (33%)	5 (10%)	3 (6%)	12 (19%)	3 (5%)	3 (5%)
	Missing	1 (2%)	19 (37%)	21 (40%)	1 (2%)	15 (23%)	23 (36%)
	***B**	237 (134)	187 (76)	160 (86)	238 (114)	190 (60)	169 (64)
	Low	1 (2%)	0 (0)	4 (7%)	0 (0)	0 (0)	1 (2%)
	Normal	40 (71%)	35 (62%)	28 (50%)	41 (69%)	38 (64%)	31 (53%)
	High	14 (25%)	5 (9%)	2 (4%)	16 (27%)	3 (5%)	1 (2%)
	Missing	2 (4%)	16 (29%)	22 (39%)	2 (3%)	18 (31%)	26 (44%)
	***C**	270 (203)	217 (170)	197 (101)	250 (142)	167 (64)	153 (46)
	Low	0 (0)	1 (3%)	0 (0)	1(2%)	4 (6%)	2 (3%)
	Normal	32 (68%)	26 (55%)	23 (92%)	43 (69%)	46 (70%)	43 (65%)
	High	12 (26%)	4 (9%)	2 (8%)	18 (29%)	3 (5%)	0(0)
	Missing	3 (6%)	16 (34%)	22 (47%)	4 (6%)	13 (20%)	21 (32%)

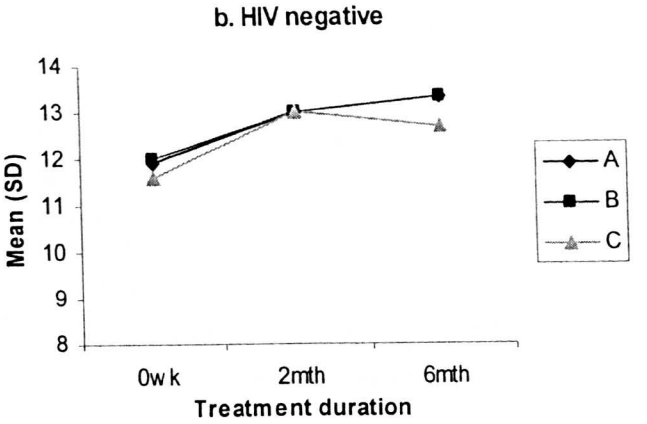
* Mean (SD); Underlined P ≤0.20; Bolded P ≤0.05;

Figure 10.8.1 Haemoglobin concentration (a and b) and ESR (c and d) by study group and HIV status

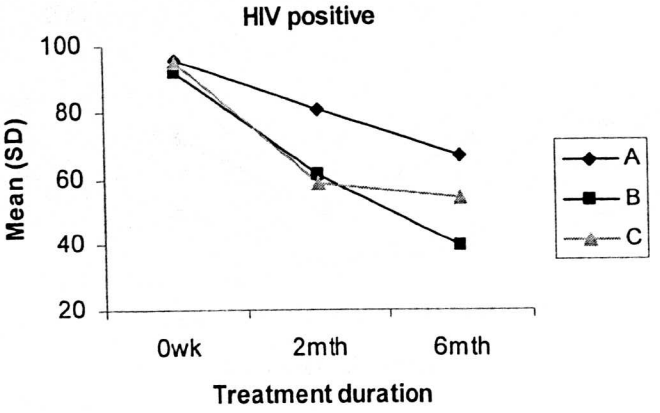
a



b



c



d

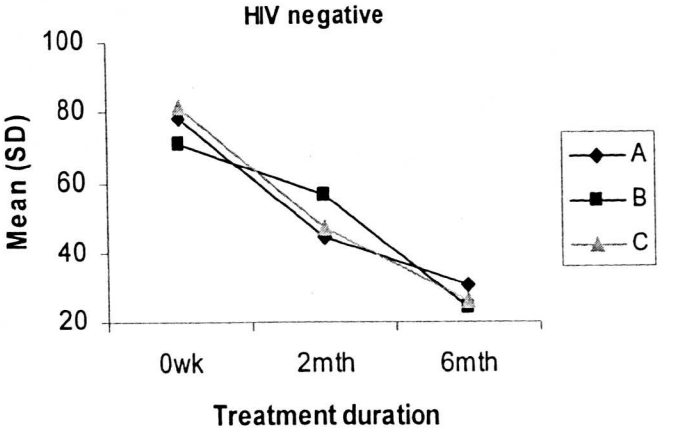
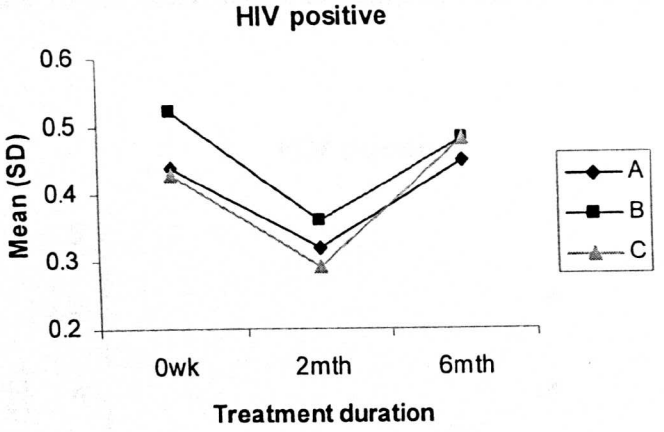
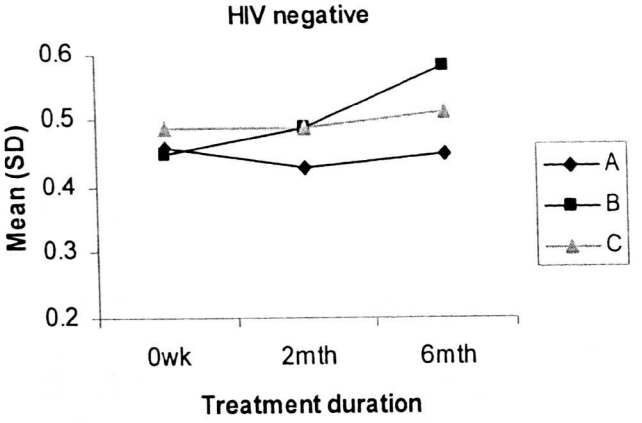


Figure 10.8.2 Total (a and b) and conjugated (c and d) bilirubin by study group and HIV status

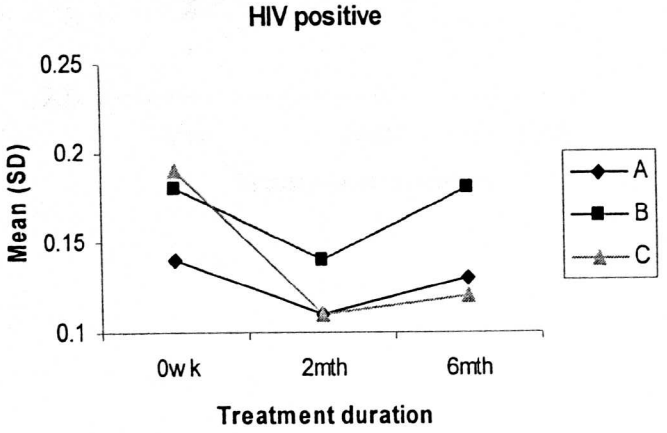
a)



b)



c)



d)

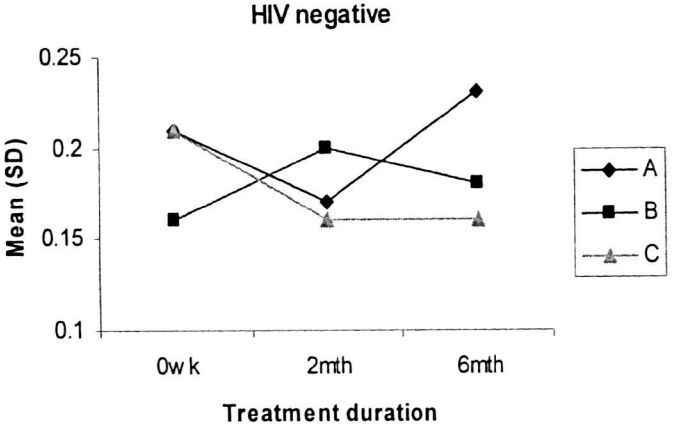


Figure 10.8.3 Mean serum albumin by study group and HIV status

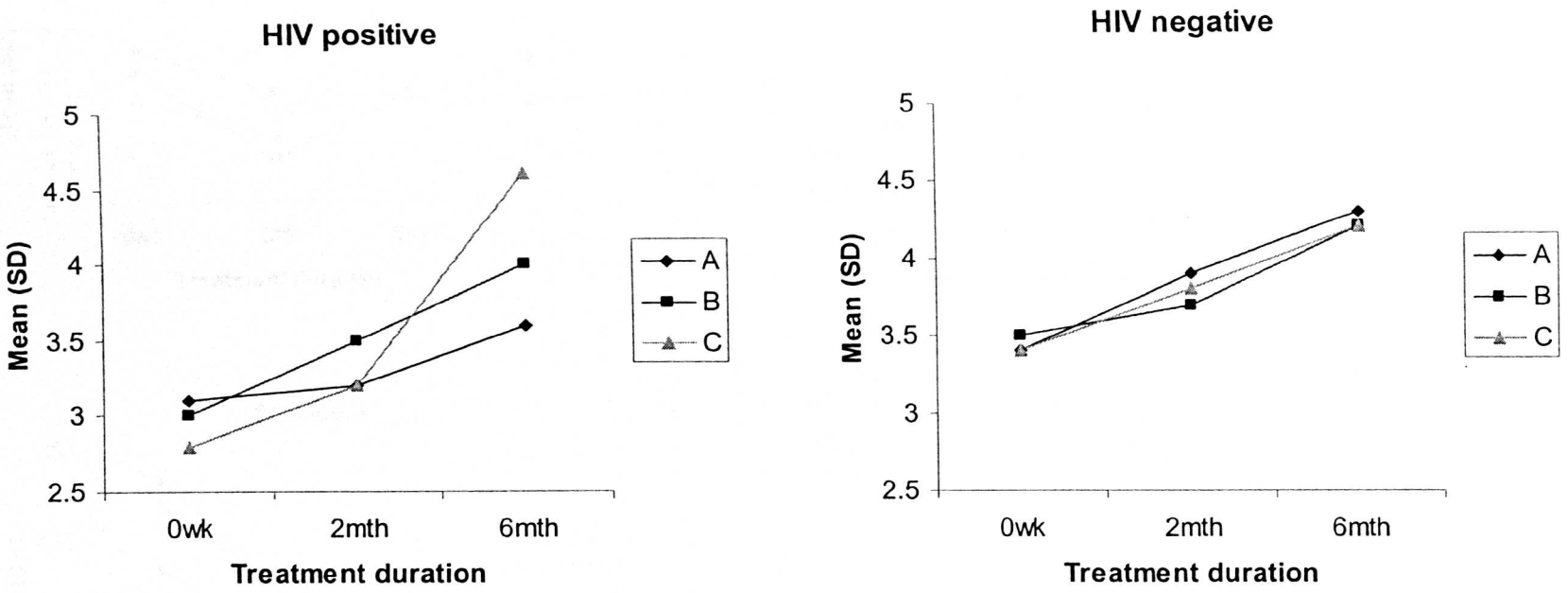
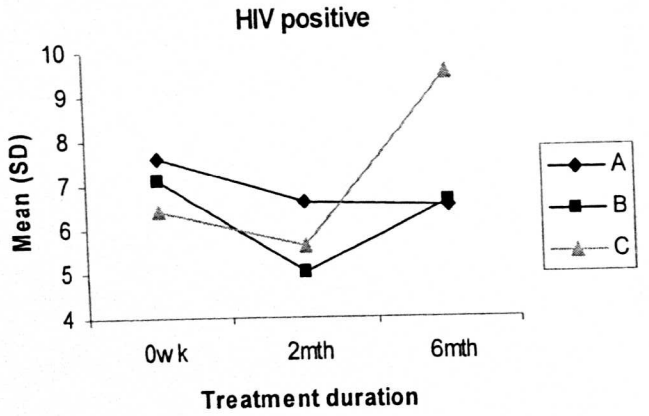
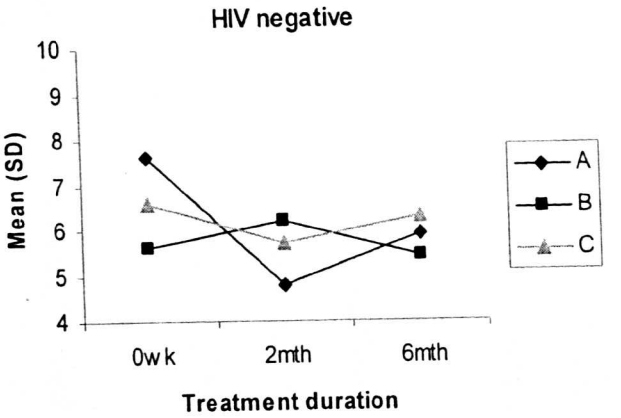


Figure 10.8.4 Mean SGOT (a and b) and alkaline phosphatase (c and d) by study group and HIV status

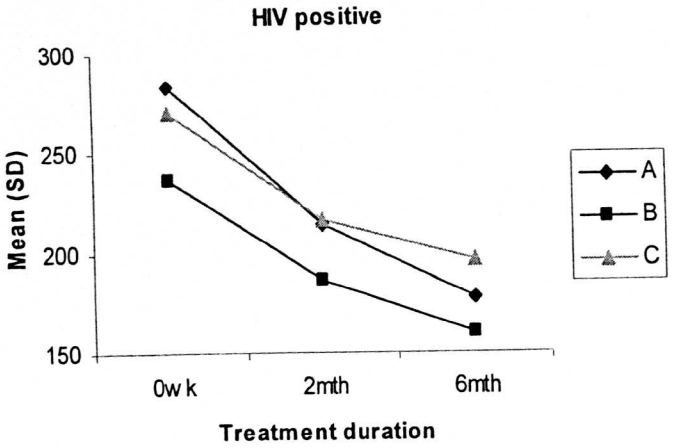
a)



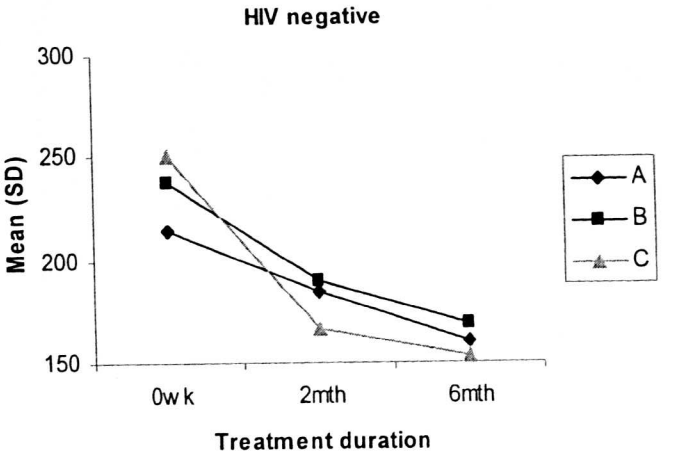
b)



c)



d)



10.9.1 Radiological results by their HIV status and treatment groups

All patients had their X-rays taken at the time of enrolment. These included 48 patients in group A, 45 in group B and 43 in group C who were HIV positive patients and 57 patients in group A, 53 in group B and 57 who were HIV negative. Among HIV positive patients, 11 (21%) in group A, 9 (16%) in group B and 9 (19%) in group C did not have cavities at enrolment. Among HIV negative patients, 9 (14%) in group A, 8 (14%) in group B and 7 (11%) in group C did not have cavities on enrolment.

By the 2nd month, 11 (21%) patients in group A, 10 (19%) in group B and 10 (21%) in group C among HIV positive patients and 19 (30%) in group A, 15 (25%) in group B and 15 (23%) among HIV negative patients did not have cavities in their chest X-rays.

At the end of the 6 months, the number of patients who did not have cavities had risen to 12 (23%) in group A, 13 (23%) in B and 14 (29%) in group C among HIV positive patients and 20 (31%) in group A, 16 (27%) in group B and 15 (23%) in group C among HIV negative patients.

Table 10.9.1 Radiological results by HIV status of patients in the three groups showing clearance of cavities with treatment

Treatment Duration	Patients groups	Cavity grading									
		HIV positive					HIV negative				
		0	1	2	3	Missing	0	1	2	3	Missing
0 month	A	11 (21)	11 (21)	18 (35)	8 (15)	4 (8%)	9 (14)	12 (19)	27 (42)	9 (14)	7 (11%)
	B	9 (16)	10 (18)	17 (30)	9 (16)	11 (20%)	8 (14)	10 (17)	25 (42)	10 (17)	6 (10%)
	C	9 (19)	10 (21)	16 (34)	8 (17)	4 (9%)	7 (11)	12 (18)	22 (33)	16 (24)	9 (14%)
2nd month	A	11 (21)	10 (19)	9 (17)	2 (4)	20 (38%)	19 (30)	14 (22)	4 (6)	2 (3)	25 (39%)
	B	10 (19)	8 (14)	5 (9)	2 (4)	31 (55%)	15 (25)	9 (15)	8 (14)	2 (3)	25 (42%)
	C	10 (21)	6 (13)	6 (13)	1 (2)	24 (51%)	15 (23)	9 (14)	10 (15)	2 (3)	30 (45%)
6th month	A	12 (23)	5 (10)	4 (8)	0 (0)	31 (59%)	20 (31)	4 (6)	1 (2)	0 (0)	39 (60%)
	B	13 (23)	3 (5)	0 (0)	1 (2)	40 (71%)	16 (27)	3 (5)	0 (0)	0 (0)	40 (68%)
	C	14 (30)	5 (11)	1 (2)	0 (0)	27 (57%)	15 (23)	4 (6)	4 (6)	0(0)	43 (65%)

* Percentages are calculated as a proportion of the 116/117 patients initially enrolled in groups A, B and C

10.9.2 Radiological results by HIV status, showing clearance of the disease lungs in the three treatment groups

The same X-rays were used to assess the extent of disease as earlier described in this chapter. Among HIV positive patients, only 1 (2%) patient in group A, 2 (4%) in group B and 1 (2%) in group C had all lung lobes clear (a score= 0). Among HIV negative patients, only 2 (4%) patients in group C had extent of disease lungs graded zero among HIV negative patients and most patients had 1 or 2 lobes affected (47% of cases) in groups A and C as shown in table 10.9.3.

By 2nd month of treatment, 5 (10%) patients in group A, 2 (4%) in group B and 3 (6%) in group C among HIV positive patients and 2 (3%) patients in group A, 2 (3%) in groups B and 3 (5%) in group C who were HIV negative had all their lung lobes read as normal (graded 0). The mean (SD) number of abnormal lobes among HIV positive was 3.1 (1.6) for group A, 2.4 (1.3) for group B and 2.5 (1.3) for group C (table 10.9.2). As for HIV negative patients, the mean (SD) number of abnormal lobes were 2.3 (1.2), 2.5 (1.4) and 2.2 (1.4) for groups A, B and C respectively (table 10.9.3). At the end of the 6th month, 4 (8%) patients in group A, 6 (11%) in group B and 7 (15%) in group C who were co-infected with HIV were graded 0 and 68 (13%) patients in group A, 6 (10%) in group B and 5 (8%) in group C who were HIV negative were graded as 0. The meam (SD) number of abnormal lobes among HIV positive patients was 1.6 (1.1) for group A, 1.0 (1.0) for group B and 1.1 (1.0) for group C (P= 0.1). As for HIV negative patients, the mean (SD) number of abnormal lobes were 1.2 (1.1), 1.1 (1.0) and 1.4 (1.3) for groups A, B and C respectively (P= 0.5).

Table 10.9.2 Radiological results in HIV positive patients among the 3 groups showing improvement in diseased lungs with treatment

Treatment Duration	Patients groups	Means (SD) abnormal lobes	Extent of diseased lung grading-HIV positive								
			0	1	2	3	4	5	6	Missing	Total
0 month	**A**	3.1 (1.6)	1 (2%)	7 (14%)	11 (21%)	9 (17%)	9 (17%)	8 (15%)	3 (6%)	4 (8%)	52
	B	3.0 (1.4)	2 (4%)	5 (9%)	9 (16%)	12 (21%)	11 (20%)	6 (11%)	1 (2%)	10 (18%)	56
	C	3.0 (1.3) P= 0.9	1 (2%)	4 (9%)	11 (23%)	11 (23%)	10 (21%)	5 (11%)	1 (2%)	4 (9%)	47
2nd month	**A**	2.6 (1.6)	5 (10%)	8 (15%)	5 (10%)	5 (10%)	6 (12%)	1 (2%)	3 (6%)	19 (37%)	52
	B	2.4 (1.3)	2 (4%)	7 (13%)	10 (18%)	6 (11%)	5 (9%)	2 (4%)	0 (0)	24 (43%)	56
	C	2.5 (1.3) P= 0.8	3 (6%)	8 (17%)	4 (9%)	6 (13%)	6 (13%)	1 (2%)	0 (0)	19 (40%)	47
6th month	**A**	1.6 (1.1)	4 (8%)	7 (13%)	6 (12%)	4 (8%)	1 (2%)	0 (0)	0 (0)	30 (58%)	52
	B	1.0 (1.0)	6 (11%)	10 (18%)	4 (7%)	1 (2%)	0 (0)	0 (0)	0 (0)	35 (63%)	56
	C	1.1 (1.0) P= 0.1	7 (15%)	7 (15%)	4 (9%)	2 (4%)	0 (0)	0 (0)	0 (0)	27 (57%)	47

* Percentages are calculated as a proportion of the 116/117 patients initially enrolled in groups A, B and C

Table 10.9.3 Radiological results in HIV negative patients among the 3 groups showing improvement in diseased lungs with treatment

Treatment Duration	Patients groups	Means (SD) abnormal lobes	Extent of diseased lung grading-HIV negative								
			0	1	2	3	4	5	6	Missing	Total
0 month	A	3.2 (1.4)	0 (0)	6 (9%)	13 (20%)	17 (27%)	9 (14%)	7 (11%)	5 (8%)	7 (11%)	64
	B	3.1 (1.3)	0 (0)	8 (14%)	8 (14%)	16 (27%)	15 (25%)	5 (8%)	2 (3%)	5 (8%)	59
	C	3.0 (1.3) P= 0.7	2 (3%)	3 (5%)	16 (24%)	18 (27%)	11 (17%)	4 (6%)	3 (5%)	9 (14%)	66
2nd month	A	2.3 (1.2)	2 (3%)	13 (20%)	11 (17%)	12 (19%)	4 (6%)	1 (2%)	0 (0)	21 (32%)	64
	B	2.5 (1.4)	2 (3%)	7 (12%)	12 (20%)	7 (12%)	7 (12%)	1 (2%)	1 (2%)	22 (37%)	59
	C	2.2 (1.4) P= 0.6	3 (5%)	11 (17%)	9 (14%)	8 (12%)	4 (6%)	2 (3%)	0 (0)	29 (44%)	66
6th month	A	1.2 (1.1)	8 (13%)	9 (14%)	6 (9%)	4 (6%)	0 (0)	0 (0)	0 (0)	37 (58%)	64
	B	1.1 (1.0)	6 (10%)	8 (14%)	4 (7%)	0 (0)	1 (2%)	0 (0)	0 (0)	40 (68%)	59
	C	1.4 (1.3) P= 0.5	5 (8%)	12 (18%)	3 (5%)	3 (5%)	1 (2%)	1 (2%)	0 (0)	41 (62%)	66

* Percentages are calculated as a proportion of the 116/117 patients initially enrolled in groups A, B and C

10.10.1 Clearance of bacilli by treatment group

All 116 patients in group A, 117 in group B and 117 in group C had 3 sputum specimens examined on enrolment. Patients were re-examined weekly for the first 8 weeks of treatment and then at monthly intervals for the next 4 months of treatment. There was general improvement in the clearance of bacilli in the three groups with treatment irrespective of the supplementation given (table 10.10.1). Sputum examinations on follow up were done in 2 sputum specimens, collected as morning and on-the-spot specimens. Patients were declared to be sputum negative for a given follow up if both smears were available and were read as negative.

By the end of the first week of treatment, 12 (10%) patients from group A, 13 (11%) from group B and 9 (8%) from group C had cleared bacilli from their sputa. Eleven (9%) patients in group A, 12 (10%) from group B and 9 (8%) from group C did not complete or submit sputum samples. By the 4th week a higher proportion of patients in group B (41, 35%) and group C (37, 32%) had cleared bacilli from their sputa compared to patients in group A (27, 23%) (P= 0.05). Thirty-three (28%) patients in group A (27, 23%) in group B and 26 (22%) in group C did not complete or submit sputum samples. At 6 weeks, patients in group C (47, 40%) had the highest proportion of patients who had cleared the bacilli from their sputum specimens, followed by patients in group B (45, 38%) and patients in group A (41, 35%) but these differences were not ststistically significant.

At the 2nd month follow up, the proportion of patients in groups A and B who had cleared the bacilli from their sputum had increased to 71 (61%) and 70 (60%)

respectively, while the proportion of patients in group C was 64 (55%) (P> 0.4). Twenty-five (21%) patients in group A, 25 (21%) in group B and 30 (26%) in group C did not complete or submit sputum samples.

At the end of the 6th month, 78 (67%) patients in group B, 73 (63%) in group A and 66 (56%) in group C had no bacilli seen in their sputa. Thirty-six (31%) patients in group A, 37 (32%) in group B and 42 (36%) of patients did not complete or submit sputum samples.

The time required for sputum clearance, (in weeks) by smear examination and the cumulative number of patients who had cleared the bacilli from their sputa for the three groups of patients are shown in table 10.10.2 and figure 10.10.1. The mean (SD) time required to clear the bacilli for the groups are shown in table 10.10.3. The mean (SD) time required for sputum clearance by smear examination for patients was 4.1 (3.1) weeks in group A, 3.3 (2.4) weeks in group B and 3.5 (2.6) weeks in group C. The mean (SD) sputum time clearance for patients in group A was significantly longer than the time for sputum clearance for patients in groups B and C (P= 0.03). The mean (SD) sputum clearance time for patients in group B and C however were not significantly different (P= 0.47). In other words, zinc supplement, wether single or in combination with vitamin A resulted in a mean one-week reduction in the time required for sputum clearance. The addition of vitamin A to zinc did not seem to enhance this reduction in time.

Table 10.10.1 ZN Smear microscopy results by treatment group

Duration	Groups	Smears				
		Negative	1 positive	2 positives	Missing	Total
0 week	A	0	0	116 (100%)	0	116
	B	0	0	117 (100%)	0	117
	C	0	0	117 (100%)	0	117
1st week	A	12 (10%)	23 (20%)	70 60%)	11 (9%)	116
	B	13 (11%)	33 (28%)	59 (50%)	12 (10%)	117
	C	9 (8%)	25 (21%)	74 (63%)	9 (8%)	117
2nd week	A	19 (16%)	26 (22%)	53 (46%)	18 (16%)	116
	B	24 (21%)	21 (18%)	49 (42%)	23 (20%)	117
	C	29 (25%)	27 (23%)	45 (38%)	16 (14%)	117
4th week	A	27 (23%)	28 (24%)	28 (24%)	33 (28%)	116
	B	41 35%)	15 (13%)	34 (29%)	27 (23%)	117
	C	37 (32%)	27 (23%)	27 (23%)	26 (22%)	117
6th week	A	41 (35%)	21 (18%)	18 (16%)	36 (31%)	116
	B	45 (38%)	14 (12%)	19 (16%)	39 (33%)	117
	C	47 (40%)	16 (14%)	20 (17%)	34 (29%)	117
2nd month	A	71 (61%)	11 (9%)	9 (8%)	25 (21%)	116
	B	70 (60%)	15 (13%)	7 (6%)	25 (21%)	117
	C	64 (55%)	10 (9%)	13 (11%)	30 (26%)	117
6th month	A	73 (63%)	6 (5%)	1 (1%)	36 (31%)	116
	B	78 (67%)	1 (1%)	1 (1%)	37 (32%)	117
	C	66 (56%)	5 (4%)	4 (3%)	42 (36%)	117

* All patients at enrolment were positive as per selection criteria

Table 10.10.2 Time required for bacilli clearance from sputum (in weeks) by treatment group

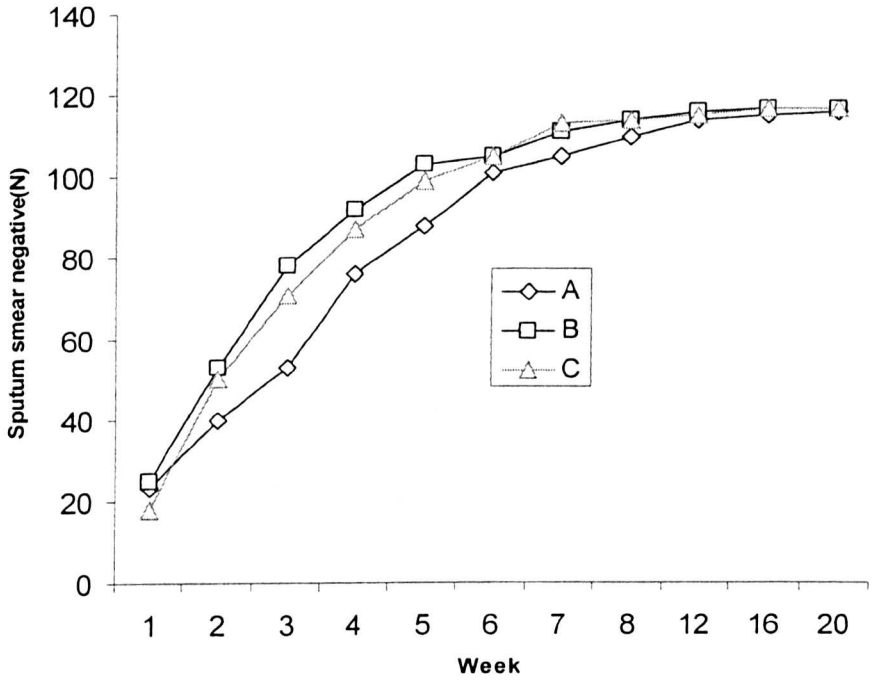
Sputum clearance	Treatment group					
(weeks)	A		B		C	
	N	N Cum	N	N Cum	N	N Cum
1	23	23	25	25	18	18
2	17	40	28	53	32	50
3	13	53	25	78	21	71
4	23	76	14	92	16	87
5	12	88	11	103	12	99
6	13	101	2	105	6	105
7	4	105	6	111	8	113
8	5	110	3	114	1	114
12	4	114	2	116	1	115
16	1	115	1	117	2	117
20	1	116	0	117	0	117

N= Number negative on follow up; N Cum= Cumulative number with negative microscopy

Table 10.10.3 Mean (SD) sputum clearance time by study group

Group	Mean (SD) sputum clearance time in weeks
A	4.1 (3.1)
B	3.3 (2.4)
C	3.5 (2.6)
B+C	3.4 (2.5)

Figure 10.10.1 Sputum bacilli clearance time by smear microscopy in the 3 treatment group



10.10.2 Time required for sputum clearance by smear examination by treatment group and HIV status

The time required for sputum clearance, (in weeks) by smear examination and the cumulative number of patients who had cleared the bacilli from their sputa for the three groups of patients in HIV positive and negative patients are shown in tables 10.10.4 and 10.10.6 and figures 10.10.2 and 10.10.3 respectively. The mean (SD) time required to clear the bacilli for the groups in HIV positive patient is shown in table 10.10.5 and in HIV negative patients in table 10.10.7.

The time for sputum clearance in HIV negative-patients shows that patients in groups B and C cleared bacilli from their sputa faster than in patients in group A, although this was only marginally significant (P= 0.07). The mean (SD) time required for

sputum clearance by smear examination in HIV positive patients was shorter in patients in groups B and C than in patients in group A (P=0.06).

Table 10.10.4 Bacilli clearance by treatment group in HIV positive patients

Sputum clearance	**Treatment group**					
(weeks)	**A**		**B**		**C**	
	N	**N Cum**	**N**	**N Cum**	**N**	**N Cum**
1	15	15	10	10	11	11
2	11	26	12	22	10	21
3	2	28	10	32	10	31
4	7	35	6	38	7	38
5	7	42	4	42	4	42
6	7	49	1	43	2	44
7	2	51	1	44	5	49
8	3	54	1	45	0	49
12	2	56	0	45	0	49
16	0	56	0	45	0	49
20	1	57	0	45	0	49

N= Number negative on follow up; N Cum= Cumulative number with negative microscopy; P= 0.13

Table 10.10.5 Mean (SD) sputum clearance time by study group for HIV positive patient

Group	Mean (SD) sputum clearance time in weeks
A	4.0 (3.4)
B	2.9 (1.7)
C	3.2 (1.9)

P= 0.06

Figure 10.10.2 Sputum bacilli clearance time by treatment group in HIV positive patients

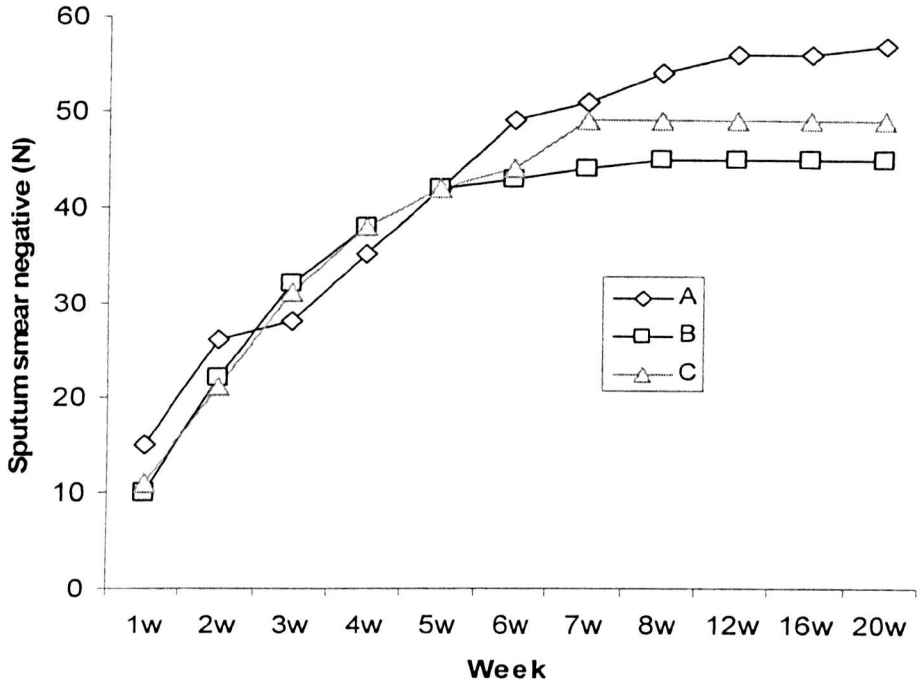


Table 10.10.6 Bacilli clearance by treatment group in HIV negative patients

Sputum clearance (weeks)	Treatment group					
	A		B		C	
	N	N Cum	N	N Cum	N	N Cum
1	8	8	13	13	6	6
2	5	13	12	25	18	24
3	7	20	15	40	10	34
4	15	35	6	46	8	42
5	5	40	3	49	7	49
6	3	43	0	49	4	53
7	1	44	5	54	1	54
8	2	46	2	56	1	55
12	2	48	2	58	1	56
16	1	49	1	59	1	57
20	0	49	0	59	0	57

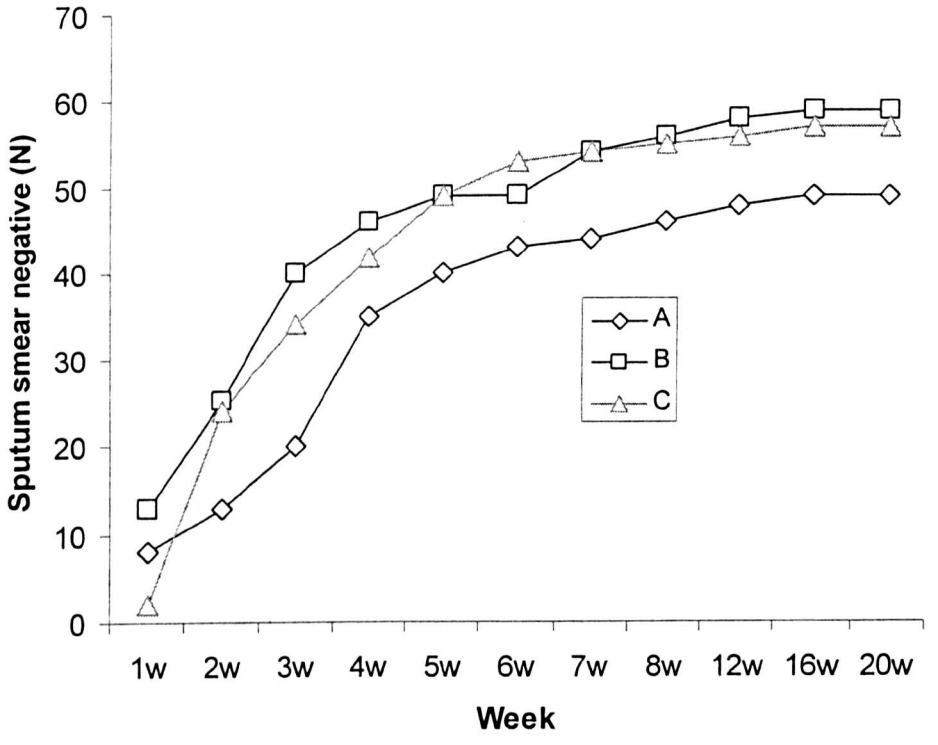
N= Number negative on follow up; N Cum= Cumulative number with negative microscopy; P= 0.07

Table 10.10.7 Mean (SD) sputum clearance time by treatment group in HIV negative patients

Group	Mean (SD) sputum clearance time in weeks
A	4.1 (3.0)
B	3.6 (3.0)
C	3.6 (2.6)

P= 0.5

Figure 10.10.3 Sputum bacilli clearance time by treatment group in HIV negative patients



10.10.3 Clearance of bacilli by culture by treatment group

All patients in group A (107, 100%), but only 104 (98%) of the 117 patients in group B and 103 (95%) in group C were culture positive on enrolment (table 10.10.8 and figure 10.10.4). By the end of the 2nd month of treatment, 27 (25%) patients in group A, 35 (33%) in group B and 35 (32%) in group A, had become culture negative.

By the 6th month of treatment, 40 (37%) patients in group A, 46 (43%) in group B and 42 (39%) of patients in group C had become culture negative. These proportions were not statistically different.

Among the HIV positive patients, 47 (100%) patients in group A, 50 (96%) in group B and 43 (96%) in group C were culture positive, while 60 (100%) patients in group A, 52 (100%) in group B and 43 (96%) in group C who were HIV negative were also culture positive. By the end of the 2nd month of treatment, 9 (19%) patients in group A, 12 (23%) in group B and 8 (18%) in group C among HIV positive patients had become culture negative (P= 0.7). Among HIV negative patients, 18 (30%) in group A, 23 (44%) in group B and 25 (42%) in group C had become culture negative (P= 0.05).

At the 6th month of treatment, 16 (34%) patients in group A, 20 (38%) in group B and 13 (28%) in group C had become culture negative among HIV positive patients (P= 0.8), while 24 (40%) patients in group A, 26 (50%) in group B and 29 (49%) in group C who were HIV negative had become culture negative (P= 0.2) (table 10.10.8 and figures 10.10.5, a and b).

Table 10.10.8 Culture results by treatment group at enrolment, 2nd and 6th month follow up

Treatment	Patients	HIV status: Positive		Negative		Total	
		BACTEC Culture					
		Positive	Negative	Positive	Negative	Positive	Negative
0 month	A	47 (100%)	0 (0)	60 (100%)	0 (0)	107 (100%)	0 (0)
	B	50 (96%)	2 (4%)	52 (100%	0 (0)	104 (98%)	2 (2%)
	C	43 (96%)	2 (4%)	56 (95%)	3 (5%)	103 (95%)	5 (5%)
2nd month	A	25 (53%)	9 (19%)	**36 (60%)**	**18 (30%)**	61 (57%)	27 (25%)
	B	26 (50%)	12 (23%)	**22 (42%)**	**23 (44%)**	48 (45%)	35 (33%)
	C	27 (60%)	8 (18%)	**19 (32%)**	**25 (42%)**	46 (43%)	35 (32%)
6th month	A	16 (34%)	16 (34%)	23 (38%)	24 (40%)	39 (36%)	40 (37%)
	B	15 (29%)	20 (38%)	15 (29%)	26 (50%)	30 (28%)	46 (43%)
	C	13 (28%)	13 (28%)	13 (22%)	29 (49%)	26 (24%)	42 (39%)

Figure 10.10.4 Proportion of patients with negative culture by treatment group

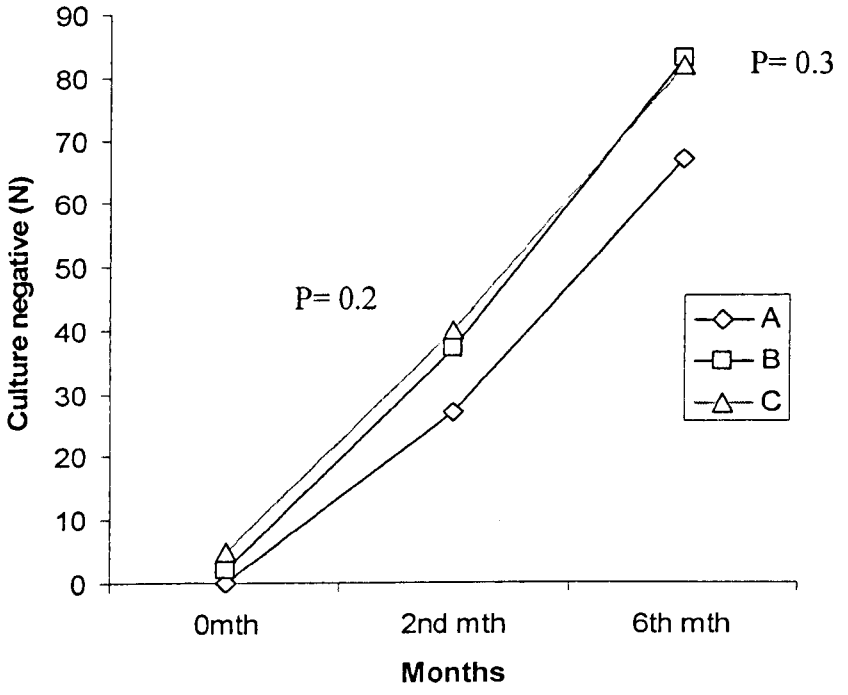
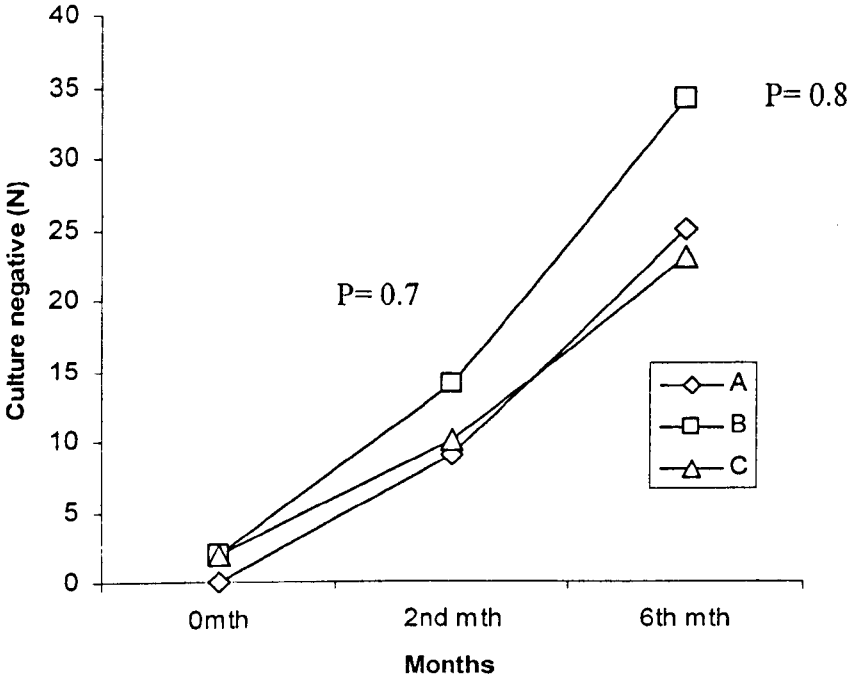
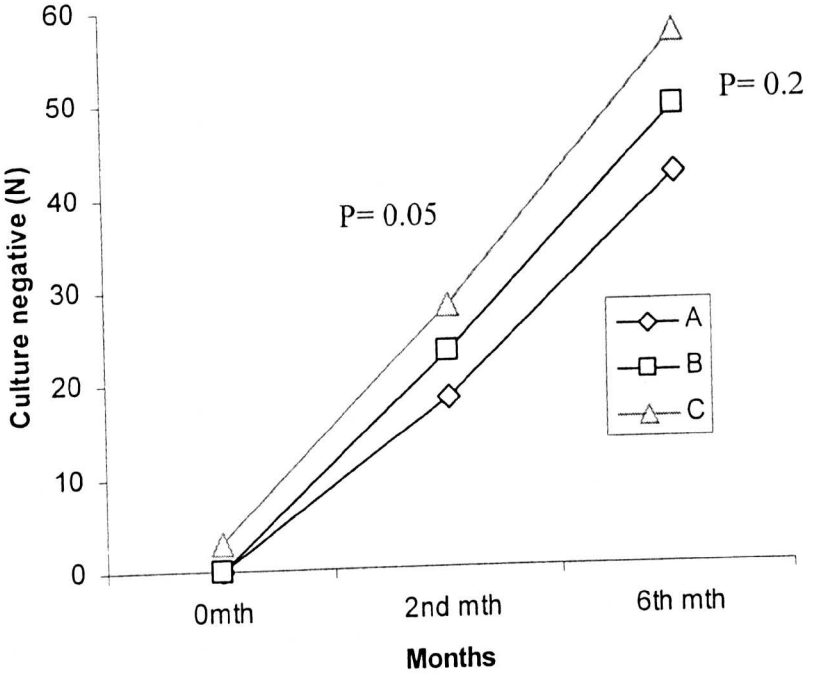


Figure 10.10.5 Proportion of patients with negative culture by treatment group and HIV status

a. HIV positive



b. HIV negative



10.11 Discussion

Of the 350 patients enrolled for the micronutrient trial, 116 were allocated to receive placebos (group A), 117 received weekly zinc supplements (group B) and 117 received weekly zinc plus vitamin A (group C). A total of 261 (75%) of the 350 patients enrolled completed the trial. Of these, 91 (76%) were in group A, 89 (76%) in group B and 81 (69%) in group C. Twenty-four (21%) patients in group A, 19 (16%) in group B and 27 (23%) in group C were lost to follow up. As expected in a highly mobile urban population as Abuja, the dropout rate was substantial. Of the 350 patients enrolled for the trial, 283 (81%) attended the 2^{nd} month follow up but only 261 (75%) attended the 6^{th} month follow up, resulting in for a drop out rate of 25%. Fourteen (4%) of the patients were dead by the 2^{nd} month and 19 (5.4%) by the end of the trial. The high dropout rate may have also been due to the mass movement of patients to new locations in and out of Abuja as a result of the demolision exercise embarked upon by the government in the shantytowns situated at the outskirts of town where most of our patients lived. Because of the poor economic situation of most of the patients, a number of them could not continue with the follow up as most of their residences were far from the participating hospitals. Finally, a number of patients who could not produce sputum for examination felt they did not need to continue attending the follow up clinics. In Tanzania, 91% of enrolled patients were alive at the end of 2-month follow up and continued with treatment (Range et al., 2005) while in Indonesia, 74% in the micronutrient group and 71% in the placebo group completed the study (Karyadi et al., 2002). The death rate in our study was low and the majority of those who died were HIV positive. Only one patient died from the group that received placebo while 9 patients died from each group that received zinc and zinc

plus vitamin A and this was statistically significant. It is also important to note here that patients who were supplemented with micronutrients in this study had significantly increasingly higher bilirubin concentration than patients not supplemented. Though not statistically significant, the CRP in patients not supplemented improved better than those supplemented as treatment progressed. The questions that can be asked will be; could zinc be provoking (a) more reaction to MTB, (b) enhanced HIV viremia (c) auto-immune reconstitution or (d) side effects/toxicity. Future research activities will be required to identify the toxic effect of zinc on the body, before its general use for supplementation in TB treatment could be encouraged. Our patients with HIV infection were not given any anti-retroviral drugs during the course of anti-TB and micronutrient therapy in order to prevent the drug reaction frequently reported between anti-retroviral drugs and anti-TB drugs. Despite this, considerable clinical improvement was observed in all the patients. Two hundred and fourteen (61%) patients attended the required 8 visits, 53 (15%) attended 7, 25 (7%) attended 6 and the rest attended ≤ 5.

An important finding was the statistically significant difference in the number of deaths of 1 (1%) patient in group A compared to 9 (8%) in each of groups B and C. Most of the deaths occurred during the early stages of treatment and in patients with HIV co-infections. Side effects of zinc and vitamin A supplementation in trial of other infectious diseases have rarely reported an increase in mortality and these supplements are generally considered safe at standard nutritional doses. One study in Bangladesh however, reported that malnourished children who received zinc supplementation in doses several times the recommended daily requirements had a higher mortality (Doherty et al., 1998).

Observational studies that examine the relationship between individual micronutrients and pregnancy outcome in HIV-infected women have described that low plasma concentration of vitamin A are associated with a higher risk of mother to child transmission of HIV (Fawzi et al., 2004). Most prospective randomised trials however, have failed to demonstrate a beneficial effect in the transmission of HIV, although other beneficial effects have been noted (Fawzi et al., 2005). Mothers receiving supplements did not have an increase in side effects, with the notable exception of a study in Tanzania, where mothers receiving supplements were more likely to transmit HIV to their babies (Wiysonge et al., 2005; Fawzi et al., 1999).

There are fewer studies regarding zinc in pregnant women. However, an observational study in the USA described that high dietary zinc intake was associated with a higher risk of progression to AIDS and mortality (Kupka et al., 2002), although other studies have reported a positive association with CD4 counts. There are clinical trials assessing its efficacy and safety in pregnancy at the time of completion of this thesis. Besides pregnant women, observational studies have provided confliting results on the role of zinc and HIV progression (Kupka et al., 2002), but the data is difficult to interpret because there are no clinical trials published to date and low plasma zinc may be a worker that is not in the causal path of disease progression.

A further possibility is an immunological reconstitution due to a combination of micronutrients with TB treatment. Zinc and vitamin A have a prominent role in CMI and other immunological mechanisms. It has recently been reported that patients with HIV who receive TB treatment have a deterioration of their clinical symptoms due to improvement of their immunity. The immune reconstitution syndrome was recognised

before the the discovery of HIV in patients with TB (Goebel 2005), and has become more frequent in patients receiving ART and may be related to a patient's improved capacity to mount an inflammatory response. Our monitoring safety committee was not provided with HIV information, as the databases were unlinked at the time of their monitoring. The increased mortality pattern only became clear when deaths were analysed by subgroup.

Social and economic characteristics of the patients were similar across the groups. Clinical symptoms of patients in the three groups improved with treatment. Faster resolution of cough, headache, night sweats, dypsnoea and chest pains occurred in patients in group C compared to patients in groups B and A and patients in group B had faster resolution of symptoms than patients in group A. However these changes were not statistically significant. Similarly, while general improvement in clinical signs occurred in all the three groups, patients in group C had significant improvement in chest anormalies and together, patients in group B had better Karnofsky scores than patients in group A. Patients in group B had significantly improved BMI and higher weight increases than patients in groups A and C as treatment progressed.

Laboratory results also improved as treatment progressed in the three groups. More patients in groups B and C had values for most laboratory results within the normal ranges as treatment progressed. However, these results were not significant. Of note is the observation that a higher proportion of patients in group A had better improved total and conjugated bilirubin than patients in groups B and C with treatment and this was statistically significant for conjugated bilirubin.

The proportion of patients with faster resolution of symptoms and signs as treatment progressed was higher among HIV negative patients compared to HIV positive patients irrespective of the treatment group. Patients in groups B and C had quicker resolution of their cough than patients in group A and patients in group C had earlier resolution of all other clinical symptoms than patients in groups B and A among both HIV positive and HIV negative patients, though these are not statistically significant. Similarly, patients in groups C and B had better-improved chest pathology and mean Kanofsky score than patients in group A, while improvement in BMI and weight increases were more prominent in patients in group B compared to patients in groups A and C. There was a general improvement in the laboratory results of patients with and without HIV infection in the three groups of patients as treatment progressed. Better results in the laboratory tests were observed in patients in groups B and C compared to patients in group A. Weight gain and BMI improvement with zinc supplementation have been reported in previous studies (Pant et al., 1987) and our findings are in agreement with Karyadi's study who reported significant increases in weight gained and BMI level in patients with TB receiving zinc plus vitamin A.

No significant differences however were observed in the chest X-rays of patients in the 3 study groups for the resolution of cavities or the improvement of lung disease with duration of treatment irrespective of their HIV status, compared to observations made in Karyadi et.al., (Karyadi et al., 2002) and Pant et al., (Pant et al., 1987).

Of 116 patients in group A, 117 in group B and 117 in group C who had their sputa examined at enrolment and subsequently, a higher proportion of patients in groups B

and C had significantly cleared bacilli from their sputa compared to patients in group A by 8 weeks. The mean (SD) time of clearance of bacilli from sputum by microscopy was 4.1 (3.1) weeks in group A, 3.3 (2.4) weeks in group B and 3.5 (2.6) weeks in group C. The combination of the mean sputum time clearance for patients in Groups B and C was significantly lower than the mean sputum time clearance for patients in group A, however, no significant difference was observed with comparison of the mean (SD) sputum time clearance for patients in group B against patients in group C. About a third of the patients in the three groups did not complete or submit sputum samples at 6^{th} month follow up.

Only few studies are available on the effect of supplementation with zinc or zinc plus vitamin A on the outcome of treatment with anti-TB drugs. In a double-blind placebo-controlled study of vitamin A and zinc supplementation in persons with TB in Indonesia, Karyadi et al., (Karyadi et al., 2002) described improvement in the effect of TB medication after 2 months of anti-TB therapy and earlier sputum smear conversion after supplementation. Also in an earlier small study on supplementation of anti-TB therapy with zinc in TB patients in India, Pant et al., (Pant et al., 1987) described a more rapid improvement of the general conditions of the patients, significantly greater weight gain and more rapid sputum conversion when compared to those receiving anti-TB therapies alone. In the most recent study of 499 PTB patients in Mwanza, Tanzania, on the effect of zinc and multi-micronutrient supplementation on treatment outcome, in patients with PTB, the authors found no effect on sputum conversion and X- ray resolution area as reported by Karyadi et al. (Range et al., 2005). While the primary outcome in the study by Range et al., was culture conversion, the primary outcome in the study by Kayardi et al., and Pant et al.,

was sputum smear conversion. Range et al., (Range et al., 2005) argued that culture conversion is a more reliable outcome because at some point, patients with TB undergoing treatment do not excrete viable bacilli. Our study while duplicating the work of Karyadi et al., (Karyadi et al., 2002) with some modifications, went further to compare the effect of supplementation with zinc plus vitamin A against zinc alone on anti-TB therapy in a randomised placebo-controlled intervention study in a setting with high HIV prevalence using both microscopy and culture for identification of bacilli.

Our study showed that the groups that received supplementation with zinc or zinc plus vitamin A had earlier resolution of clinical symptoms and signs on anti-TB therapy and showed faster sputum conversion time than those without supplementation. Karyadi et al., (Karyadi et al., 2002) described improvement in the effect of TB medication after 2 months of anti-TB therapy and earlier sputum smear conversion after supplementation. We observed no difference in resolution of X-ray features in the three groups. At 6 months, the proportion of patients without chest cavities was higher in patients in group A than in patients in groups B and C. The differences however, were not statistically significant. No significant differences was observed in the extent of disease in the lungs of the 3 treatment groups.The proportion of patients in group C without cavities in HIV positive patients at 6 months was higher than in groups B and C and the proportion of patients in group B among HIV negative patients without cavities was higher than in groups A and C. These differences were also not statistically significant. Our X-ray results did not show earlier resolution in patients with micronutrient supplementation as reported by Karyardi et al., (Karyadi et al., 2002). However, Range et al., (Range et al., 2005) did not observe such resolution

in their study. The difference observed between the X-ray results in our study and Range et al., as opposed to Karyadi's results could be due to the nature of the study population. While Karyadi's study was based in Indonesia, our study and that of Range et al., were based in Africa where patients are more likely to seek medical attention late, as seen in our study where duration of symptoms had been for a year or more, and had more severe chest pathology. Most of the X-rays seen in our study still showed lesions even when the patients had become smear negative.

In our study, both culture and sputum smear conversion methods were used. While smear conversion time was significantly reduced in patients supplemented with zinc and zinc plus vitamin A compared to those not supplemented, the culture conversion time was equally faster for the supplemented groups but this was not statistically significant for all patients. However, a significant reduction was observed at the 2^{nd} month in HIV negative patients whose sputa were cultured. The difference in conclusions by Karyadi et al., and Range et al., could result from the HIV status of the different locations. Indonesia, like China and Bangladesh belong to the WHO Western Pacific Region where the HIV prevalence is 1% (Dye et al., 1999; Dye et al., 2005), whereas Tanzania, like most countries in sub Saharan Africa has a high HIV prevalence (Simooya et al., 1991; Elliott et al., 1990). Our study suggested that without the effect of HIV, even when culture is used, patients supplemented with zinc or zinc plus vitamin A may be more likely to convert to culture-negative than patients not supplemented but larger studies would be required. Karyardi, et al., and Range et al., however limited their studies to the first two months while our study spanned through the 6 months of treatment and this may also explain the differences.

We did not observe any difference between patients supplemented with zinc plus vitamin A or with zinc alone. Zinc is an important micronutrient. Its role in human nutrition, health and disease has been extensively documented (Prasad et al., 1971a; Pories et al., 1967). Zinc pivotally influences the actions of hundreds of enzymes, stabilizes cell membrane and modulates humoral and CMI (Thurnham et al., 2000) which play a major role in the host response to tubercule bacilli. Even though studies have reported improvement in the clinical status of patients supplemented with zinc and vitamin A in diseased patients (Smith 1980; Christian et al., 2001; Karyadi et al., 2002), data on interaction between the two however are limited in humans and results of some of the studies are inconclusive. Vitamin A supplementation in conjunction with anti-TB treatment was shown to have some negative effects on clinical response in South African children with TB (Hanekom et al., 1997) and our study did not find any additional benefit in supplementation wth the combination of vitamin A and zinc compared to zinc alone.

Current treatment of TB lasts a minimum of 6 months and requires patients swallowing minimum of 4 tablets daily in the DOTS strategy. There have been many attempts to introduce shorter regimens, which could reduce the dosage and frequency of administration of anti-TB drugs. Introduction of such new drugs could lead to better drug compliance. Drugs that can facilitate faster sputum conversion could also reduce the risk of tuberculosis transmission, which is a major benefit to the community. Our study found some clinical improvement in patients supplemented with zinc and zinc plus vitamin A and a significantly faster conversion of culture-positive sputum to culture-negative sputum at 2 months in patients supplemented compared to those not supplemented. However, the mean time for clearance of bacilli by smear microscopy

was a week shorter in patients supplemented than those not supplemented and larger proportion of patients among those without HIV cleared their sputa faster in patients in groups B and C compared to patients in group A. It might be tempting to recommend inclusion of zinc into the current TB treatment but the evidence for such inclusion is not conclusive, as for instance, the difference in the mean time of clearance of bacilli between those supplemented and those not supplemented is only 1 week and this shorter time would not be of clinical or public health importance. In addition, most of the better-improved clinical presentations in patients supplemented were not statistically significant. However, in areas of low HIV prevalence, supplementation with zinc as an adjuvant to anti-TB therapy may be useful. Further investigations must be carried out to determine the effects of zinc in combination with anti-TB drugs before its inclusion in TB treatment.

CHAPTER ELEVEN

General Discussion

The study comprised of patients attending eight district hospitals in Abuja, Nigeria with a clinical suspicion of tuberculosis. A research laboratory at Zankli Medical Centre, Abuja, Nigeria performed sputum microscopy, culture and blood analysis and an X-ray department in the same centre conducted chest-X-rays for patients who were enrolled into the micronutrient trial.

During the study period from September 2003 to April 2005, 1321 patients were screened for TB after compliance with the inclusion and exclusion criteria for enrolment into the project, discussion with the nurses and completion of the consent form. Seven hundred and seventy-four (59%) were male and 547 (41%) were female. The mean (SD) age for all patients was 34 (12) years, males 35 (11) years and females 33 (12) years. Three hundred and ninety-nine (30%) of the 1321 patients screened fulfilled the WHO definition for positive TB ($\geq$ 2 positive direct smears) of which 350 were enrolled into the micronutrient trial. One thousand, two hundred and eighty-six (97%) sputum samples from the 1321 patients were cultured on the BACTEC 960. Of this, 731 (62%) were culture positive and 455 (38%) were culture negative. A total of 1045 (79%) of the 1321 patients were screened for HIV. Five hundred and sixty-six (54%) were positive for HIV and 479 (46%) were negative. Of those positive, 317 (56%) were male and 249 (44%) were female (P= 0.003).

The cornerstone of global TB control strategies is the rapid identification and treatment of smear-positive patients. The direct examination of sputum is still the most important method of making a diagnosis of PTB. Though direct smear microscopy is relatively insensitive for the diagnosis of TB, simple digestion of sputum with household bleach prior to the smear preparation has been reported to improve its sensitivity even in HIV positive patients. This method however had not been validated against culture. Chapter 5 described a study that aimed to validate the bleach technique against culture in a sub-Saharan African setting by comparing bleach digested sputum stained with ZN with smears prepared the standard method and culture. For this purpose, 756 consecutive patients with symptoms suggestive of pulmonary TB (PTB) were asked to submit 3 sputum specimens to prepare direct smears. One specimen was selected at random for culture and another specimen was digested with bleach to prepare a further smear. After counselling, patients were tested for HIV. Four hundred and fifty-five (60%) of the 756 patients were culture positive. Two hundred and thirty-five (31%) had "definite" PTB based on the WHO case definition for smear-positive PTB (≥2 positive direct smears or one positive smear and positive culture). A further 223 (29%) patients were "very likely" to have PTB (positive culture but three negative direct smears). The WHO case definition identified 51% (235/458) of the patients with "definite" or "very likely" PTB. One digested smear detected 219 (93%) of the 235 patients with "definite" PTB and 10 patients with "very likely" PTB. A single digested smear identified 229 (50%) of the cases with PTB. Therefore, similar number of patients were identified by a single digested smear and the 3-sputum smear strategy with confirmatory culture of singleton positives with similar sensitivity and specificity (95%CI) of 50% (229/458; 45%-55%) and 99% (97%-100%) respectively. The positive and negative predictive

values for one digested smear were 98% (95%-99%) and 56% (52%-60%) respectively, which were not different ($p>0.5$) to the values for the WHO case definition (100% and 57% respectively). Our study therefore demonstrated that a single, bleach-digested smear is as sensitive and specific as 3 direct smears for the diagnosis of PTB. The method has the potential to improve access to a quality-assured TB diagnosis, particularly for poorer patients.

In addition, the digestion of sputum in bleach has not been studied in population with a high prevalence of HIV. Chapter 6 therefore described a study to assess the performance of bleach-digested smears in diagnosing PTB among patients with and without HIV. Four hundred and fifty-five (60%) of the 756 patients, included in chapter 5, had PTB and 230 (56%) of the 413 patients screened for HIV were positive. One hundred and sixteen (50%) of the 225 HIV-positive and 113 (62%) of the 181 HIV-negative patients with PTB were smear-positive. In comparison, one digested smear identified 111 (49%) of the 225 HIV-positive and 108 (60%) of the 181 HIV-negative patients with PTB. Three direct smears identified 235 (51%) and one digested smear identified 229 (50%) of the 458 patients with PTB. As both methods were less sensitive in HIV-positive than in HIV-negative patients, one digested smear could still improve the detection rate and efficiency of PTB diagnosis independently of the prevalence of HIV.

In addition to modifying the yield of sputum, several investigators have suggested that smears with a small number of bacilli (scanty) should be considered positive. Very few study however have assessed if smears with scanty bacilli have a lower specificity. Chapter 7 therefore described a study to verify if sputum smears graded as

scanty according to the IYATLD classification were false positive among TB suspects attending hospitals in Abuja, Nigeria. For this purpose sputum smears from 1068 patients were graded by the IUATLD classification. Of these, 824 (26%) smears were positive, 137 (4%) scanty and 2243 (70%) negative. Of the 1068 cultures, 680 (64%) were positive. One hundred and thirty (95%) scanty and 809 (98%) positive-smears were culture-positive. Twelve of the 18 patients with single scanty smear and 51 of 52 with $\geq$ 2 scanty smears were culture-positive. Fewer than 5% scanty results, < 1% of the patients treated for TB, were false positive. These findings suggest that with adequate quality control, smears reported as "scanty" are more likely to be true than false positive.

This thesis also described the clinical presentation and risk factors for TB in patients with and without HIV. Patients $\geq$ 15 years with clinical suspicion of TB had a physical examination on enrolment and blood samples taken for biochemical and HIV serological studies. Of 1186 patients who had their sputum cultured, 731 (62%) were culture-positive and 455 (38%) culture-negative. Factors significantly associated with an increased risk of culture-positive TB were weight loss, a BMI $\leq$ 18.5, the presence of anaemia and conjugated bilirubin $\geq$ 0.2 mg/dl and a granulocyte count $\geq$ 65%. Those marginally associated were the presence of haemoptysis and a Kanorfsky score < 60. Factors positively associated with HIV among culture-positive patients were anorexia, BMI $\leq$ 18.5, anaemia, hypoalbuminaemia, raised ESR and SGOT. Factors positively associated with HIV among culture-negative patients were anaemia and hypoalbuminaemia. In conclusion, a BMI < 18.5 plus weight loss and the presence of anaemia, conjugated bilirubin $\geq$ 0.2 mg/dl and a granulocyte count $\leq$ 65% could independently predict TB. In addition, being a female, having anorexia plus a BMI <

18.5 in the presence of anaemia, hypoalbuminaemia, raised ESR, and high SGOT were independent predictors of TB and HIV.

The early identification of patients with PTB, whether they are smear-positive or smear-negative is desirable, both to allow for appropriate control procedures and to provide a basis for early therapy. Chapter 9 describes the clinical presentation of patients with smear-positive and smear-negative TB with and without HIV. Of the 731 culture-positive patients, 353 (48%) were smear-positive and 378 (52%) smear-negative. Two hundred and twenty-eight (65%) of smear-positive and 208 (55%) of smear-negative patients were male. Smear-positive patients were younger than smear-negatives (P= 0.001). A history of contact with patients with PTB, clinical symptoms of chest pains, the presence of rhonchi, lymphocytosis and hypoalbuminaemia were positive predictive factors for smear-positive TB. Six hundred and twenty-five (86%) of the 731 culture-positive TB patients were tested for HIV. Of 340 smear-positive and 285 smear-negative patients, 158 (47%) and 171 (60%) tested positive for HIV, respectively (P= 0.001). Only haemoptysis was negatively and anaemia positively associated with HIV in smear-positive patients while weight loss, anorexia, BMI $\leq$ 18.5, anaemia, hypoalbuminaemia together with lower ESR, reduced lymphocyte count were independently associated with HIV among smear-negative patients.

The link between malnutrition and TB has long been recognised. Low plasma levels of zinc had been associated with impaired immune function, thus affecting host defences and predisposing to increases in infection and morbidity rates. Conversely, zinc supplementation has been observed to substantially improve the immune defence in individuals with infectious diseases by preventing the dismantling of the immune

system. A fall in plasma zinc concentration in patients with active PTB has been observed in micronutrient studies and supplementation with zinc has been found to improve clinical presentations of patients. Vitamin A also plays a major role in the immune status of patients and its deficiency has been linked with severity of several infectious diseases especially in children. Supplementation with vitamin A has been associated with reduction in morbidity and mortality of many diseases. Several cross-sectional studies have suggested that patients with TB suffer from vitamin A deficiency. Studies have shown that supplementation of anti-TB drugs with zinc or zinc and vitamin A improve clinical symptoms and signs and show early clearance of bacilli from sputum of patients with PTB.

Chapter 10 therefore compared whether weekly zinc and weekly zinc plus vitamin A supplementation as adjunct for the treatment of PTB increased the efficacy of anti-TB treatment. This was a double-blinded, placebo-controlled supplementation clinical trial where patients were allotted by block randomisation into three groups and given zinc, zinc plus vitamin A or placebos together with anti-TB therapy. Patients newly diagnosed as having active PTB as per the WHO definition of smear-positive PTB from the 1321 patients who attended the eight participating hospitals with a history of cough of more than 3 weeks or symptoms suspicious of PTB were enrolled. Enrolled patients were examined and monitored weekly for the first 2 months and monthly for the last 6 months of treatment.

Of the 350 patients enrolled into the micronutrient study, 116 were allocated into the placebo group (group A), 117 into the group receiving weekly zinc (group B) and 117 into the group receiving zinc plus vitamin A (group C). A total of 261 (75%) patients

out of the 350 enrolled completed the trial. Clinical symptoms and signs and laboratory results improved with treatment in the 3 treatment groups but faster resolution of symptoms and improvement in signs and laboratory results were observed to occur faster in patients in groups B and C compared to patients in group A. Changes observed in symptoms were however not statistically significant. No significant differences were observed in X-ray changes within the treatment groups. A higher proportion of patients in groups B and C significantly cleared bacilli from their sputa faster than patients in group A by both microscopic examination and culture. Only a mean difference of 1 week sputum time clearance was observed between patients in groups B and C and patients in group A. No significant differences were observed between the mean sputum time clearance in patients in group B or C. The evidence available is not conclusive to recommend the inclusion of zinc into the current TB treatment in high HIV prevalence population. However, in areas of low HIV prevalence, supplementation with zinc as an adjuvant to anti-TB treatment may be useful.

Further studies are required as follow up to the results arrived at in this thesis. Our findings indicate that, although morning samples from TB suspects were more likely to have higher smear grades, the proportion of slides classified as negative was independent of whether the sample was collected as an on the spot or as a morning sputum. Although this may be setting specific and be associated with later presentation and more advanced disease in our study population, further studies to examine the number of sputum specimens that are required to diagnose a patient as smear-positive in other locations will be required.

Smear examinations were carried out independently by two microscopists and reviewed by a third under strict research setting, allowing for higher sensitivity than would normally have occurred under normal conditions. Studies under normal laboratory conditions as occur outside research laboratories will be required as this may reveal different results for this setting.

Our results confirmed the relatively low sensitivity of both direct and digested sputum smear microscopy. The digestion method may benefit from some further development. A number of digested smears were unavailable due to laboratory mishap during this study. In every case, this mishap was the observed loss of the entire (heat-fixed) smear during the staining process as a result of the bleach digesting the proteinaceous material required for adherence of the clinical material to the glass slide. Previous studies have shown Bovine serum albumin to promote adherence of sodium hydroxide treated sputum to microscope slides, but is an expensive option for resource-poor settings. The use of alternatives such as sterile skimmed milk may be worth investigating. The effects of age and storage conditions of bleach on the performance of the method also need to be explored. Further, the effect of improving the sensitivity of smear results by bleach digestion of 2 or 3 sputum smears can also be exployed and reduction of visits to hospital which can be reduced by same day 2 sputum samples strategy.

Our study showed that sputum smears identified as scanty-smears are more than likely to be positive smears in areas of high HIV prevalence. The sample size in the study was inadequate to give the study the power necessary to arrive at a definite

conclusion, as such, further prospective studies should be conducted in high incidence countries to confirm these findings.

In the micronutrient study, our resuts showed a higher mortality in patients supplemented with zinc and zinc and vitamin A compared to those not supplemented. Further investigations on the effect of zinc in combination with anti-TB drugs on patients with TB will be required for its inclusion into TB treatment regimens.

References

Aber, VR; Allen, BW; Mitchison, DA; Ayuma, P; Edwards, EA Keyes, AB. Quality control in tuberculosis bacteriology. 1. Laboratory studies on isolated positive cultures and the efficiency of direct smear examination. Tubercle 1980: 61(3): 123-33.

Abouya, YL; Beaumel, A; Lucas, S; Dago-Akribi, A; Coulibaly, G; N'Dhatz, M; Konan, JB; Yapi, A De Cock, KM. Pneumocystis carinii pneumonia. An uncommon cause of death in African patients with acquired immunodeficiency syndrome. Am Rev Respir Dis 1992: 145(3): 617-20.

Abujacity.com pages, wac-k-C-S. (2004). "Abuja city, Nigeria, capital,information on the region,Africa and Nigerian Newspapers, links."

Abul, HT; Abul, AT; al-Athary, EA; Behbehani, AE; Khadadah, ME Dashti, HM. Interleukin-1 alpha (IL-1 alpha) production by alveolar macrophages in patients with acute lung diseases: the influence of zinc supplementation. Mol Cell Biochem 1995: 146(2): 139-45.

Ackah, AN; Coulibaly, D; Digbeu, H; Diallo, K; Vetter, KM; Coulibaly, IM; Greenberg, AE De Cock, KM. Response to treatment, mortality, and CD4 lymphocyte counts in HIV-infected persons with tuberculosis in Abidjan, Cote d'Ivoire. Lancet 1995: 345(8950): 607-10.

Aerts, D Jobim, R. The epidemiological profile of tuberculosis in southern Brazil in times of AIDS. Int J Tuberc Lung Dis 2004: 8(6): 785-91.

Aghokeng, AF; Ewane, L; Awazi, B; Nanfack, A; Delaporte, E; Peeters, M Zekeng, L. Evaluation of Four Simple/Rapid Assays and Two Fourth-Generation ELISAs for the Identification of HIV Infection on a Serum Panel Representing the HIV-1 Group M Genetic Diversity in Cameroon. J Acquir Immune Defic Syndr 2004: 37(5): 1632-40.

Ahmad, P; Garg, R Salahuddin, A. Serum zinc and copper in tuberculosis. Indian Pediatr 1985: 22(10): 786-8.

Ahmed, N; Caviedes, L; Alam, M; Rao, KR; Sangal, V; Sheen, P; Gilman, RH Hasnain, SE. Distinctiveness of Mycobacterium tuberculosis genotypes from human immunodeficiency virus type 1-seropositive and -seronegative patients in Lima, Peru. J Clin Microbiol 2003: 41(4): 1712-6.

Aktogu, S; Yorgancioglu, A; Cirak, K; Kose, T Dereli, SM. Clinical spectrum of pulmonary and pleural tuberculosis: a report of 5,480 cases. Eur Respir J 1996: 9(10): 2031-5.

al-Haddad, MK; Baig, BZ Ebrahim, RA. Epidemiology of HIV and AIDS in Bahrain. J Commun Dis 1997: 29(4): 321-8.

Alarcon, JO; Johnson, KM; Courtois, B; Rodriguez, C; Sanchez, J; Watts, DM Holmes, KK. Determinants and prevalence of HIV infection in pregnant Peruvian women. Aids 2003: 17(4): 613-8.

Alarcon, K; Kolsteren, PW; Prada, AM; Chian, AM; Velarde, RE; Pecho, IL Hoeree, TF. Effects of separate delivery of zinc or zinc and vitamin A on hemoglobin response, growth, and diarrhea in young Peruvian children receiving iron therapy for anemia. Am J Clin Nutr 2004: 80(5): 1276-82.

Alausa, KO; Osoba, AO; Montefiore, D Sogbetun, OA. Laboratory diagnosis of tuberculosis in a developing country 1968-1975. Afr J Med Med Sci 1977: 6(2): 103-8.

Albert, MJ; Qadri, F; Wahed, MA; Ahmed, T; Rahman, AS; Ahmed, F; Bhuiyan, NA; Zaman, K; Baqui, AH; Clemens, JD Black, RE. Supplementation with zinc, but not vitamin A, improves seroconversion to vibriocidal antibody in children given an oral cholera vaccine. J Infect Dis 2003: 187(6): 909-13.

Alseda Graells Pere, M Godoy Garcia, P. [Factors related to the tuberculous patient and the study of contacts.]. Rev Clin Esp 2004: 204(1): 3-8.

Alvarez-Suarez, Y; Marin-Lopez, A; Lobato-Mendizabal, E Galindo-Rodriguez, C. [Paid blood donors: a new risk group for the development of AIDS in Mexico]. Salud Publica Mex 1989: 31(5): 642-4.

Anderson, RM May, RM Infectious diseases of humans: dynamics and control (1991). Oxford University Press.

Angeby, KA; Hoffner, SE Diwan, VK. Should the 'bleach microscopy method' be recommended for improved case detection of tuberculosis? Literature review and key person analysis. Int J Tuberc Lung Dis 2004: 8(7): 806-15.

Ani, MN Agwale, SM. Human Immunodeficiency Virus Infection in Nigeria. Braz J Infect Dis 1998: 2(3): 143-59.

Anteyi, EA; Idoko, JA; Ukoli, CO Bello, CS. Clinical pattern of human immunodeficiency virus infection (HIV) in pulmonary tuberculosis patients in Jos, Nigeria. Afr J Med Med Sci 1996: 25(4): 317-21.

Antonucci, G; Girardi, E; Raviglione, MC Ippolito, G. Risk factors for tuberculosis in HIV-infected persons. A prospective cohort study. The Gruppo Italiano di Studio Tubercolosi e AIDS (GISTA). Jama 1995: 274(2): 143-8.

Arkhipova, OP. [Effect of tuberculosis infection and antibacterial preparations on thiamine metabolism]. Vopr Med Khim 1975: 21(5): 485-9.

ATS. Control of tuberculosis in the United States. American Thoracic Society. Am Rev Respir Dis 1992: 146(6): 1623-33.

Awoyemi, OB; Ige, OM Onadeko, BO. Prevalence of active pulmonary tuberculosis in human immunodeficiency virus seropositive adult patients in University College Hospital, Ibadan, Nigeria. Afr J Med Med Sci 2002: 31(4): 329-32.

Bahl, R; Bhandari, N; Hambidge, KM Bhan, MK. Plasma zinc as a predictor of diarrheal and respiratory morbidity in children in an urban slum setting. Am J Clin Nutr 1998: 68(2 Suppl): 414S-7S.

Banavaliker, J; R, G; DC, S; MK, G; S, K; Gupta, R Goel, MK. HIV seropositivity in hospitalised pulmonary tuberculosis patients in Delhi. Indian Journal of Tuberculosis. 1997: 44(1): 17-20.

Bandele, EO Osadiaye, JA. Attitudes and smoking habits of physicians at the Lagos University Teaching Hospital. J Natl Med Assoc 1987: 79(4): 430-2.

Barnes, PF; Bloch, AB; Davidson, PT Snider, DE, Jr. Tuberculosis in patients with human immunodeficiency virus infection. N Engl J Med 1991: 324(23): 1644-50.

Barnes, PF; Le, HQ Davidson, PT. Tuberculosis in patients with HIV infection. Med Clin North Am 1993: 77(6): 1369-90.

Bates, CJ; Evans, PH; Dardenne, M; Prentice, A; Lunn, PG; Northrop-Clewes, CA; Hoare, S; Cole, TJ; Horan, SJ; Longman, SC et al. A trial of zinc

supplementation in young rural Gambian children. Br J Nutr 1993: 69(1): 243-55.

Bayer, R Wilkinson, D. Directly observed therapy for tuberculosis: history of an idea. Lancet 1995: 345(8964): 1545-8.

Beck-Sague, C; Dooley, SW; Hutton, MD; Otten, J; Breeden, A; Crawford, JT; Pitchenik, AE; Woodley, C; Cauthen, G Jarvis, WR. Hospital outbreak of multidrug-resistant Mycobacterium tuberculosis infections. Factors in transmission to staff and HIV-infected patients. Jama 1992: 268(10): 1280-6.

Beck, FW; Prasad, AS; Kaplan, J; Fitzgerald, JT Brewer, GJ. Changes in cytokine production and T cell subpopulations in experimentally induced zinc-deficient humans. Am J Physiol 1997: 272(6 Pt 1): E1002-7.

Beck, MA Levander, OA. Host nutritional status and its effect on a viral pathogen. J Infect Dis 2000: 182 Suppl 1: S93-6.

Beelaert, G; Vercauteren, G; Fransen, K; Mangelschots, M; De Rooy, M; Garcia-Ribas, S van der Groen, G. Comparative evaluation of eight commercial enzyme linked immunosorbent assays and 14 simple assays for detection of antibodies to HIV. J Virol Methods 2002: 105(2): 197-206.

Behr, MA; Warren, SA; Salamon, H; Hopewell, PC; Ponce de Leon, A; Daley, CL Small, PM. Transmission of Mycobacterium tuberculosis from patients smear-negative for acid-fast bacilli. Lancet 1999: 353(9151): 444-9.

Best, M; Sattar, SA; Springthorpe, VS Kennedy, ME. Efficacies of selected disinfectants against Mycobacterium tuberculosis. J Clin Microbiol 1990: 28(10): 2234-9.

Bhandari, N; Bahl, R; Taneja, S; Strand, T; Molbak, K; Ulvik, RJ; Sommerfelt, H Bhan, MK. Effect of routine zinc supplementation on pneumonia in children aged 6 months to 3 years: randomised controlled trial in an urban slum. Bmj 2002b: 324(7350): 1358.

Bhandari, N; Bahl, R; Taneja, S; Strand, T; Molbak, K; Ulvik, RJ; Sommerfelt, H Bhan, MK. Substantial reduction in severe diarrheal morbidity by daily zinc supplementation in young north Indian children. Pediatrics 2002a: 109(6): e86.

Bhutta, ZA; Bird, SM; Black, RE; Brown, KH; Gardner, JM; Hidayat, A; Khatun, F; Martorell, R; Ninh, NX; Penny, ME; Rosado, JL; Roy, SK; Ruel, M; Sazawal, S Shankar, A. Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials. Am J Clin Nutr 2000: 72(6): 1516-22.

Bhutta, ZA; Black, RE; Brown, KH; Gardner, JM; Gore, S; Hidayat, A; Khatun, F; Martorell, R; Ninh, NX; Penny, ME; Rosado, JL; Roy, SK; Ruel, M; Sazawal, S Shankar, A. Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials. Zinc Investigators' Collaborative Group. J Pediatr 1999: 135(6): 689-97.

Binka, FN; Ross, DA; Morris, SS; Kirkwood, BR; Arthur, P; Dollimore, N; Gyapong, JO Smith, PG. Vitamin A supplementation and childhood malaria in northern Ghana. Am J Clin Nutr 1995: 61(4): 853-9.

Bitarakwate, E; Mworozi, E Kekitiinwa, A. Serum zinc status of children with persistent diarrhoea admitted to the diarrhoea management unit of Mulago Hospital, Uganda. Afr Health Sci 2003: 3(2): 54-60.

Blumberg, HM. The "new tuberculosis" and its changing epidemiology. Nosocomial transmission and hospital discharge standards. J Med Assoc Ga 1995: 84(5): 209-14.

Blumberg, HM. Tuberculosis and infection control: what now? Infect Control Hosp Epidemiol 1997: 18(8): 538-41.

Bogden, JD; Lintz, DI; Joselow, MM; Charles, J Salaki, JS. Effect of pulmonary tuberculosis on blood concentrations of copper and zinc. Am J Clin Pathol 1977: 67(3): 251-6.

Brandli, O. The clinical presentation of tuberculosis. Respiration 1998: 65(2): 97-105.

Brown, N Roberts, C. Vitamin A for acute respiratory infection in developing countries: a meta-analysis. Acta Paediatr 2004: 93(11): 1437-42.

Bruchfeld, J; Aderaye, G; Palme, IB; Bjorvatn, B; Kallenius, G Lindquist, L. Sputum concentration improves diagnosis of tuberculosis in a setting with a high prevalence of HIV. Trans R Soc Trop Med Hyg 2000: 94(6): 677-80.

BTS. Chemotherapy and management of tuberculosis in the United Kingdom: recommendations 1998. Joint Tuberculosis Committee of the British Thoracic Society. Thorax 1998: 53(7): 536-48.

Cambau, E; Wichlacz, C; Truffot-Pernot, C Jarlier, V. Evaluation of the new MB redox system for detection of growth of mycobacteria. J Clin Microbiol 1999: 37(6): 2013-5.

Cantwell, MF Binkin, NJ. Tuberculosis in sub-Saharan Africa: a regional assessment of the impact of the human immunodeficiency virus and National Tuberculosis Control Program quality. Tuber Lung Dis 1996: 77(3): 220-5.

Carcillo, JA. Reducing the global burden of sepsis in infants and children: a clinical practice research agenda. Pediatr Crit Care Med 2005: 6(3 Suppl): S157-64.

Castillo-Duran, C; Garcia, H; Venegas, P; Torrealba, I; Panteon, E; Concha, N Perez, P. Zinc supplementation increases growth velocity of male children and adolescents with short stature. Acta Paediatr 1994: 83(8): 833-7.

Chandra, RK. Interactions between early nutrition and the immune system. Ciba Found Symp 1991: 156: 77-89; discussion -92.

Chandra, RK Kumari, S. Effects of nutrition on the immune system. Nutrition 1994a: 10(3): 207-10.

Chandra, RK Kumari, S. Nutrition and immunity: an overview. J Nutr 1994b: 124(8 Suppl): 1433S-5S.

Chaudhary, S; Verma, M; Dhawan, V; Nain, CK; Kumar, R; Kumar, L Ganguly, NK. Plasma vitamin A, zinc and selenium concentrations in children with acute and persistent diarrhoea. J Diarrhoeal Dis Res 1996: 14(3): 190-3.

Chretien, J. Tuberculosis and HIV. The cursed duet. Bull Int Union Tuberc Lung Dis 1990: 65(1): 25-8.

Christian, P; Khatry, SK; Katz, J; Pradhan, EK; LeClerq, SC; Shrestha, SR; Adhikari, RK; Sommer, A West, KP, Jr. Effects of alternative maternal

micronutrient supplements on low birth weight in rural Nepal: double blind randomised community trial. Bmj 2003: 326(7389): 571.

Christian, P; Khatry, SK; Yamini, S; Stallings, R; LeClerq, SC; Shrestha, SR; Pradhan, EK West, KP, Jr. Zinc supplementation might potentiate the effect of vitamin A in restoring night vision in pregnant Nepalese women. Am J Clin Nutr 2001: 73(6): 1045-51.

Ciftci, TU; Ciftci, B; Yis, O; Guney, Y; Bilgihan, A Ogretensoy, M. Changes in serum selenium, copper, zinc levels and cu/zn ratio in patients with pulmonary tuberculosis during therapy. Biol Trace Elem Res 2003: 95(1): 65-71.

Cohen, R; Muzaffar, S; Capellan, J; Azar, H Chinikamwala, M. The validity of classic symptoms and chest radiographic configuration in predicting pulmonary tuberculosis. Chest 1996: 109(2): 420-3.

Colebunders, RL; Lebughe, I; Nzila, N; Kalunga, D; Francis, H; Ryder, R Piot, P. Cutaneous delayed-type hypersensitivity in patients with human immunodeficiency virus infection in Zaire. J Acquir Immune Defic Syndr 1989: 2(6): 576-8.

Colebunders, RL; Ryder, RW; Nzilambi, N; Dikilu, K; Willame, JC; Kaboto, M; Bagala, N; Jeugmans, J; Muepu, K; Francis, HL et al. HIV infection in patients with tuberculosis in Kinshasa, Zaire. Am Rev Respir Dis 1989: 139(5): 1082-5.

Collins, HL Kaufmann, SH. Prospects for better tuberculosis vaccines. Lancet Infect Dis 2001: 1(1): 21-8.

Corbett, EL; Watt, CJ; Walker, N; Maher, D; Williams, BG; Raviglione, MC Dye, C. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. Arch Intern Med 2003: 163(9): 1009-21.

Corper, H CR, N. Methods for concentrating acid-fast bacilli. Am. J. Clin. Pathol. 1949: (19): 269-73.

Cuevas, LE; Almeida, LM; Mazunder, P; Paixao, AC; Silva, AM; Maciel, L; Hart, CA Coulter, JB. Effect of zinc on the tuberculin response of children exposed to adults with smear-positive tuberculosis. Ann Trop Paediatr 2002: 22(4): 313-9.

D'Arcy Hart, P Payling Wright, G. Tuberculosis and social conditions in England with special reference to young adults. London: National Association for the prevention of Tuberculosis 1939.

Daniel, OJ; Salako, AA; Oluwole, FA; Alausa, OK Oladapo, OT. HIV sero-prevalence among newly diagnosed adult pulmonary tuberculosis patients in Sagamu. Niger J Med 2004: 13(4): 393-7.

Daniel, TM. Rapid diagnosis of tuberculosis: laboratory techniques applicable in developing countries. Rev Infect Dis 1989: 11 Suppl 2: S471-8.

Davies, P Clinical Tuberculosis (2003a). London, Arnold Publishers.

Davies, PD. The world-wide increase in tuberculosis: how demographic changes, HIV infection and increasing numbers in poverty are increasing tuberculosis. Ann Med 2003: 35(4): 235-43.

Davies, PD; De Cock, KM; Leese, J Moore-Gillon, J. Tuberculosis 2000. J R Soc Med 1996: 89(8): 431-5.

Dax, EM Vandenbelt, TA. HIV antibody testing in Australia. J Acquir Immune Defic Syndr 1993: 6 Suppl 1: S24-8.

De Cock, KM. Tuberculosis control in resource-poor settings with high rates of HIV infection. Am J Public Health 1996: 86(8 Pt 1): 1071-3.

De Cock, KM; Gnaore, E; Adjorlolo, G; Braun, MM; Lafontaine, MF; Yesso, G; Bretton, G; Coulibaly, IM; Gershy-Damet, GM; Bretton, R et al. Risk of tuberculosis in patients with HIV-I and HIV-II infections in Abidjan, Ivory Coast. Bmj 1991: 302(6775): 496-9.

Dey, SK; Datta, PK; Chakraborty, MS Niyogi, DK. Epidemiology of HIV infection among tuberculosis patients in Calcutta. Indian J Public Health 2003: 47(1): 37-8, 0.

Dijkhuizen, MA; Wieringa, FT; West, CE Muhilal. Zinc plus beta-carotene supplementation of pregnant women is superior to beta-carotene supplementation alone in improving vitamin A status in both mothers and infants. Am J Clin Nutr 2004: 80(5): 1299-307.

Doherty, CP; Sarkar, MA; Shakur, MS; Ling, SC; Elton, RA Cutting, WA. Zinc and rehabilitation from severe protein-energy malnutrition: higher-dose regimens are associated with increased mortality. Am J Clin Nutr 1998: 68(3): 742-8.

Douthwaite, S; Yassin, MA; Squire, SB; A, I Cuevas, LE. Bleach-digested sputum smears for the diagnosis of tuberculosis in HIV-infected individuals. Trop Doct (In press) 2006.

Driessen, C; Hirv, K; Rink, L Kirchner, H. Induction of cytokines by zinc ions in human peripheral blood mononuclear cells and separated monocytes. Lymphokine Cytokine Res 1994: 13(1): 15-20.

Duggan, C; MacLeod, WB; Krebs, NF; Westcott, JL; Fawzi, WW; Premji, ZG; Mwanakasale, V; Simon, JL; Yeboah-Antwi, K Hamer, DH. Plasma zinc concentrations are depressed during the acute phase response in children with falciparum malaria. J Nutr 2005: 135(4): 802-7.

Dutt, AK Stead, WW. Smear-negative pulmonary tuberculosis. Semin Respir Infect 1994: 9(2): 113-9.

Dutta, P; Mitra, U; Datta, A; Niyogi, SK; Dutta, S; Manna, B; Basak, M; Mahapatra, TS Bhattacharya, SK. Impact of zinc supplementation in malnourished children with acute watery diarrhoea. J Trop Pediatr 2000: 46(5): 259-63.

Dye, C; Scheele, S; Dolin, P; Pathania, V Raviglione, MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. Jama 1999: 282(7): 677-86.

Dye, C; Watt, CJ; Bleed, DM; Hosseini, SM Raviglione, MC. Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence, and deaths globally. Jama 2005: 293(22): 2767-75.

El'kin, AV; Ariel, BM Nechaev, VV. [Diagnosis of liver diseases in patients with pulmonary tuberculosis]. Probl Tuberk 1992: (7-8): 15-8.

El-Sony, AI; Mustafa, SA; Khamis, AH; Sobhi, S; Enarson, DA; Baraka, OZ Bjune, G. Symptoms in patients attending services for diagnosis of pulmonary tuberculosis in Sudan. Int J Tuberc Lung Dis 2003: 7(6): 550-5.

Elliott, AM; Halwiindi, B; Hayes, RJ; Luo, N; Tembo, G; Machiels, L; Bem, C; Steenbergen, G; Pobee, JO; Nunn, PP 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): 1-11.

Elliott, AM; Halwiindi, B; Hayes, RJ; Luo, N; Tembo, G; Machiels, L; Bem, C; Steenbergen, G; Pobee, JO; Nunn, PP et al. The impact of human immunodeficiency virus on presentation and diagnosis of tuberculosis in a cohort study in Zambia. J Trop Med Hyg 1993d: 96(1): 1-11.

Elliott, AM; Hayes, RJ; Luo, N; Pobee, JO McAdam, KP. Tuberculosis and immunodeficiency in HIV-1-infected patients in Africa. Lancet 1993a: 342(8878): 1053.

Elliott, AM; Luo, N; Tembo, G; Halwiindi, B; Steenbergen, G; Machiels, L; Pobee, J; Nunn, P; Hayes, RJ McAdam, KP. Impact of HIV on tuberculosis in Zambia: a cross sectional study. Bmj 1990: 301(6749): 412-5.

Elliott, AM; Namaambo, K; Allen, BW; Luo, N; Hayes, RJ; Pobee, JO McAdam, KP. Negative sputum smear results in HIV-positive patients with pulmonary tuberculosis in Lusaka, Zambia. Tuber Lung Dis 1993c: 74(3): 191-4.

Ellner, JJ. Regulation of the human immune response during tuberculosis. J Lab Clin Med 1997: 130(5): 469-75.

Ellner, JJ; Hinman, AR; Dooley, SW; Fischl, MA; Sepkowitz, KA; Goldberger, MJ; Shinnick, TM; Iseman, MD Jacobs, WR, Jr. Tuberculosis symposium: emerging problems and promise. J Infect Dis 1993: 168(3): 537-51.

Elwood, RK; Cook, VJ Hernandez-Garduno, E. Risk of tuberculosis in children from smear-negative source cases. Int J Tuberc Lung Dis 2005: 9(1): 49-55.

Enarson, DA. The International Union Against Tuberculosis and Lung Disease model National Tuberculosis Programmes. Tuber Lung Dis 1995: 76(2): 95-9.

Enarson, DA; el Sony, A Bam, DS. Response from IUATLD. Scott Med J 2000: 45(5 Suppl): 60-2.

Enarson, DA; Rieder, HL; T, A A, T. A guide for low income countries. IUATLD 2000: (ISBN-2-914365-00-4): 1-91.

Eriki, PP; Okwera, A; Aisu, T; Morrissey, AB; Ellner, JJ Daniel, TM. The influence of human immunodeficiency virus infection on tuberculosis in Kampala, Uganda. Am Rev Respir Dis 1991: 143(1): 185-7.

Erlich, P. Aus dem verein fur innere Medicin zu Berlin. Sitzung vom 1. Dtsch Med Wochenschr 1882: 8: 269-70.

Evans, DI Attock, B. Folate deficiency in pulmonary tuberculosis: relationship to treatment and to serum vitamin A and beta-carotene. Tubercle 1971: 52(4): 288-94.

Ewer, K; Deeks, J; Alvarez, L; Bryant, G; Waller, S; Andersen, P; Monk, P Lalvani, A. Comparison of T-cell-based assay with tuberculin skin test for diagnosis of Mycobacterium tuberculosis infection in a school tuberculosis outbreak. Lancet 2003: 361(9364): 1168-73.

Fairchild, AL Oppenheimer, GM. Public health nihilism vs pragmatism: history, politics, and the control of tuberculosis. Am J Public Health 1998: 88(7): 1105-17.

Farnia, P; Mohammadi, F; Zarifi, Z; Tabatabee, DJ; Ganavi, J; Ghazisaeedi, K; Farnia, PK; Gheydi, M; Bahadori, M; Masjedi, MR Velayati, AA. Improving sensitivity of direct microscopy for detection of acid-fast bacilli

in sputum: use of chitin in mucus digestion. J Clin Microbiol 2002: 40(2): 508-11.
Fauci, AS; Pantaleo, G; Stanley, S Weissman, D. Immunopathogenic mechanisms of HIV infection. Ann Intern Med 1996: 124(7): 654-63.
Fawzi, W Msamanga, G. Micronutrients and adverse pregnancy outcomes in the context of HIV infection. Nutr Rev 2004: 62(7 Pt 1): 269-75.
Fawzi, W; Msamanga, G; Spiegelman, D Hunter, DJ. Studies of vitamins and minerals and HIV transmission and disease progression. J Nutr 2005: 135(4): 938-44.
Fawzi, WW; Chalmers, TC; Herrera, MG Mosteller, F. Vitamin A supplementation and child mortality. A meta-analysis. Jama 1993: 269(7): 898-903.
Fawzi, WW; Mbise, RL; Fataki, MR; Herrera, MG; Kawau, F; Hertzmark, E; Spiegelman, D Ndossi, G. Vitamin A supplementation and severity of pneumonia in children admitted to the hospital in Dar es Salaam, Tanzania. Am J Clin Nutr 1998: 68(1): 187-92.
Fawzi, WW; Mbise, RL; Hertzmark, E; Fataki, MR; Herrera, MG; Ndossi, G Spiegelman, D. A randomized trial of vitamin A supplements in relation to mortality among human immunodeficiency virus-infected and uninfected children in Tanzania. Pediatr Infect Dis J 1999: 18(2): 127-33.
Flynn, JL; Chan, J; Triebold, KJ; Dalton, DK; Stewart, TA Bloom, BR. An essential role for interferon gamma in resistance to Mycobacterium tuberculosis infection. J Exp Med 1993: 178(6): 2249-54.
Flynn, JL; Goldstein, MM; Chan, J; Triebold, KJ; Pfeffer, K; Lowenstein, CJ; Schreiber, R; Mak, TW Bloom, BR. Tumor necrosis factor-alpha is required in the protective immune response against Mycobacterium tuberculosis in mice. Immunity 1995: 2(6): 561-72.
Flynn, N; Jain, S; Keddie, EM; Carlson, JR; Jennings, MB; Haverkos, HW; Nassar, N; Anderson, R; Cohen, S Goldberg, D. In vitro activity of readily available household materials against HIV-1: is bleach enough? J Acquir Immune Defic Syndr 1994: 7(7): 747-53.
Fraker, PJ; King, LE; Laakko, T Vollmer, TL. The dynamic link between the integrity of the immune system and zinc status. J Nutr 2000: 130(5S Suppl): 1399S-406S.
Galan, P; Samba, C; Luzeau, R Amedee-Manesme, O. Vitamin A deficiency in pre-school age Congolese children during malarial attacks. Part 2: Impact of parasitic disease on vitamin A status. Int J Vitam Nutr Res 1990: 60(3): 224-8.
Gebre-Selassie, S. Evaluation of the concentration sputum smear technique for the laboratory diagnosis of pulmonary tuberculosis. Trop Doct 2003: 33(3): 160-2.
Gebre, N; Karlsson, U; Jonsson, G; Macaden, R; Wolde, A; Assefa, A Miorner, H. Improved microscopical diagnosis of pulmonary tuberculosis in developing countries. Trans R Soc Trop Med Hyg 1995: 89(2): 191-3.
Gilbert, C Awan, H. Blindness in children. Bmj 2003: 327(7418): 760-1.
Gilks, CF Balbirnie, E. Testing sputum samples for HIV antibodies, using a rapid, saliva-card test system. Ann Trop Med Parasitol 1997: 91(1): 115-7.
Gilks, CF; Brindle, RJ; Otieno, LS; Bhatt, SM; Newnham, RS; Simani, PM; Lule, GN; Okelo, GB; Watkins, WM; Waiyaki, PG et al.

Extrapulmonary and disseminated tuberculosis in HIV-1-seropositive patients presenting to the acute medical services in Nairobi. Aids 1990: 4(10): 981-5.

Gnaore, E; Sassan-Morokro, M; Kassim, S; Ackah, A; Yesso, G; Adjorlolo, G; Digbeu, H; Coulibaly, D; Coulibaly, IM; Doorly, R et al. A comparison of clinical features in tuberculosis associated with infection with human immunodeficiency viruses 1 and 2. Trans R Soc Trop Med Hyg 1993: 87(1): 57-9.

Goebel, FD. Immune reconstitution inflammatory syndrome (IRIS)--another new disease entity following treatment initiation of HIV infection. Infection 2005: 33(1): 43-5.

Gordin, FM; Cohn, DL; Matts, JP; Chaisson, RE O'Brien, RJ. Hepatotoxicity of rifampin and pyrazinamide in the treatment of latent tuberculosis infection in HIV-infected persons: is it different than in HIV-uninfected persons? Clin Infect Dis 2004: 39(4): 561-5.

Grange, J; Story, A Zumla, A. Tuberculosis in disadvantaged groups. Curr Opin Pulm Med 2001a: 7(3): 160-4.

Grange, JM; Gandy, M; Farmer, P Zumla, A. Historical declines in tuberculosis: nature, nurture and the biosocial model. Int J Tuberc Lung Dis 2001b: 5(3): 208-12.

Grant, AD De Cock, KM. The growing challenge of HIV/AIDS in developing countries. Br Med Bull 1998: 54(2): 369-81.

Groopman, JE; Chen, FW; Hope, JA; Andrews, JM; Swift, RL; Benton, CV; Sullivan, JL; Volberding, PA; Sites, DP; Landesman, S et al. Serological characterization of HTLV-III infection in AIDS and related disorders. J Infect Dis 1986: 153(4): 736-42.

Grzybowski, S. Tuberculosis in the Third World. Thorax 1991: 46(10): 689-91.

Grzybowski, S; Barnett, GD Styblo, K. Contacts of cases of active pulmonary tuberculosis. Bull Int Union Tuberc 1975: 50(1): 90-106.

Guerrant, RL; Lima, AA Davidson, F. Micronutrients and infection: interactions and implications with enteric and other infections and future priorities. J Infect Dis 2000: 182 Suppl 1: S134-8.

Guryleva, ME Vizel, AA. [Statistical analysis of liver dysfunction in patients with pulmonary tuberculosis using the data base control system]. Probl Tuberk 1993: (3): 36-7.

Habeenzu, C; Lubasi, D Fleming, AF. 'Improved sensitivity of direct microscopy for detection of acid-fast bacilli in sputum in developing countries. Trans R Soc Trop Med Hyg 1998: 92(4): 415-6.

Halsted, JA Smith, JC, Jr. Plasma-zinc in health and disease. Lancet 1970: 1(7642): 322-4.

Hanekom, WA; Potgieter, S; Hughes, EJ; Malan, H; Kessow, G Hussey, GD. Vitamin A status and therapy in childhood pulmonary tuberculosis. J Pediatr 1997: 131(6): 925-7.

Hanks, J; Clark, H H, F. Concentration of tubercule bacilli from sputum by chemical flocculation methods. J Lab Clin Med 1938: 23: 736-46.

Hargreaves, NJ; Kadzakumanja, O; Phiri, S; Nyangulu, DS; Salaniponi, FM; Harries, AD Squire, SB. What causes smear-negative pulmonary tuberculosis in Malawi, an area of high HIV seroprevalence? Int J Tuberc Lung Dis 2001a: 5(2): 113-22.

Hargreaves, NJ; Kadzakumanja, O; Whitty, CJ; Salaniponi, FM; Harries, AD Squire, SB. 'Smear-negative' pulmonary tuberculosis in a DOTS programme: poor outcomes in an area of high HIV seroprevalence. Int J Tuberc Lung Dis 2001b: 5(9): 847-54.

Harries, AD. Tuberculosis and human immunodeficiency virus infection in developing countries. Lancet 1990: 335(8686): 387-90.

Harries, AD. Tuberculosis in Africa: clinical presentation and management. Pharmacol Ther 1997: 73(1): 1-50.

Harries, AD. Tuberculosis in HIV-infected persons with special emphasis on sub-Saharan Africa. J Infect 1998: 37(3): 205-9.

Harries, AD; Chilewani, N Dzinyemba, W. Diagnosing tuberculosis in a resource-poor setting: the value of sputum concentrations. Trans R Soc Trop Med Hyg 1998: 92(1): 123.

Harries, AD; Maher, D; Mvula, B Nyangulu, D. An audit of HIV testing and HIV serostatus in tuberculosis patients, Blantyre, Malawi. Tuber Lung Dis 1995: 76(5): 413-7.

Havlir, DV Barnes, PF. Tuberculosis in patients with human immunodeficiency virus infection. N Engl J Med 1999: 340(5): 367-73.

Heifets, L. Dilemmas and realities of rapid diagnostic tests for tuberculosis. Chest 2000: 118(1): 4-5.

Herrera, MG; Nestel, P; el Amin, A; Fawzi, WW; Mohamed, KA Weld, L. Vitamin A supplementation and child survival. Lancet 1992: 340(8814): 267-71.

Herzog, C Birkhauser, MH. [Tuberculin screening tests. Open comparative trial of Tine-Test and Monotest versus Mantoux 1:1000 (10 TU) in 119 outpatients]. Schweiz Med Wochenschr 1980: 110(48): 1817-21.

Hira, SK; Dore, GJ Sirisanthana, T. Clinical spectrum of HIV/AIDS in the Asia-Pacific region. Aids 1998: 12 Suppl B: S145-54.

Hira, SK; Ngandu, N; Wadhawan, D; Nkowne, B; Baboo, KS; Macuacua, R; Kamanga, J; Mpoko, B; Heiba, IM Perine, PL. Clinical and epidemiological features of HIV infection at a referral clinic in Zambia. J Acquir Immune Defic Syndr 1990: 3(1): 87-91.

HMSO. Mortality statistics. Office for National Statistics 1988-2001: Series DH2: 15-28.

Houston, S; Ray, S; Mahari, M; Neill, P; Legg, W; Latif, AS; Emmanuel, J; Bassett, M; Pozniak, A; Tswana, S et al. The association of tuberculosis and HIV infection in Harare, Zimbabwe. Tuber Lung Dis 1994: 75(3): 220-6.

Hsieh, SM; Hung, CC; Chen, MY; Chang, SC; Hsueh, PR; Luh, KT Chuang, CY. Clinical features of tuberculosis associated with HIV infection in Taiwan. J Formos Med Assoc 1996: 95(12): 923-8.

Huebner, RE; Schein, MF Bass, JB, Jr. The tuberculin skin test. Clin Infect Dis 1993: 17(6): 968-75.

Huebner, RE; Schein, MF; Cauthen, GM; Geiter, LJ; Selin, MJ; Good, RC O'Brien, RJ. Evaluation of the clinical usefulness of mycobacterial skin test antigens in adults with pulmonary mycobacterioses. Am Rev Respir Dis 1992: 145(5): 1160-6.

Hussey, G. Managing measles. Bmj 1997: 314(7077): 316-7.

Hussey, GD Klein, M. A randomized, controlled trial of vitamin A in children with severe measles. N Engl J Med 1990: 323(3): 160-4.

Idigbe, EO; Nasidi, A; Anyiwo, CE; Onubogu, C; Alabi, S; Okoye, R; Ugwu, O John, EK. Prevalence of human immunodeficiency virus (HIV) antibodies in tuberculosis patients in Lagos, Nigeria. J Trop Med Hyg 1994: 97(2): 91-7.

Ipuge, YA; Rieder, HL Enarson, DA. The yield of acid-fast bacilli from serial smears in routine microscopy laboratories in rural Tanzania. Trans R Soc Trop Med Hyg 1996: 90(3): 258-61.

Iseman, MD; Cohn, DL Sbarbaro, JA. Directly observed treatment of tuberculosis. We can't afford not to try it. N Engl J Med 1993: 328(8): 576-8.

Jain, SK; Aggarwal, JK; S., R U, B. Prevalence of HIV infection among TB patients in a Delhi-A sentinel surveilance study. Ind. J. Tub., 47,21 2000: 21-6.

Jasmer, RM Daley, CL. Rifampin and pyrazinamide for treatment of latent tuberculosis infection: is it safe? Am J Respir Crit Care Med 2003: 167(6): 809-10.

Jayaswal, R; P.N., A B.N., P. HIV in tuberculosis. Medical Journal Armed Forces. India 1995: 51, 259.

Kanaya, AM; Glidden, DV Chambers, HF. Identifying pulmonary tuberculosis in patients with negative sputum smear results. Chest 2001: 120(2): 349-55.

Karstaedt, AS; Jones, N; Khoosal, M Crewe-Brown, HH. The bacteriology of pulmonary tuberculosis in a population with high human immunodeficiency virus seroprevalence. Int J Tuberc Lung Dis 1998: 2(4): 312-6.

Karyadi, E; Schultink, W; Nelwan, RH; Gross, R; Amin, Z; Dolmans, WM; van der Meer, JW; Hautvast, JG West, CE. Poor micronutrient status of active pulmonary tuberculosis patients in Indonesia. J Nutr 2000: 130(12): 2953-8.

Karyadi, E; West, CE; Schultink, W; Nelwan, RH; Gross, R; Amin, Z; Dolmans, WM; Schlebusch, H van der Meer, JW. A double-blind, placebo-controlled study of vitamin A and zinc supplementation in persons with tuberculosis in Indonesia: effects on clinical response and nutritional status. Am J Clin Nutr 2002: 75(4): 720-7.

Katila, ML; Katila, P Erkinjuntti-Pekkanen, R. Accelerated detection and identification of mycobacteria with MGIT 960 and COBAS AMPLICOR systems. J Clin Microbiol 2000: 38(3): 960-4.

Katz, P; Goldstein, RA Fauci, AS. Immunoregulation in infection caused by Mycobacterium tuberculosis: the presence of suppressor monocytes and the alteration of subpopulations of T lymphocytes. J Infect Dis 1979: 140(1): 12-21.

Kelly, P; Burnham, G Radford, C. HIV seropositivity and tuberculosis in a rural Malawi hospital. Trans R Soc Trop Med Hyg 1990: 84(5): 725-7.

Kemp, EB; Belshe, RB Hoft, DF. Immune responses stimulated by percutaneous and intradermal bacille Calmette-Guerin. J Infect Dis 1996: 174(1): 113-9.

Kent, P Kubica, G Public health Mycobacteriology: Guide for the Level III Laboratory. US Department of Health and Human Services, Centres for Disease Control, USA. (1985). Atlanta.

Kenyon, TA; Mwasekaga, MJ; Huebner, R; Rumisha, D; Binkin, N Maganu, E. Low levels of drug resistance amidst rapidly increasing tuberculosis and human immunodeficiency virus co-epidemics in Botswana. Int J Tuberc Lung Dis 1999: 3(1): 4-11.

Khan, EA Starke, JR. Diagnosis of tuberculosis in children: increased need for better methods. Emerg Infect Dis 1995: 1(4): 115-23.

Kim, TC; Blackman, RS; Heatwole, KM; Kim, T Rochester, DF. Acid-fast bacilli in sputum smears of patients with pulmonary tuberculosis. Prevalence and significance of negative smears pretreatment and positive smears post-treatment. Am Rev Respir Dis 1984: 129(2): 264-8.

Kimerling, ME; Schuchter, J; Chanthol, E; Kunthy, T; Stuer, F; Glaziou, P Ee, O. Prevalence of pulmonary tuberculosis among HIV-infected persons in a home care program in Phnom Penh, Cambodia. Int J Tuberc Lung Dis 2002: 6(11): 988-94.

Kjolhede, CL; Chew, FJ; Gadomski, AM Marroquin, DP. Clinical trial of vitamin A as adjuvant treatment for lower respiratory tract infections. J Pediatr 1995: 126(5 Pt 1): 807-12.

Klein, M Hussey, GD. Vitamin A reduces morbidity and mortality in measles. S Afr Med J 1990: 78(2): 56-8.

Kobashi, Y; Matsushima, T; Kimura, M; Yoneyama, H Yano, T. [Comparison of the clinical features of smear-positive and culture-positive tuberculous patients with smear-negative and culture-positive patients in an education hospital]. Kekkaku 1995: 70(12): 679-84.

Koch, R. Die aetiologie de tuvberculose. Berl Klin Wochenschr 1882: 19: 221-30.

Koch, R. The Etiolpgy of Tuberculosis. Am Rev Tuberc 1932: 202(114.65.51).

Kochi, A. The global tuberculosis situation and the new control strategy of the World Health Organization. Tubercle 1991: 72(1): 1-6.

Kool, HE; Bloemkolk, D; Reeve, PA Danner, SA. HIV seropositivity and tuberculosis in a large general hospital in Malawi. Trop Geogr Med 1990: 42(2): 128-32.

Koyanagi, A; Kuffo, D; Gresely, L; Shenkin, A Cuevas, LE. Relationships between serum concentrations of C-reactive protein and micronutrients, in patients with tuberculosis. Ann Trop Med Parasitol 2004: 98(4): 391-9.

Kramer, F; Modilevsky, T; Waliany, AR; Leedom, JM Barnes, PF. Delayed diagnosis of tuberculosis in patients with human immunodeficiency virus infection. Am J Med 1990: 89(4): 451-6.

Krishnan, S; Krishnan, AD; Mustafa, AS; Talwar, GP Ramalingaswami, V. Effect of vitamin A and undernutrition on the susceptibility of rodents to a malarial parasite Plasmodium berghei. J Nutr 1976: 106(6): 784-91.

Kritski, A; Dalcolmo, M; del Bianco, R; del Melo, FF; Pinto, WP; Schechther, M Castelo, A. [Association of tuberculosis and HIV infection in Brazil]. Bol Oficina Sanit Panam 1995: 118(6): 542-54.

Kupka, R Fawzi, W. Zinc nutrition and HIV infection. Nutr Rev 2002: 60(3): 69-79.

Lalvani, A Hill, AV. Cytotoxic T-lymphocytes against malaria and tuberculosis: from natural immunity to vaccine design. Clin Sci (Lond) 1998: 95(5): 531-8.

Lesourd, BM. Nutrition and immunity in the elderly: modification of immune responses with nutritional treatments. Am J Clin Nutr 1997: 66(2): 478S-84S.

Levy, H; Feldman, C; Sacho, H; van der Meulen, H; Kallenbach, J Koornhof, H. A reevaluation of sputum microscopy and culture in the diagnosis of pulmonary tuberculosis. Chest 1989: 95(6): 1193-7.

Liberato, IR; de Albuquerque Mde, F; Campelo, AR de Melo, HR. Characteristics of pulmonary tuberculosis in HIV seropositive and seronegative patients in a Northeastern region of Brazil. Rev Soc Bras Med Trop 2004: 37(1): 46-50.

Liebeschuetz, S; Bamber, S; Ewer, K; Deeks, J; Pathan, AA Lalvani, A. Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. Lancet 2004: 364(9452): 2196-203.

Liu, YC; Huang, TS Huang, WK. Comparison of a nonradiometric liquid-medium method (MB REDOX) with the BACTEC system for growth and identification of mycobacteria in clinical specimens. J Clin Microbiol 1999: 37(12): 4048-50.

Long, R. Smear-negative pulmonary tuberculosis in industrialized countries. Chest 2001: 120(2): 330-4.

Long, R; Scalcini, M; Manfreda, J; Carre, G; Philippe, E; Hershfield, E; Sekla, L Stackiw, W. Impact of human immunodeficiency virus type 1 on tuberculosis in rural Haiti. Am Rev Respir Dis 1991: 143(1): 69-73.

Loudon, RG; Williamson, J Johnson, JM. An analysis of 3,485 tuberculosis contacts in the city of Edinburgh during 1954-1955. Am Rev Tuberc 1958: 77(4): 623-43.

Mackaness, GB. The immunology of antituberculous immunity. Am Rev Respir Dis 1968: 97(3): 337-44.

Maher, D; Blanc, L Raviglione, M. WHO policies for tuberculosis control. Lancet 2004: 363(9424): 1911.

Markkanen, T; Levanto, A; Sallinen, V Virtanen, S. Folic acid and vitamin B12 in tuberculosis. Scand J Haematol 1967: 4(4): 283-91.

Mathur, P; Sacks, L; Auten, G; Sall, R; Levy, C Gordin, F. Delayed diagnosis of pulmonary tuberculosis in city hospitals. Arch Intern Med 1994: 154(3): 306-10.

Mazurek, GH Villarino, ME. Guidelines for using the QuantiFERON-TB test for diagnosing latent Mycobacterium tuberculosis infection. Centers for Disease Control and Prevention. MMWR Recomm Rep 2003: 52(RR-2): 15-8.

McGowan, JE, Jr. Nosocomial tuberculosis: new progress in control and prevention. Clin Infect Dis 1995: 21(3): 489-505.

McLeod, MM Silverthorn, KG. AIDS and the ICU nurse. Can Nurse 1988: 84(7): 28-30.

Meduri, GU Stein, DS. Pulmonary manifestations of acquired immunodeficiency syndrome. Clin Infect Dis 1992: 14(1): 98-113.

Meltzer, MS; Nakamura, M; Hansen, BD; Turpin, JA; Kalter, DC Gendelman, HE. Macrophages as susceptible targets for HIV infection, persistent viral reservoirs in tissue, and key immunoregulatory cells that control levels of virus replication and extent of disease. AIDS Res Hum Retroviruses 1990: 6(8): 967-71.

Miasnikov, VG. [Several indices of vitamin B6 metabolism in middle-aged and elderly patients with pulmonary tuberculosis]. Vrach Delo 1969: 3: 77-9.

Michaelsen, KF; Samuelson, G; Graham, TW Lonnerdal, B. Zinc intake, zinc status and growth in a longitudinal study of healthy Danish infants. Acta Paediatr 1994: 83(11): 1115-21.

Migliori, GB; Borghesi, A; Adriko, C; Manfrin, V; Okware, S; Naamara, W; Bartoloni, A; Neri, M Acocella, G. Tuberculosis and HIV infection association in a rural district of northern Uganda: epidemiological and clinical considerations. Tuber Lung Dis 1992: 73(5): 285-90.

Miki, F; Sakai, T; Wariishi, M Kaji, M. Measurement of zinc, copper, manganese, and iron concentrations in hair of pituitary dwarfism patients using flameless atomic absorption spectrophotometry. Biol Trace Elem Res 2002: 85(2): 127-36.

Miorner, H; Ganlov, G; Yohannes, Z Adane, Y. Improved sensitivity of direct microscopy for acid-fast bacilli: sedimentation as an alternative to centrifugation for concentration of tubercle bacilli. J Clin Microbiol 1996: 34(12): 3206-7.

Miorner, H; Gebre, N; Karlsson, U; Jonsson, G; Macaden, R; Wolde, A Assefa, A. Diagnosis of pulmonary tuberculosis. Lancet 1994: 344(8915): 127.

Montenegro, SH; Gilman, RH; Sheen, P; Cama, R; Caviedes, L; Hopper, T; Chambers, R Oberhelman, RA. Improved detection of Mycobacterium tuberculosis in Peruvian children by use of a heminested IS6110 polymerase chain reaction assay. Clin Infect Dis 2003: 36(1): 16-23.

Morrison, SA; Russell, RM; Carney, EA Oaks, EV. Zinc deficiency: a cause of abnormal dark adaptation in cirrhotics. Am J Clin Nutr 1978: 31(2): 276-81.

Moses, AE; Adelowo, KA Ajayi, BB. Prevalence of HIV-1 infection among patients with leprosy and pulmonary tuberculosis in a semi-arid region, Nigeria. J R Soc Health 2003: 123(2): 117-9.

Mugusi, FM; Rusizoka, O; Habib, N Fawzi, W. Vitamin A status of patients presenting with pulmonary tuberculosis and asymptomatic HIV-infected individuals, Dar es Salaam, Tanzania. Int J Tuberc Lung Dis 2003: 7(8): 804-7.

Muhilal; Permeisih, D; Idjradinata, YR; Muherdiyantiningsih Karyadi, D. Vitamin A-fortified monosodium glutamate and health, growth, and survival of children: a controlled field trial. Am J Clin Nutr 1988: 48(5): 1271-6.

Mukadi, Y; Perriens, JH; St Louis, ME; Brown, C; Prignot, J; Willame, JC; Pouthier, F; Kaboto, M; Ryder, RW; Portaels, F et al. Spectrum of immunodeficiency in HIV-1-infected patients with pulmonary tuberculosis in Zaire. Lancet 1993: 342(8864): 143-6.

Muller, O; Becher, H; van Zweeden, AB; Ye, Y; Diallo, DA; Konate, AT; Gbangou, A; Kouyate, B Garenne, M. Effect of zinc supplementation on malaria and other causes of morbidity in west African children: randomised double blind placebo controlled trial. Bmj 2001: 322(7302): 1567.

Mundy, CJ; Bates, I; Nkhoma, W; Floyd, K; Kadewele, G; Ngwira, M; Khuwi, A; Squire, SB Gilks, CF. The operation, quality and costs of a district hospital laboratory service in Malawi. Trans R Soc Trop Med Hyg 2003: 97(4): 403-8.

Narain, JP Lo, YR. Epidemiology of HIV-TB in Asia. Indian J Med Res 2004: 120(4): 277-89.

Narain, JP; Raviglione, MC Kochi, A. HIV-associated tuberculosis in developing countries: epidemiology and strategies for prevention. Tuber Lung Dis 1992: 73(6): 311-21.

Narain, R; Nair, SS; Naganna, K; Chandrasekhar, P; Rao, GR Lal, P. Problems in defining a "case" of pulmonary tuberculosis in prevalence surveys. Bull World Health Organ 1968: 39(5): 701-29.

Narain, R; Rao, MS; Chandrasekhar, P Pyarelal. Microscopy positive and microscopy negative cases of pulmonary tuberculosis. Am Rev Respir Dis 1971: 103(6): 761-73.

Nash, DR Douglass, JE. Anergy in active pulmonary tuberculosis. A comparison between positive and negative reactors and an evaluation of 5 TU and 250 TU skin test doses. Chest 1980: 77(1): 32-7.

Ndjoyi-Mbiguino, A Belec, L. [Evaluation of a panel of commercial kits for the detection of serum HIV-specific antibodies, and choice of alternative strategies for the serological diagnosis in Libreville (Gabon)]. Sante 2005: 15(1): 23-9.

Neu, N; Saiman, L; San Gabriel, P; Whittier, S; Knirsch, C; Ruzal-Shapiro, C Della-Latta, P. Diagnosis of pediatric tuberculosis in the modern era. Pediatr Infect Dis J 1999: 18(2): 122-6.

Newman, GW; Kelley, TG; Gan, H; Kandil, O; Newman, MJ; Pinkston, P; Rose, RM Remold, HG. Concurrent infection of human macrophages with HIV-1 and Mycobacterium avium results in decreased cell viability, increased M. avium multiplication and altered cytokine production. J Immunol 1993: 151(4): 2261-72.

Niyongabo, T; Mlika-Cabanne, N; Barihuta, T; Henzel, D; Aubry, P Larauze, B. Malnutrition, tuberculosis and HIV infection in Burundi. Aids 1994: 8(6): 851-2.

NTBLCP (2004). NTBLCP programme: Guidelines for the clinical management of TB and HIV/AIDS related conditions in Abuja, Nigeria. Federal Ministry of Health, Abuja, Nigeria. Abuja.

NTBLCP (2004). Workers Manual 4th Edition. Federal Ministry of Health Dept of Public Health, Abuja, Nigeria: 1-164.

Nunn, P; Gicheha, C; Hayes, R; Gathua, S; Brindle, R; Kibuga, D; Mutie, T; Kamunyi, R; Omwega, M; Were, J et al. Cross-sectional survey of HIV infection among patients with tuberculosis in Nairobi, Kenya. Tuber Lung Dis 1992: 73(1): 45-51.

Nwachokor, FN Thomas, JO. Tuberculosis in Ibadan, Nigeria--a 30 year review. Cent Afr J Med 2000: 46(11): 287-92.

Nwobu.; Okodua, M Tatfeng, Y. Comparative Study Of HIV Associated Pulmonary Tuberculosis In Chest Clinics From Two Regions Of Edo State, Nigeria. Online J Health Allied Scs 2004: 3(3): 1-7.

Nwobu, G; MA, O YM, T. Comparative Study Of HIV Associated Pulmonary Tuberculosis In Chest Clinics From Two Regions Of Edo State, Nigeria. Online J Health Allied Scs 2004: 3(3): 1-7.

Okogun, G; Okodua M; Tatfeng YM; Nwobu GO; Isibor JO NW., D. HealthPoint Prevalence of PulmonaryTuberculosis (PTB) Associated HumanImmunodeficiency Virus (HIV) inWestern Nigeria. Int. J. Expt. Hlth &Human Devpt. 2002: 3(2.): 49-53.

Onipede, AO; Idigbe, O; Ako-Nai, AK; Omojola, O; Oyelese, AO; Aboderin, AO; Akinosho; Komolafe, AO Wemambu, SN. Sero-prevalence of HIV

antibodies in tuberculosis patients in Ile-Ife, Nigeria. East Afr Med J 1999: 76(3): 127-32.

Onorato, IM McCray, E. Prevalence of human immunodeficiency virus infection among patients attending tuberculosis clinics in the United States. J Infect Dis 1992: 165(1): 87-92.

Onwubalili, JK; Nwosu, CM Onuigbo, WI. Deaths from AIDS-like illnesses in west Africans. East Afr Med J 1988: 65(12): 867-73.

Ouattara, S; D, D Meite, M. Epidemiologie des infections par le virus de l'immunodeficience humaine (VIH-1 et VIH-2 en Cote d'Ivoire. Med Trop (Mars) 1988: 48: 375-9.

Pai, M. Alternatives to the tuberculin skin test: Interferon-gamma assays in the diagnosis of mycobacterium tuberculosis infection. Indian J Med Microbiol 2005: 23(3): 151-8.

Pai, M; Riley, LW Colford, JM, Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. Lancet Infect Dis 2004: 4(12): 761-76.

Palmieri, F; Girardi, E; Pellicelli, AM; Rianda, A; Bordi, E; Rizzi, EB; Petrosillo, N Ippolito, G. Pulmonary tuberculosis in HIV-infected patients presenting with normal chest radiograph and negative sputum smear. Infection 2002: 30(2): 68-74.

Pant, K; Biswas, SK; Chawla, R; Shah, A Singh, MM. Zinc in active pulmonary tuberculosis. Indian J Chest Dis Allied Sci 1987: 29(3): 144-9.

Paterson, R. Human trials start for new tuberculosis vaccine. Lancet Infect Dis 2001: 1(5): 291.

Penny, ME; Peerson, JM; Marin, RM; Duran, A; Lanata, CF; Lonnerdal, B; Black, RE Brown, KH. Randomized, community-based trial of the effect of zinc supplementation, with and without other micronutrients, on the duration of persistent childhood diarrhea in Lima, Peru. J Pediatr 1999: 135(2 Pt 1): 208-17.

Perkins, MD. New diagnostic tools for tuberculosis. Int J Tuberc Lung Dis 2000: 4(12 Suppl 2): S182-8.

Perry, RT Halsey, NA. The clinical significance of measles: a review. J Infect Dis 2004: 189 Suppl 1: S4-16.

Petran, E Perry, C. The routine use of concentration of tubercule bacilli in microscopically negative sputa and other body fluids. J Lab Clin Med 1939: 24: 539-43.

Pories, WJ; Henzel, JH; Rob, CG Strain, WH. Acceleration of wound healing in man with zinc sulphate given by mouth. Lancet 1967: 1(7482): 121-4.

Prasad, AS. Recognition of zinc-deficiency syndrome. Nutrition 2001: 17(1): 67-9.

Prasad, AS; Fitzgerald, JT; Bao, B; Beck, FW Chandrasekar, PH. Duration of symptoms and plasma cytokine levels in patients with the common cold treated with zinc acetate. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 2000: 133(4): 245-52.

Prasad, AS; Miale, A, Jr.; Farid, Z; Sandstead, HH Schulert, AR. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypognadism. J Lab Clin Med 1963: 61: 537-49.

Prasad, AS Oberleas, D. Changes in activities of zinc-dependent enzymes in zinc-deficient tissues of rats. J Appl Physiol 1971a: 31(6): 842-6.

Prasad, AS; Oberleas, D; Miller, ER Luecke, RW. Biochemical effects of zinc difficiency: changes in activities of zinc-dependent enzymes and ribonucleic acid and deoxyribonucleic acid content of tissues. J Lab Clin Med 1971b: 77(1): 144-52.

Prasad, R; Saini, JK; Gupta, R; Kannaujia, RK; Sarin, S; Suryakant; Kulshreshth, R; Nag, VL Tripathi, AK. A comparative study of clinico-radiological spectrum of tuberculosis among HIV seropositive and HIV seronegative patients. Indian J Chest Dis Allied Sci 2004: 46(2): 99-103.

Rahman, MM; Wahed, MA; Fuchs, GJ; Baqui, AH Alvarez, JO. Synergistic effect of zinc and vitamin A on the biochemical indexes of vitamin A nutrition in children. Am J Clin Nutr 2002: 75(1): 92-8.

Ramachandran, G; Santha, T; Garg, R; Baskaran, D; Iliayas, SA; Venkatesan, P; Fathima, R Narayanan, PR. Vitamin A levels in sputum-positive pulmonary tuberculosis patients in comparison with household contacts and healthy 'normals'. Int J Tuberc Lung Dis 2004: 8(9): 1130-3.

Range, N; Andersen, AB; Magnussen, P; Mugomela, A Friis, H. The effect of micronutrient supplementation on treatment outcome in patients with pulmonary tuberculosis: a randomized controlled trial in Mwanza, Tanzania. Trop Med Int Health 2005: 10(9): 826-32.

Raviglione, MC. The TB epidemic from 1992 to 2002. Tuberculosis (Edinb) 2003: 83(1-3): 4-14.

Raviglione, MC; Harries, AD; Msiska, R; Wilkinson, D Nunn, P. Tuberculosis and HIV: current status in Africa. Aids 1997: 11 Suppl B: S115-23.

Raviglione, MC Luelmo, F. Update on the global epidemiology of tuberculosis. Curr Issues Public Health 1996: 2(4): 192-7.

Raviglione, MC; Narain, JP Kochi, A. HIV-associated tuberculosis in developing countries: clinical features, diagnosis, and treatment. Bull World Health Organ 1992: 70(4): 515-26.

Raviglione, MC; Rieder, HL; Styblo, K; Khomenko, AG; Esteves, K Kochi, A. Tuberculosis trends in eastern Europe and the former USSR. Tuber Lung Dis 1994: 75(6): 400-16.

Raviglione, MC; Snider, DE, Jr. Kochi, A. Global epidemiology of tuberculosis. Morbidity and mortality of a worldwide epidemic. Jama 1995: 273(3): 220-6.

Ray, M; Kumar, L Prasad, R. Plasma zinc status in Indian childhood tuberculosis: impact of antituberculosis therapy. Int J Tuberc Lung Dis 1998: 2(9): 719-25.

Ray, SK; Mallik, S; Munsi, AK; Mitra, SP; Baur, B Kumar, S. Epidemiological study of measles in slum areas of Kolkata. Indian J Pediatr 2004: 71(7): 583-6.

Rieder, HL. Epidemiology of tuberculosis in Europe. Eur Respir J Suppl 1995: 20: 620s-32s.

Rose, CE, Jr.; Zerbe, GO; Lantz, SO Bailey, WC. Establishing priority during investigation of tuberculosis contacts. Am Rev Respir Dis 1979: 119(4): 603-9.

Roy, SK; Tomkins, AM; Akramuzzaman, SM; Behrens, RH; Haider, R; Mahalanabis, D Fuchs, G. Randomised controlled trial of zinc supplementation in malnourished Bangladeshi children with acute diarrhoea. Arch Dis Child 1997: 77(3): 196-200.

Roy, SK; Tomkins, AM; Haider, R; Behren, RH; Akramuzzaman, SM; Mahalanabis, D Fuchs, GJ. Impact of zinc supplementation on subsequent growth and morbidity in Bangladeshi children with acute diarrhoea. Eur J Clin Nutr 1999: 53(7): 529-34.

Rwangabwoba, JM; Fischman, H Semba, RD. Serum vitamin A levels during tuberculosis and human immunodeficiency virus infection. Int J Tuberc Lung Dis 1998: 2(9): 771-3.

Saha, K Rao, KN. Undernutrition in lepromatous leprosy. V. Severe nutritional deficit in lepromatous patients co-infected with pulmonary tuberculosis. Eur J Clin Nutr 1989: 43(2): 117-28.

Samb, B; Sow, PS; Kony, S; Maynart-Badiane, M; Diouf, G; Cissokho, S; Ba, D; Sane, M; Klotz, F; Faye-Niang, MA; Mboup, S; Ndoye, I; Delaporte, E; Hane, AA; Samb, A; Coulaud, JP; Coll-Seck, AM; Larouze, B Murray, JF. Risk factors for negative sputum acid-fast bacilli smears in pulmonary tuberculosis: results from Dakar, Senegal, a city with low HIV seroprevalence. Int J Tuberc Lung Dis 1999: 3(4): 330-6.

Schag, CC; Heinrich, RL Ganz, PA. Karnofsky performance status revisited: reliability, validity, and guidelines. J Clin Oncol 1984: 2(3): 187-93.

Scott, GM Holton, J. Transmission of tuberculosis by patients with HIV infection. Bmj 1994: 309(6967): 1515.

Scrimshaw, NS. The new paradigm of public health nutrition. Am J Public Health 1995: 85(5): 622-4.

Scrimshaw, NS. Rhoades Lecture. Effect of infection on nutrient requirements. JPEN J Parenter Enteral Nutr 1991: 15(6): 589-600.

Scrimshaw, NS SanGiovanni, JP. Synergism of nutrition, infection, and immunity: an overview. Am J Clin Nutr 1997: 66(2): 464S-77S.

Semba, RD. Vitamin A and immunity to viral, bacterial and protozoan infections. Proc Nutr Soc 1999: 58(3): 719-27.

Sempertegui, F; Estrella, B; Correa, E; Aguirre, L; Saa, B; Torres, M; Navarrete, F; Alarcon, C; Carrion, J; Rodriguez Griffiths, JK. Effects of short-term zinc supplementation on cellular immunity, respiratory symptoms, and growth of malnourished Equadorian children. Eur J Clin Nutr 1996: 50(1): 42-6.

Shams, H; Weis, SE; Klucar, P; Lalvani, A; Moonan, PK; Pogoda, JM; Ewer, K Barnes, PF. Enzyme-Linked Immunospot and Tuberculin Skin Testing to Detect Latent Tuberculosis Infection. Am J Respir Crit Care Med 2005.

Shaw, JB Wynn-Williams, N. Infectivity of pulmonary tuberculosis in relation to sputum status. Am Rev Tuberc 1954: 69(5): 724-32.

Shor-Posner, G; Miguez, MJ; Pineda, LM; Rodriguez, A; Ruiz, P; Castillo, G; Burbano, X; Lecusay, R Baum, M. Impact of selenium status on the pathogenesis of mycobacterial disease in HIV-1-infected drug users during the era of highly active antiretroviral therapy. J Acquir Immune Defic Syndr 2002: 29(2): 169-73.

Simooya, OO; Maboshe, MN; Kaoma, RB; Chimfwembe, EC; Thurairajah, A Mukunyandela, M. HIV infection in newly diagnosed tuberculosis patients in Ndola, Zambia. Cent Afr J Med 1991: 37(1): 4-7.

Sklan, D; Melamed, D Friedman, A. The effect of varying levels of dietary vitamin A on immune response in the chick. Poult Sci 1994: 73(6): 843-7.

Smith, JC, Jr. The vitamin A-zinc connection: a review. Ann N Y Acad Sci 1980: 355: 62-75.

Smurova, TF Prokop'ev, DI. [Vitamin A and carotene levels in the blood of patients with pulmonary tuberculosis and diabetes mellitus]. Probl Tuberk 1969: 47(11): 50-5.

Sodhi, A; Gong, J; Silva, C; Qian, D Barnes, PF. Clinical correlates of interferon gamma production in patients with tuberculosis. Clin Infect Dis 1997: 25(3): 617-20.

Solomon, S; Anuradha, S Rajasekaran, S. Trend of HIV infection in patients with pulmonary tuberculosis in south India. Tuber Lung Dis 1995: 76(1): 17-9.

Sommer, A; Katz, J Tarwotjo, I. Increased risk of respiratory disease and diarrhea in children with preexisting mild vitamin A deficiency. Am J Clin Nutr 1984: 40(5): 1090-5.

Sommer, A; Tarwotjo, I; Djunaedi, E; West, KP, Jr.; Loeden, AA; Tilden, R Mele, L. Impact of vitamin A supplementation on childhood mortality. A randomised controlled community trial. Lancet 1986: 1(8491): 1169-73.

Somoskovi, A; Kodmon, C; Lantos, A; Bartfai, Z; Tamasi, L; Fuzy, J Magyar, P. Comparison of recoveries of mycobacterium tuberculosis using the automated BACTEC MGIT 960 system, the BACTEC 460 TB system, and Lowenstein-Jensen medium. J Clin Microbiol 2000: 38(6): 2395-7.

Stanford, JL; Grange, JM Pozniak, A. Is Africa lost? Lancet 1991: 338(8766): 557-8.

Strand, TA; Chandyo, RK; Bahl, R; Sharma, PR; Adhikari, RK; Bhandari, N; Ulvik, RJ; Molbak, K; Bhan, MK Sommerfelt, H. Effectiveness and efficacy of zinc for the treatment of acute diarrhea in young children. Pediatrics 2002: 109(5): 898-903.

Styblo, K. The impact of HIV infection on the global epidemiology of tuberculosis. Bull Int Union Tuberc Lung Dis 1991: 66(1): 27-32.

Sudre, P; ten Dam, G Kochi, A. Tuberculosis: a global overview of the situation today. Bull World Health Organ 1992: 70(2): 149-59.

Suffys, PN; da Silva Rocha, A; de Oliveira, M; Campos, CE; Barreto, AM; Portaels, F; Rigouts, L; Wouters, G; Jannes, G; van Reybroeck, G; Mijs, W Vanderborght, B. Rapid identification of Mycobacteria to the species level using INNO-LiPA Mycobacteria, a reverse hybridization assay. J Clin Microbiol 2001: 39(12): 4477-82.

Tabone, MD; Muanza, K; Lyagoubi, M; Jardel, C; Pied, S; Amedee-Manesme, O; Grau, GE Mazier, D. The role of interleukin-6 in vitamin A deficiency during Plasmodium falciparum malaria and possible consequences for vitamin A supplementation. Immunology 1992: 75(3): 553-4.

Taneja, DP. Observations on serum zinc in patients of pulmonary tuberculosis. J Indian Med Assoc 1990: 88(10): 280-1, 75.

Tattevin, P; Casalino, E; Fleury, L; Egmann, G; Ruel, M Bouvet, E. The validity of medical history, classic symptoms, and chest radiographs in predicting pulmonary tuberculosis: derivation of a pulmonary tuberculosis prediction model. Chest 1999: 115(5): 1248-53.

Thu, BD; Schultink, W; Dillon, D; Gross, R; Leswara, ND Khoi, HH. Effect of daily and weekly micronutrient supplementation on micronutrient deficiencies and growth in young Vietnamese children. Am J Clin Nutr 1999: 69(1): 80-6.

Thurnham, DI; Northrop-Clewes, CA; McCullough, FS; Das, BS Lunn, PG. Innate immunity, gut integrity, and vitamin A in Gambian and Indian infants. J Infect Dis 2000: 182 Suppl 1: S23-8.

Tsao; Chung; Yang Chiou. (2004). "Significant difference of clinical manifestations between smear negative and smear positive PTB." CHEST, 2004.

Ulrichs, T; Munk, ME; Mollenkopf, H; Behr-Perst, S; Colangeli, R; Gennaro, ML Kaufmann, SH. Differential T cell responses to Mycobacterium tuberculosis ESAT6 in tuberculosis patients and healthy donors. Eur J Immunol 1998: 28(12): 3949-58.

Van Deun, A; Maug, AK; Cooreman, E; Hossain, MA; Chambuganj, N; Rema, V; Marandi, H; Kawria, A Portaels, F. Bleach sedimentation method for increased sensitivity of sputum smear microscopy: does it work? Int J Tuberc Lung Dis 2000: 4(4): 371-6.

Van Deun, A; Salim, AH; Cooreman, E; Daru, P; Das, AP; Aung, KJ Rieder, HL. Scanty AFB smears: what's in a name? Int J Tuberc Lung Dis 2004: 8(7): 816-23.

Van Deun, A; Salim, AH; Cooreman, E; Hossain, MA; Rema, A; Chambugonj, N; Hye, MA; Kawria, A Declercq, E. Optimal tuberculosis case detection by direct sputum smear microscopy: how much better is more? Int J Tuberc Lung Dis 2002: 6(3): 222-30.

van Lettow, M; Fawzi, WW Semba, RD. Triple trouble: the role of malnutrition in tuberculosis and human immunodeficiency virus co-infection. Nutr Rev 2003: 61(3): 81-90.

van Lettow, M; Harries, AD; Kumwenda, JJ; Zijlstra, EE; Clark, TD; Taha, TE Semba, RD. Micronutrient malnutrition and wasting in adults with pulmonary tuberculosis with and without HIV co-infection in Malawi. BMC Infect Dis 2004a: 4(1): 61.

Van Lettow, M; Kumwenda, JJ; Harries, AD; Whalen, CC; Taha, TE; Kumwenda, N; Kang'ombe, C Semba, RD. Malnutrition and the severity of lung disease in adults with pulmonary tuberculosis in Malawi. Int J Tuberc Lung Dis 2004: 8(2): 211-7.

Ventura, MT; Crollo, R Lasaracina, E. In vitro zinc correction of natural killer (NK) activity in the elderly. Clin Exp Immunol 1986: 64(1): 223-4.

Vijayaraghavan, K; Radhaiah, G; Prakasam, BS; Sarma, KV Reddy, V. Effect of massive dose vitamin A on morbidity and mortality in Indian children. Lancet 1990: 336(8727): 1342-5.

Villarino, ME; Geiter, LJ Simone, PM. The multidrug-resistant tuberculosis challenge to public health efforts to control tuberculosis. Public Health Rep 1992: 107(6): 616-25.

Walker, GM. Metals in yeast fermentation processes. Adv Appl Microbiol 2004: 54: 197-229.

Walley, JD; Khan, MA; Newell, JN Khan, MH. Effectiveness of the direct observation component of DOTS for tuberculosis: a randomised controlled trial in Pakistan. Lancet 2001: 357(9257): 664-9.

Wellinghausen, N; Martin, M Rink, L. Zinc inhibits interleukin-1-dependent T cell stimulation. Eur J Immunol 1997: 27(10): 2529-35.

West, KP, Jr.; Pokhrel, RP; Katz, J; LeClerq, SC; Khatry, SK; Shrestha, SR; Pradhan, EK; Tielsch, JM; Pandey, MR Sommer, A. Efficacy of vitamin

A in reducing preschool child mortality in Nepal. Lancet 1991: 338(8759): 67-71.

WHO. Global tuberculosis Control, Geneva. World Health Organization, 2001 2001.

WHO (2005). Global Tuberculosis Control, Surveilance, Planning, Financing: 108-11.

WHO. TB-a global emergency. WHO TB programme, Geneva, Switzerland 1994: WHO/TB/94.177: 1-28.

WHO (2000b). Tuberculosis. Fact Sheet 104. Geneva: WHO,2000.

WHO (2002). WHO report 2002. Global tuberculosis Control, Surveilance, Planning, Financing. WHO/CDS/TB/2002.295 Geneva: WHO 2002.

WHO (2000a). World Health Organization-Programme of Tropical Diseases Research. Why tuberculosis research? Geneva:WHO,2000.

Wilkinson, D Sturm, AW. Diagnosing tuberculosis in a resource-poor setting: the value of sputum concentration. Trans R Soc Trop Med Hyg 1997: 91(4): 420-1.

Wilkinson, RJ; Llewelyn, M; Toossi, Z; Patel, P; Pasvol, G; Lalvani, A; Wright, D; Latif, M Davidson, RN. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. Lancet 2000: 355(9204): 618-21.

Wiysonge, C; Shey, M; Sterne, J; Brocklehurst, P Wiysonge, CS. Vitamin A supplementation for reducing the risk of mother-to-child transmission of HIV infection. Cochrane Database Syst Rev 2005: (4): CD003648.

Woodring, JH; Vandiviere, HM; Fried, AM; Dillon, ML; Williams, TD Melvin, IG. Update: the radiographic features of pulmonary tuberculosis. AJR Am J Roentgenol 1986: 146(3): 497-506.

Yassin, MA; Cuevas, LE; Gebrexabher, H Squire, SB. Efficacy and safety of short-term bleach digestion of sputum in case-finding for pulmonary tuberculosis in Ethiopia. Int J Tuberc Lung Dis 2003: 7(7): 678-83.

Yassin, MA; Takele, L; Gebresenbet, S; Girma, E; Lera, M; Lendebo, E Cuevas, LE. HIV and tuberculosis coinfection in the southern region of Ethiopia: a prospective epidemiological study. Scand J Infect Dis 2004: 36(9): 670-3.

Yew, WW; Chau, CH; Lee, J Leung, CK. Is inhaled corticosteroid useful as adjunctive management in tuberculous pyrexia? Drugs Exp Clin Res 1999: 25(4): 179-84.

Ziehl, F. Zur Farbung des tuberkelbacillus. Dtsch Med Wochenschr 1982: 8: 451.

Appedices

Name---------- Study Number ☐☐☐

DIAGNOSIS OF TUBERCULOSIS AND THE ROLE OF MICRONUTRIENTS IN THE TREATMENT OF PULMONARY TUBERCULOSIS IN NIGERIA

Patient initials _____

ENTRY CRITERIA

Patients can be recruited for the study only when all the boxes below can be ticked NO.

	Yes	No
1) Patient's age is under 15years.	☐	☐
2) Patient had moderate to severe abdominal surgery during the previous month.	☐	☐
3) Patient has a history of diabetes mellitus or severe cardiovascular, liver or renal disease.	☐	☐
4) Patient has been treated in the past for tuberculosis.	☐	☐
5) Patient has taken corticosteroids or zinc supplements during the previous month.	☐	☐
6) Patient is pregnant, lactating or taking oral contraceptives.	☐	☐

Signature ——————————————— Date: ———————————

Diagnosis of tuberculosis and the role of micronutrients in the treatment of pulmonary tuberculosis - Consent Form

Good morning/afternoon. I am Dr/Mr/Mrs/
Miss.....Tuberculosis is an important lung infection that can kill if not properly treated. This infection, which is difficult to diagnose, is on the increase worldwide and some of the bacteria are becoming difficult to treat. I will like to know if you will be willing to participate in a study we are carrying out here to try to find newer and better techniques to detect and treat tuberculosis.

We are studying the effect of a mineral called zinc. Zinc is contained in everyday diet, and I would like to know whether the level of zinc in patients with tuberculosis is low or not. Also, because zinc is known to improve the body responses to infections, we would like to know whether giving zinc would improve the treatment of tuberculosis. Zinc is a micronutrient available in the food, and so, taking zinc supplements is unlikely to have adverse effects. It is now known that patients with some other infections such as diarrhoea and measles have improved faster when the usual drugs are given together with these nutrients.

We intend to add zinc and or Vitamin A to your routine TB drugs and lookout for improvement during your follow up. You would be asked to take two extra capsules once a day. The capsules will contain zinc, vitamin A or lactose. We will not tell you which treatment you are receiving, as we need to be as objective as possible to assess if there is any beneficial effect.

We also know that majority of patients suffering from tuberculosis also suffer from HIV. We would like to determine how many patients, in this hospital, who are suffering from TB, are infected with the virus.

To carry out the study we will require a chest X-ray and the collection of three samples of sputum and taking some blood from you for investigations during your first and your follow up visits. This would normally have been collected and taken from you even if you are not taking part in the study. We would like to take the blood samples to see how much zinc is there in the blood at the beginning of the study and at 2 and 8 weeks after initiation of treatment. During the follow up, we would ask you to bring your morning sputum and give us a sputum sample every week for the first 2 months and until the sputum has become negative for the TB germ. These samples will be taken when you come to the hospital to receive your weekly treatment. We will see how many germs causing tuberculosis remain in your sputum. We would measure your height and weight at the beginning and after 2 months to see if there are any changes.

We will require your permission to be able to include you in our study. Once we receive your consent, you will not have to pay for any drug or investigations during the course of this study. The results of the HIV test will not be disclosed to anyone. We can tell you your HIV results if you wish to know. If you would like

us to tell you, we will ask you to attempt pre and post-test counselling to help you decide.

We hope you would be willing to participate, but if you do not feel like taking part in this study, it will not affect, in any way, the treatment you will receive from the hospital. You can also stop participating at any stage, without detriment to you or your relationship with the doctors in the hospital. You do not need to explain to us why you do not want to participate or withdraw from the trial.

Please ask us any questions you might have.

I understand the content, and I willingly give my consent.

Name .....

Address .....

Signature .....

QUESTIONAIRE Date of interview ☐☐☐☐☐☐

Name: _____ Age ☐☐

Address: _____ Sex: ☐

Occupation: _____

Tribe: _____

A. Background

Education: *(none=0, primary=1, secondary=2, university=4* ☐

How many people sleep in the same room with you? *(Number)* ☐☐

Have you ever smoked? *(Yes=1, no=2)* ☐

If yes, for how long? *(Years)* ☐☐

How many sticks per day do you smoke? *(Number)* ☐☐☐

B. Contact Data

Do you have anybody with a cough of 3wks or more, in your house? *(Yes=1, no=2, not known=3)* ☐

If yes, for how long have you lived with this person? *(Years)* ☐

Do you have anybody with a fever of 3wks or more in your house? *(Yes=1, no=2, not known=3)* ☐

Have you been in close contact with anyone diagnosed with TB in the past two years? ☐

(Yes=1, no=2, not known=3)

If yes, for how long? *(Years)* ☐☐

Is he/she currently on treatment? *(Yes=1, no=2, not know=3)* ☐

C. Clinical Data

If yes, for how long?

Have you had BCG at birth or at any other time?*(Yes=1, no=2, not kno* ☐ *(Years)* ☐☐

Have you had cough recently? *(Yes=1,no=2)* ☐ *(Weeks)* ☐☐

Have you ever been diagnosed with TB in the past? *(Yes=1, no=2, not known=3* ☐ *(Weeks)* ☐☐

Have you recently had fever or sweating without exercise? ☐ *(Yes=1, no* ☐☐ *(Weeks)*

Have you had any blood in your coughed out sputum?*(Yes=1, no=2* ☐ *(Weeks)* ☐☐

Have you been breathless in the past few weeks? *(Yes=1, no=2* ☐ *(Weeks)* ☐☐

Have you had any chest pains the past few weeks? *(Yes=1, no=2)* ☐ *(Weeks)* ☐☐

Have you been losing weight recently? *(Yes=1, no=2)* ☐ *(Weeks)* ☐☐

Have you had night sweats? *(Yes=1, no=2)* ☐ *(W* ☐☐

Have you had loss of appetite? *(Yes=1,no=2)* ☐ *(Weeks)* ☐☐

Have you noticed any swelling of the tip of your fingers (finger clubbir *(Yes=1, no=2)* ☐ *(Weeks)* ☐☐

Have you had any firm swellings (lymph nodes) around your neck? *(Yes* ☐ *2)* *(Weeks)* ☐☐

D. Examinations

Presence of BCG scar (yes=1, no=2) ☐ BCG scar siz ☐☐

Weight *(kg)* ☐☐☐ Height *(cm)* ☐☐☐

Temperature (°C) ☐☐☐ Karnofsky score ☐☐☐

Chest examination *(normal=1, abnormal=2)* ☐

Wheeze *(yes=1, no=2)* ☐ Bronchial breath sounds *(yes=1, no=2)* ☐

☐

Rhonchi *(yes=1, no=2)* Crepitations *(yes=1, no=2)*

Diagnosis: 1 _____ 2 _____ 3 _____

E. Investigations

PPD *(Done=1, not done=2)* ☐ PPD *(size in mm)* ☐

Chest X-ray (*Done=1, not done=2)* ☐

F. Laboratory examinations - Taken? *(yes=1, no=2)* ☐

Date of next appointment ☐☐☐☐☐☐

1st sputum (on the spot)? ☐ Morning sputum taken? ☐

3rd sputum (on the spot) ☐ Was blood taken? ☐

Follow up week 1

	YES	NO		
Was patient followed?	☐	☐	If yes, date attended	☐☐☐☐☐☐
			If no, date for home follow up	_____
Did you take the treatment?	☐	☐	If NO, which one did you miss?	_____

What symptoms are you suffering from today? If YES, are you better or worse than last week?

	YES	NO	Better	same	worse
Cough	☐	☐	☐	☐	☐
Fever	☐	☐	☐	☐	☐
Night sweats	☐	☐	☐	☐	☐
Hemoptysis	☐	☐	☐	☐	☐
Headache	☐		☐	☐	☐
Dyspnea	☐		☐	☐	☐
Chest pains	☐		☐	☐	☐
			☐	☐	☐

Loss of appetite ☐

Chest exam (normal, abnormal) ☐

Other ☐ Specify _____

How do you describe your cough today? (see codes at the bott☐)1

Weight (kg) ☐☐☐ Height (cm) ☐☐☐

Temperature (oC) ☐☐☐ Karnofsky scale ☐☐☐

Laboratory examinations - Taken? *(yes=1, no=2)*

1st sputum (on the spot)? ☐ Morning sputum taken? ☐

3rd sputum (on the spot) ☐

Date of next appointment ☐☐☐☐☐☐

1(No cough at all=0; Occasional hems=1; Mild, isolated cough, accompanied by chest discomfort=2;

Moderate, paroxymal cough without additional symptoms=3; Severe strenuous cough, accompanied by chest discomfort=

Follow up week 2

	YES	NO	
Was patient followed?	☐	☐	If yes, date atte☐☐☐☐☐☐
			If no, date for home follow up _____
Did you take the treatment?	☐	☐	If NO, which one did you miss? _____

What symptoms are you suffering from today? If YES, are you better or worse than last week?

	YES	NO	Better	same	worse
Cough			☐	☐	☐
Fever			☐	☐	☐
Night sweats			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

Hemoptysis

Headache

Dyspnea

Chest pains

Loss of appetite

Chest exam (normal, abnormal)

Other Specify_____

How do you describe your cough today? (see codes at the bott)1

Weight (kg) Height (cm)

Temperature (oC) Karnofsky scale

Laboratory examinations - Taken? *(yes=1, no=2)*

1st sputum (on the spot)? Morning sputum taken?

3rd sputum (on the spot)

Date of next appointment

1(No cough at all=0; Occasional hems=1;

Mild, isolated cough, accompanied by chest discomfort=2;

Moderate, paroxymal cough without additional symptoms=3;

Severe strenuous cough, accompanied by chest discomfort=4

Follow up week 3

	YES	NO	
Was patient followed?			If yes, date attended
			If no, date for home follow up _____
Did you take the treatment?			If NO, which one did you miss?_____

What symptoms are you suffering from today? If YES, are you better or worse than last week?

	YES	NO	Better	same	worse
Cough					
Fever					
Night sweats					
Hemoptysis					
Headache					
Dyspnea					
Chest pains					
Loss of appetite					
Chest exam (normal, abnormal)					
Other					

Specify_____

How do you describe your cough today? (see codes at the bot)1

Weight (kg)

Height (cm)

Temperature (oC)

Karnofsky scale

Laboratory examinations - Taken? *(yes=1, no=2)*

1st sputum (on the spot)?

Morning sputum taken

3rd sputum (on the spot)

Date of next appointment

1(No cough at all=0; Occasional hems=1;

Mild, isolated cough, accompanied by chest discomfort=2;

Moderate, paroxymal cough without additional symptoms=3;

Severe strenuous cough, accompanied by chest discomfort=4

Follow up week 4, 3rd month

YES NO

Was patient followed?

If yes, date atte

If no, date for home follow up _____

Did you take the treatment? ☐ ☐ If NO, which one did you miss? _____

What symptoms are you suffering from today? If YES, are you better or worse than last week?

	YES	NO	Better	same	worse
Cough			☐	☐	☐
Fever	☐	☐	☐	☐	☐
Night sweats	☐	☐	☐	☐	☐
Hemoptysis	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Dyspnea		☐	☐	☐	☐
Chest pains	☐	☐	☐	☐	☐
Loss of appetite	☐	☐	☐	☐	☐
Chest exam (normal, abnormal)	☐	☐	☐	☐	☐
Other	☐	☐			

Specify _____

How do you describe your cough today? (see codes at the bottom)1 ☐

Weight (kg) ☐☐☐ Height (cm) ☐☐☐

Temperature (oC) ☐☐☐ Karnofsky scale ☐☐☐

Laboratory examinations - Taken? *(yes=1, no=2)*

Date of next appointment ☐☐☐☐☐☐

1st sputum (on the spot)? ☐ Morning sputum taken? ☐

3rd sputum (on the spot) ☐ Was blood taken? ☐

1(No cough at all=0; Occasional hems=1; Mild, isolated cough, accompanied by chest discomfort=2;

Moderate, paroxymal cough without additional symptoms=3; Severe strenuous cough, accompanied by chest discomfort=4

Follow up week 5

	YES	NO	
Was patient followed?	☐	☐	If yes, date atte ☐☐☐☐☐☐
			If no, date for home follow up _____
Did you take the treatment?	☐	☐	If NO, which one did you miss? _____

What symptoms are you suffering from today? | If YES, are you better or worse than last week?

	YES	NO	Better	same	worse
Cough			☐	☐	☐
Fever	☐	☐	☐	☐	☐
Night sweats	☐	☐	☐	☐	☐
Hemoptysis	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Dyspnea		☐	☐	☐	☐
Chest pains	☐	☐	☐	☐	☐
Loss of appetite	☐	☐	☐	☐	☐
Chest exam (normal, abnormal)	☐	☐	☐	☐	☐
Other	☐	☐			

Specify _____

How do you describe your cough today? (see codes at the bottom) ☐

Weight (kg) ☐☐☐ Height (cm) ☐☐☐

Temperature (oC) ☐☐☐ Karnofsky scale ☐☐☐

Laboratory examinations - Taken? *(yes=1, no=2)*

1st sputum (on the spot)? ☐ Morning sputum taken? ☐

☐

Date of next appointment

☐☐☐☐☐☐

3rd sputum (on the spot)

_____

1(No cough at all=0; Occasional hems=1; Mild, isolated cough, accompanied by chest discomfort=2;

Moderate, paroxymal cough without additional symptoms=3; Severe strenuous cough, accompanied by chest discomfort=4

Follow up week 6

	YES	NO	
Was patient followed?	☐	☐	If yes, date atte ☐☐☐☐☐☐
			If no, date for home follow up _____
Did you take the treatment?	☐	☐	If NO, which one did you miss? _____

What symptoms are you suffering from today? If YES, are you better or worse than last week?

	YES	NO	Better	same	worse
Cough			☐	☐	☐
Fever	☐	☐	☐	☐	☐
Night sweats	☐	☐	☐	☐	☐
Hemoptysis	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Dyspnea		☐	☐	☐	☐
Chest pains	☐	☐	☐	☐	☐
Loss of appetite	☐	☐	☐	☐	☐
Chest exam (normal, abnormal)	☐	☐			
Other	☐	☐	Specify _____		

How do you describe your cough today? (see codes at the bot☐)1

Weight (kg) ☐☐☐ Height (cm) ☐☐☐

Temperature (oC) ☐☐☐ Karnofsky scale ☐☐☐

Laboratory examinations - Taken? *(yes=1, no=2)*

1st sputum (on the spot)? ☐ Morning sputum taken? ☐

3rd sputum (on the spot) ☐

Date of next appointment ☐☐☐☐☐☐

1(No cough at all=0; Occasional hems=1;

Mild, isolated cough, accompanied by chest discomfort=2;

Moderate, paroxymal cough without additional symptoms=3;

Severe strenuous cough, accompanied by chest discomfort=4

Follow up week 7

	YES	NO	
Was patient followed?	☐	☐	If yes, date attended ☐☐☐☐☐☐
			If no, date for home follow up _____
Did you take the treatment?	☐	☐	If NO, which one did you miss? _____

What symptoms are you suffering from today? If YES, are you better or worse than last week?

	YES	NO	Better	same	worse
Cough			☐	☐	☐
Fever	☐	☐	☐	☐	☐
Night sweats	☐	☐	☐	☐	☐
Hemoptysis	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Dyspnea		☐	☐	☐	☐
Chest pains	☐	☐	☐	☐	☐
Loss of appetite	☐	☐	☐	☐	☐
Chest exam (normal, abnormal)	☐	☐	☐	☐	☐
Other	☐	☐			

Specify _____

☐

How do you describe your cough today? (see codes at the bottom)1

Weight (kg) ☐☐☐ Height (cm) ☐☐☐

Temperature (oC) ☐☐☐ Karnofsky scale ☐☐☐

Laboratory examinations - Taken? *(yes=1, no=2)*

1st sputum (on the spot)? ☐ Morning sputum taken ☐

3rd sputum (on the spot) ☐

Date of next appointment

☐☐☐☐☐☐

1(No cough at all=0; Occasional hems=1;

Mild, isolated cough, accompanied by chest discomfort=2;

Moderate, paroxymal cough without additional symptoms=3;

Severe strenuous cough, accompanied by chest discomfort=

Follow up 2nd month

YES NO

Was patient followed? ☐ ☐ If yes, date atte ☐☐☐☐☐☐

If no, date for home follow up _____

Did you take the treatment? ☐ ☐ If NO, which one did you miss? _____

What symptoms are you suffering from today? If YES, are you better or worse than last week?

	YES	NO	Better	same	worse
Cough			☐	☐	☐
Fever			☐	☐	☐
Night sweats			☐	☐	☐
Hemoptysis			☐	☐	☐
Headache	☐		☐	☐	☐
Dyspnea			☐	☐	☐
Chest pains			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

Loss of appetite

Chest exam (normal, abnormal)

Other Specify_____

How do you describe your cough today? (see codes at the bott...)1

Weight (kg) Height (cm)

Temperature (oC) Karnofsky scale

Laboratory examinations - Taken? *(yes=1, no=2)*

1st sputum (on the spot)? Morning sputum taken?

3rd sputum (on the spot) Was blood take

Was chest X-rays taken

Date of next appointment

1 (No cough at all=0; Occasional hems=1;
Moderate, paroxymal cough without additional symptoms=3;
Mild, isolated cough, accompanied by chest discomfort=2;
Severe strenuous cough, accompanied by chest discomfort=4

Follow up 3rd month YES NO

Was patient followed? If yes, date attended

If no, date for home follow up _____

Did you take the treatment? If NO, which one did you miss?_____

What symptoms are you suffering from today? If YES, are you better or worse than last week?

	YES	NO	Better	same	worse
Cough					
Fever					
Night sweats					
Hemoptysis					

Headache

Dyspnea

Chest pains

Loss of appetite

Chest exam (normal, abnormal)

Other Specify_____

How do you describe your cough today? (see codes at the bottom)1

Weight (kg) Height (cm)

Temperature (oC) Karnofsky scale

Laboratory examinations - Taken? *(yes=1, no=2)*

1st sputum (on the spot)? Morning sputum taken?

3rd sputum (on the spot) Was blood taken?

Date of next appointment

1(No cough at all=0; Occasional hems=1; Mild, isolated cough, accompanied by chest discomfort=2;

Moderate, paroxymal cough without additional symptoms=3; Severe strenuous cough, accompanied by chest discomfort=4

Follow up 4th month

	YES	NO	
Was patient followed?			If yes, date attended
			If no, date for home follow up _____
Did you take the treatment?			If NO, which one did you miss?_____

What symptoms are you suffering from today? If YES, are you better or worse than last week?

	YES	NO	Better	same	worse
Cough					
Fever					
Night sweats					
Hemoptysis					
Headache					
Dyspnea					
Chest pains					
Loss of appetite					
Chest exam (normal, abnormal)					
Other					

Specify_____

How do you describe your cough today? (see codes at the bot[ ]1

Weight (kg) [ ][ ][ ]

Height (cm) [ ][ ][ ]

Temperature (oC) [ ][ ][ ]

Karnofsky scale [ ][ ][ ]

Date of next appointment

[ ][ ][ ][ ][ ][ ]

Note: take a sputum sample
IF the patient is still coughing or
IF his last sputum was still posi
Was s sputum take[ ]

1(No cough at all=0; Occasional hems=1;
Mild, isolated cough, accompanied by chest discomfort=2;
Moderate, paroxymal cough without additional symptoms=3;
Severe strenuous cough, accompanied by chest discomfort=4

Follow up 5th month

	YES	NO		
Was patient followed?			If yes, date attended	[ ][ ][ ][ ][ ][ ]

If no, date for home follow up _____

Did you take the treatment? ☐ ☐ If NO, which one did you miss? _____

What symptoms are you suffering from today? If YES, are you better or worse than last week?

	YES	NO	Better	same	worse
Cough			☐	☐	☐
Fever	☐	☐	☐	☐	☐
Night sweats	☐	☐	☐	☐	☐
Hemoptysis	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Dyspnea		☐	☐	☐	☐
Chest pains	☐	☐	☐	☐	☐
Loss of appetite	☐	☐	☐	☐	☐
Chest exam (normal, abnormal)	☐	☐	☐	☐	☐
Other	☐	☐	Specify _____		

How do you describe your cough today? (see codes at the bott☐)1

Weight (kg) ☐☐☐ Height (cm) ☐☐☐

Temperature (oC) ☐☐☐ Karnofsky scale ☐☐☐

Date of next appointment

☐☐☐☐☐☐

Note: take a sputum sample
IF the patient is still coughing or
IF his last sputum was still positive
Was s sputum take☐

1(No cough at all=0; Occasional hems=1; Mild, isolated cough, accompanied by chest discomfort=2;
Moderate, paroxymal cough without additional symptoms=3; Severe strenuous cough, accompanied by chest discomfort=4

Follow up 6th month YES NO

Was patient followed? ☐ ☐ If yes, date attended ☐☐☐☐☐☐

If no, date for home follow up _____

Did you take the treatment? ☐ ☐ If NO, which one did you miss? _____

What symptoms are you suffering from today? If YES, are you better or worse than last week?

	YES	NO	Better	same	worse
Cough			☐	☐	☐
Fever	☐	☐	☐	☐	☐
Night sweats	☐	☐	☐	☐	☐
Hemoptysis	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Dyspnea		☐	☐	☐	☐
Chest pains	☐	☐	☐	☐	☐
Loss of appetite	☐	☐	☐	☐	☐
Chest exam (normal, abnormal)	☐	☐	☐	☐	☐
Other	☐	☐			

Specify _____

How do you describe your cough today? (see codes at the bot☐)1

Weight (kg) ☐☐☐ Height (cm) ☐☐☐

Temperature (oC) ☐☐☐ Karnofsky scale ☐☐☐

Laboratory examinations - Taken? *(yes=1, no=2)*

1st sputum (on the spot)? ☐ Morning sputum taken? ☐

3rd sputum (on the spot) ☐ Was blood taken? ☐

Was chest X-rays taken ☐

Date of next appointment ☐☐☐☐☐☐

Laboratory results

Sputum smear results

Follow up	0wk	1st wk	2nd wk	3rd wk	4th wk	5th wk	6th wk	7th wk	8th wk	6th mth
1st spot										
Morning										
2nd spot										
Bleach/ZN										

	Taken Y		Results N
Culture LJ			
Enrolment	☐	☐	_____
BACTEC			
Enrolment	☐	☐	_____
2nd mth	☐	☐	_____
6th mth	☐	☐	_____
Zinc levels			
Enrolment	☐	☐	_____
1st mth	☐	☐	_____
2nd mth	☐	☐	_____
3rd mth	☐	☐	_____
6th mth	☐	☐	_____
Vitamin D levels			
Enrolment	☐	☐	_____
1st mth	☐	☐	_____
2nd mth	☐	☐	_____
3rd mth	☐	☐	_____
6th mth	☐	☐	_____
PCR			
Enrolment	☐	☐	_____

	Taken Y		Results N
Drug resistance	☐	☐	_____
Copper levels			
Enrolment	☐	☐	_____
1st mth	☐	☐	_____
2nd mth	☐	☐	_____
3rd mth	☐	☐	_____
6th mth	☐	☐	_____
Vitamin A			
1st mth	☐	☐	_____
2nd mth	☐	☐	_____
3rd mth	☐	☐	_____
6th mth	☐	☐	_____
Selenium			
1st mth	☐	☐	_____
2nd mth	☐	☐	_____
3rd mth	☐	☐	_____
6th mth	☐	☐	_____

Hb

Enrolment ☐☐ _____

2nd mth ☐☐ _____

6th mth ☐☐ _____

WBC

Enrolment ☐☐ _____

2nd mth ☐☐ _____

6th mth ☐☐ _____

Granulocytes

Enrolment ☐☐ _____

2nd mth ☐☐ _____

6th mth ☐☐ _____

Lymphocytes

Enrolment ☐☐ _____

2nd mth ☐☐ _____

6th mth ☐☐ _____

ESR

Enrolment ☐☐ _____

2nd mth ☐☐ _____

6th mth ☐☐ _____

Bilirubin (T)

Enrolment ☐☐ _____

2nd mth ☐☐ _____

6th mth ☐☐ _____

Bilirubin (C)

☐☐

Serum Albumin

Enrolment ☐☐ _____

2nd mth ☐☐ _____

6th mth ☐☐ _____

SGPT

Enrolment ☐☐ _____

2nd mth ☐☐ _____

6th mth ☐☐ _____

SGPT

Enrolment ☐☐ _____

2nd mth ☐☐ _____

6th mth ☐☐ _____

Alk Phosphatace

Enrolment ☐☐ _____

2nd mth ☐☐ _____

6th mth ☐☐ _____

RVD 1

Enrolment ☐☐ _____

RVD 2

Enrolment ☐☐ _____

CD4 Count

Enrolment ☐☐ _____

Enrolment

2nd mth ☐☐ _____

6th mth ☐☐ _____

X-ray readings

Reader 1	**Taken?**	**Cavities?**	**If cavities, diameter (cm)**
Enrolment _____	☐☐	☐☐	☐☐☐
2nd mth _____	☐☐	☐☐	☐☐☐
6th mth _____	☐☐	☐☐	☐☐☐
Reader2			
Enrolment _____	☐☐	☐☐	☐☐☐
2nd mth _____	☐☐	☐☐	☐☐☐
6th mth _____	☐☐	☐☐	☐☐☐

LIVERPOOL SCHOOL OF TROPICAL MEDICINE (Affiliated to the University of Liverpool)

Pembroke Place
Liverpool L3 5QA
Telephone: 0151-708 9393
Fax: 0151-705 3370
http://www.liv.ac.uk/lstm/lstm.html

25 July 2003

Mr Lovett Lawson
C/o Dr L E Cuevas

Dear Mr Lawson

The research protocol **Diagnosis of Tuberculosis and the role of Micronutrients in the treatment of Pulmonary Tuberculosis in Nigeria** Reference No **03.33** was considered by the Research Ethics Committee on **5 June 2003.**

Thank you for your letter of 16 July 2003 with the clarification requested by the chair. The protocol now has formal Ethical Approval from the LSTM Research Ethics Committee.

This approval should not be seen as a substitute for Local Ethical Approval from the country/institution where the research is to be carried out and that you have undertaken to seek such approval wherever an appropriate mechanism is in place.

The Research Office (RO) maintains a Database of Local Research Committees in the countries where collaborative work is being carried out. Could you, therefore, feed back to me (via Sharda Mistry in the RO) as much information as possible on the local Committees/Review Bodies that will review (or have reviewed) this protocol. The following details would be much appreciated:

- Name
- Address
- Contact numbers or individuals (tel / fax / e-mail)
- A copy of the appropriate form or some details on the submission mechanism
- Any details you are able to obtain on
  a) number on the committee
  b) how many lay representatives sit on the committee?

Yours sincerely

Dr D Lalloo
Chair, Research Ethics Committee

MINISTRY OF FEDERAL CAPITAL TERRITORY

P.M.B. 25
ABUJA, NIGERIA

HEALTH & SOCIAL SERVICES DEPARTMENT

Our Ref:
Your Ref:

Tel:
Telex:
Date: 23/5/03

Dr. L. Lawson,
Zankli Medical Centre,
Abuja.

RE: PERMISSION TO CARRY OUT RESEARCH
IN THE F.C.T.

This is to convey the approval of the Director, Health and Social Services to you to carry out research activities in Asokoro, Wuse and Maitama Hospitals.

2. Congratulations.

DR. OGBOLE EBE
DEPUTY DIRECTOR, HOSPITAL
FOR: DIRECTION, HEALTH & SOCIAL SERVICES

23rd May, 2003.

MINISTRY OF FEDERAL CAPITAL TERRITORY

P.M.B. 25
ABUJA NIGERIA

HEALTH & SOCIAL SERVICES **DEPARTMENT**

Our Ref: **M.F.C.T /GEN/24/VOL 1**
Your Ref:

Tel:
Telex:
Date: **19th June. 2003**

Dr. L. Lawson
Chief Medical Director
Zankli Medical Centre
Plot No 1021
B.5 Shehu Yar'adua Way
Opposite Fed. Min. of Works
Utako, Abuja.

RE-REQUEST FOR PERMISSION TO EXTEND THE NUMBER OF HOSPITALS

This is to acknowledge receipt of your letter dated 18th June 2003 on the above subject matter.

2. Approval is hereby given for the extension of your research project to the under-listed hospitals as requested in your letter under reference:-

a) Kubwa Hospital
b) Nyanya Hospital
c) Gwarinpa Hospital

3. Note that the Kuje and Karu Hospitals are yet to be operational please.

4. Thank you.

Dr. M.O. AYO
Director, Health & Social Services