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# Analysis of secondary data from Mycobacterium vaccae tuberculosis clinical trial

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# UNIVERSITY OF THE WITWATERSRAND

FACULTY OF HEALTH SCIENCES

SCHOOL OF PUBLIC HEALTH

**RESEARCH REPORT**

## PROJECT TITLE

ANALYSIS OF SECONDARY DATA FROM *Mycobacterium vaccae*

TUBERCULOSIS CLINICAL TRIAL

**MUNYARADZI PAUL MAPINGURE**

A research report submitted to the Faculty of Health Sciences,

University of the Witwatersrand,

in partial fulfillment of the requirements for

The degree of Masters of Science in Medicine in the field of Epidemiology

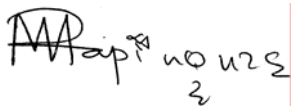
and Biostatistics

Johannesburg, South Africa 2006

Financial support for my MSc was provided by the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), to whom I am indebted

**DECLARATION**

I, Munyaradzi Paul Mapingure declare that this research report is my own work. It is being submitted for the degree of Masters of Science in Medicine in the field of Epidemiology and Biostatistics in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.



[Signature of candidate]

Full Name: MUNYARADZI PAUL MAPINGURE

27 day of April 2007

## DEDICATION

to my wife Nerisa Nyasha Dehwe-Mapingure

with everlasting love

**ABSTARCT**

**Background:** Sputum culture conversion at two months is an important indicator for the effectiveness of treatment and the infectivity of a patient with pulmonary TB. This study aimed at investigating factors that are associated with tuberculosis culture conversion at two months as well as investigating whether sputum culture conversion at two months is a reliable predictor of relapse.

**Methods**: The study makes use of data obtained from 347 newly diagnosed tuberculosis patients who participated in a randomized placebo controlled immunotherapy trial at King George V hospital in Durban. Above objectives were met by carrying out statistical analysis of the secondary data. Chi-square tests for categorical explanatory variables such as HIV status and smoking status and (b) t-tests for continuous variables such as age were used for investigating factors associated with 2-month culture conversion. Multivariate models were used to find the most important variables for predicting 2-month culture conversion. Kaplan Meier curves were used for investigating whether culture conversion at two months is a reliable predictor of relapse.

**Findings:** Of the 347 tuberculosis patients, 34 % were HIV sero-positive. Age, body mass index (BMI), smoking status and gender were found to be important variables that affect sputum culture conversion at two months. At

5 % significance level there was no evidence that those who culture convert at two months were less likely to relapse than those who had not culture converted at two months (p=0.1165). However the trend shown is striking to report as it may be of clinical significance. Among those who had not culture converted at two months, more people (40) than expected (34) relapsed an among those who had culture converted at to months, less people (19) than expected (24) relapsed.

**Interpretation and recommendations:** Some behavioral and biological factors affect two month tuberculosis culture conversion therefore successful tuberculosis management need to take into account the effect of these factors. This study did not show that the sterilizing potential of an anti-tuberculosis regimen can be obtained by evaluation of the culture conversion rates at two months and this may be due to small sample size.

**ACKNOWLEDGEMENTS**

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## ABBREVIATIONS AND ACRONYMS

| | | |
|---|---|---|
| T.D.R | : | Tropical Diseases Research |
| UNDP | : | United Nations Development Programme |
| UNICEF | : | United Nations Children's Fund |
| W.H.O | : | World Health Organization |
| TB | : | Tuberculosis |
| BMI | : | Body Mass Index |
| ESR | : | Erythrocyte Sedimentation Rate |
| HIV | : | Human Immunodeficiency Virus |

**NOMENCLATURE**

| | |
|---|---|
| *Definite tuberculosis case*: | A patient with culture positive for the *Mycobacterium tuberculosis* complex. |
| *Relapse*: | A patient previously treated for tuberculosis that has been declared cured and is diagnosed with bacteriologically positive (smear / culture) tuberculosis (1). |
| *Culture conversion*: | Two consecutive negative cultures for tuberculosis from a patient, whose sputum was initially culture positive for *Mycobacterium tuberculosis*, provided there is no subsequent positive result. |
| *Body Mass Index* | Body Mass Index (BMI) is a number calculated from a person's weight and height. BMI provides a reliable indicator of body fatness for most people and is used to screen for weight categories that may lead to health problems. |
| *Erythrocyte Sedimentation Rate* | The rate at which red blood cells settle in blood with anticoagulant; increased rates are often associated with anemia or inflammatory states. |

# CHAPTER ONE

*This chapter begins with a brief introduction of the study. Literature review includes an overview of tuberculosis disease burden in South Africa, Africa and the whole world, tuberculosis symptoms, therapy and surrogate markers for tuberculosis therapy. The chapter concludes by emphasizing the problem statement, research questions and the objectives of this study.*

## 1.1.0 INTRODUCTION

With the current high levels of infection, tuberculosis's status as an urgent public health priority is questioned no longer. Fifty years after the introduction of effective chemotherapy, tuberculosis remains, along with AIDS, the leading infectious cause of adult mortality in the world, causing up to two million deaths in a single year (2).

It is important to conduct research on factors affecting response to tuberculosis chemotherapy so as to reverse today's worrisome tuberculosis trends. This study will investigate factors associated with a tuberculosis patient's two month culture conversion as well as investigate whether two month culture conversion status is a good predictor of tuberculosis relapse and to see whether this relationship depends on HIV status, smoking and other demographic characteristics of patients. Data were obtained from newly diagnosed tuberculosis patients entered into a randomized control immunotherapy trial at King George V Hospital in Durban, South Africa.

## 1.2.0 LITERATURE REVIEW

### *1.2.1 Tuberculosis*

One out of three people worldwide is infected with tuberculosis – that is two billion people in total, mostly in developing countries where 95 % of cases occur (3). Although in the past decade, there has been substantial progress in the development and implementation of the strategies necessary for effective global tuberculosis control, the disease remains an enormous growing global health problem (4). In 2003, there were an estimated 8.8 million new cases of tuberculosis, of which 3.9 million were sputum smear-positive thus highly infectious. In the African region of the World Health Organization (WHO) the tuberculosis case rate continues to increase, both because of the HIV epidemic in Sub-Saharan countries and the poor or absent primary care services throughout the region (5). Tuberculosis currently holds seventh place in the global ranking of causes of death, and unless intensive efforts are made, is likely to maintain that position through to 2020 (6).

### *1.2.2 Tuberculosis burden in South Africa*

South Africa is burdened by one of the worst tuberculosis epidemics in the world, with disease rates more than double those observed in other developing countries and up to 60 times higher than those currently seen in the USA or Western Europe. This is mainly due to the HIV epidemic,

historical neglect and poor management systems, compounded by the legacy of fragmented health services (7). Multi-drug resistance by *Mycobacterium tuberculosis* (MDR-TB) to anti-tuberculosis drugs and the emergence of the extensively drug resistant tuberculosis (XDR-TB) is also a new threat to the control of the disease (8, 9).

Tuberculosis was declared a top health priority by the Department of Health in November 1996 and the then National Health Minister, Zuma, committed her Department to implementing a new control programme based on the DOTS strategy of the World Health Organization. Given that more than US$100 million are spent annually on tuberculosis in South Africa, in excess of US$3 billion would be required over the next 10 years if current increases in tuberculosis rates are allowed to continue unabated (10).

In 2006, the World Health Organization ranked South Africa fifth among the world's 22 high-burden TB countries. According to the *World Health Organization (WHO) Global TB Report 2006*, South Africa had nearly 340,000 new TB cases in 2004, with an incidence rate of 718 cases per 100,000 people – a major increase from 338 per 100,000 in 1998 (7 cited previously).

*1.2.3 Tuberculosis Symptoms*

Most people infected with tuberculosis have inactive disease that does not cause any symptoms. Among people with active tuberculosis, symptoms

include cough that is worse in the morning (sometimes with haemoptysis – blood in the sputum), chest pain, breathlessness, night sweats, and signs of pneumonia. In advanced disease, there may be extreme weight loss (11).

### *1.2.4 Tuberculosis Therapy*

The internationally accepted first line treatment regimen for tuberculosis is isoniazid, rifampicin, pyrazinamide and ethambutol for two months followed by a dose of isoniazid and rifampicin for four months (12). In the first few weeks of therapy, there is rapid killing of metabolically active bacilli. Thereafter, therapy is continued for months to prevent a relapse of the active infection after treatment discontinuation. The ability to prevent relapse is termed sterilizing activity because it is presumed to require killing nearly all bacilli remaining after the initial phase of therapy (13).

Treatment of tuberculosis is not only a matter of individual health, but it is a matter of public health as untreated infective tuberculosis patients may infect members of the society where they live. The goal of tuberculosis treatment is to ensure relapse-free cure while preventing the emergence of drug resistance. Treatment success is among the five principal indicators of tuberculosis burden, the other four being tuberculosis incidence, prevalence, death and case detection (14). Scientists are also looking for interventions that would shorten the treatment period and hence decrease expenditure on drugs and in-hospital care, which have the attendant danger of nosocomial transmission of different strains. Potentially therefore the *Mycobacterium*

*vaccae* immunotherapy trial, from which data for this study was obtained, represents an attempt at alleviating the logistical problems of long term drug treatment and compliance (15). The purpose of an immunotherapeutic intervention would be to markedly shorten tissue sterilization phase, through the enhancement of host immune response, which would facilitate the elimination of persister organisms (16). An understanding of how an individual's biological and behavioral factors affects response to tuberculosis chemotherapy is also needed, hence this present study.

### *1.2.5 Surrogate Markers in the Clinical Evaluation of Anti-tuberculosis chemotherapy*

The validation of surrogate markers that reliably predict relapse of tuberculosis is essential if the pace of clinical research in tuberculosis is to be accelerated. Focus is paid on outcomes that are of direct patient benefit e.g. survival, relief of pain or resolution of other signs and symptoms of disease as well as eradication of pathogen. Some current and new surrogate markers for clinical trials of new anti-tuberculosis chemotherapy include relapse rates one year after treatment, two month culture conversion, and time until sputum culture conversion, Early Bactericidal Activity (EBA) and Serial Sputum Colony Forming Unit Counts, Sputum mycobacterium antigens and Nucleic Acids, Whole Blood Bactericidal Activity and Sputum Cytokines. Sputum culture conversion at two months among patients with tuberculosis is the most important indicator for the effectiveness of treatment and the infectivity of the disease (17, 18, 19). There is need to

continuously investigate its reliability in predicting relapse in the wake of drug resistance to current and new anti-tuberculosis regimens by *M tuberculosis.*

### *1.2.6 Factors Associated with tuberculosis Chemotherapy*

In discussion of TB control, it is vital to remember that incidence, cure rates and death rates are not governed simply by drug treatment. Among other determinants of the slow dynamics of TB epidemics are cofactors such as nutritional status, tobacco and alcohol use, other infectious agents and susceptibility genes that warrant additional study (20, 21).

Factors such as HIV co-infection and smoking may affect cure rate and lead to tuberculosis relapse and jeopardize the global targets for tuberculosis control (14 cited previously). An investigation of the impact of such factors on an individual's response to tuberculosis chemotherapy will help in the management of the disease (12 cited previously).

## 1.3.0 PROBLEM STATEMENT AND JUSTIFICATION

The consequences of tuberculosis on society are immense. Presently the research activities for tuberculosis span diverse areas. Some of the important areas are treatment shortening/simplification, optimizing case management with due attention to social, behavioral, physical and biological issues necessary for optimized tuberculosis control in resource-limited settings (22) hence the need to investigate how some of these factors affect an individual's response to tuberculosis chemotherapy by looking at two month culture status. Culture conversion at two months has been used as a surrogate marker for sterilizing effect of anti-tuberculosis drugs (18 cited previously, 23).

### *1.3.1 Research Questions*

1. What are the factors (age, gender, smoking status, HIV status, BMI, ESR) that are associated with two month tuberculosis culture conversion?
2. Is two month tuberculosis culture conversion status a good predictor of relapse?
3. Are changes in tuberculosis symptoms (namely chest pain, coughing, fever, haemoptysis, night sweats, and weight loss) at two months consistent with a patient's tuberculosis status (culture positive/ culture negative) at two months?

*1.3.2 Null Hypotheses*

1. Behavioral and biological factors, notably smoking status, HIV status, gender, age, BMI, ESR do not affect two month tuberculosis culture conversion.
2. The sterilizing potential of an anti-tuberculosis regimen can not be obtained by evaluation of the culture conversion rates at two months.
3. Changes in tuberculosis symptoms at two months are not consistent with patient's tuberculosis status at two months.

**1.4.0 STUDY OBJECTIVES**

1. To determine factors that are associated with tuberculosis culture conversion at two months - and in particular to examine the role of smoking and HIV co-infection.
2. To determine whether tuberculosis culture conversion at two months is a reliable predictor of relapse – and to examine the impact of HIV co-infection and smoking on this relationship.
3. To determine whether changes in tuberculosis symptoms (namely chest pain, coughing, fever, haemoptysis, night sweats, and weight loss) are consistent with patient's culture status at two months.
4. To determine factors which are associated with time to tuberculosis symptom resolution; in particular the effect of HIV co-infection and smoking.

## CHAPTER TWO

*This chapter begins by describing the materials and methods used in the original study i.e. the Durban Immunotherapy Randomized Placebo Controlled trial. The analysis carried out on the data sets for the purpose of this research report is then detailed.*

## MATERIALS AND METHODS

### 2.1.0 ORIGINAL STUDY FROM WHICH DATA USED IN THIS STUDY WAS GENERATED

"Patients of either sex (non-pregnant females) with newly diagnosed, sputum smear-positive pulmonary tuberculosis were enrolled at King George V Hospital in Durban, South Africa, between September 1994 and March 1996. They were eligible for inclusion in the study if they were aged between 18 to 65 years, had not received treatment for tuberculosis during the two-year period prior to recruitment, weighed at least 34 kg (females) or 38 kg (males), did not suffer from tuberculosis meningitis (other forms of tuberculosis in the presence of pulmonary tuberculosis were acceptable), agreed to be hospitalized for at least two months, and consented to be tested for HIV after counseling. There were 374 patients enrolled on the *M. vaccae* study, of whom 347 were eligible for efficacy analysis (i.e. who had tuberculosis confirmed by culture and who were not resistant to isoniazid and/or rifampicin). All patients received rifampicin, isoniazid, pyrazinamide

and ethambutol daily in the first 8 weeks and 3 times a week rifampicin and isoniazid for a further 4 months. Pyridoxine 25 mg daily was taken for the full 6 months. Patients were randomly assigned to receive either *Mycobacterium vaccae* or a saline placebo on day 8 of the anti-tuberculosis regimen. Randomization of patients in balanced blocks of 20 (block randomization) was performed. According to protocol all patients were hospitalized for the first eight weeks and for a further four weeks if they were smear positive after the initial eight weeks of treatment. Early morning sputum specimens were collected on two consecutive days from every patient on admission. For the first 8 weeks sputum samples were checked by microscopy and culture at weekly intervals. Thereafter two sputum specimens were collected on consecutive days at monthly intervals up to six month from all patients able to produce sputum. Clinical Symptoms, (namely cough, chest pain, fever, haemoptysis, night sweat, weight loss) were recorded at weekly intervals from day of admission up to day 56. All patients who completed treatment at six month were followed-up at three-monthly intervals until month 24 and six-monthly intervals subsequently (16 cited previously)".

**2.2.0 STUDY DESIGN**

Randomized placebo controlled trial. Patients were randomly assigned to receive either *Mycobacterium vaccae* or a saline placebo on day 8 of the anti-tuberculosis regimen.

### 2.3.0 STUDY SAMPLE

Analysis of secondary data was done on 347 patients who participated in the *M. vaccae* randomized control trial.

### 2.4.0 DATA PROCESSING METHODS

a. A general description of study population's baseline demographic and clinical characteristics was given as well as a description of the study population by treatment arm i.e. *M. vaccae* or saline placebo.
b. For investigating factors associated with 2-month culture conversion firstly a descriptive analysis was carried out comparing the distribution of the potential explanatory variables between those who converted and those who did not convert using (a) a chi-square test for categorical explanatory variables such as HIV status and smoking status and (b) t-tests for continuous variables such as age and ESR. Thereafter multiple logistic regression models were fitted to find the most important variables for predicting 2-month culture conversion.

   *Chi square is a non parametric test for significance of the relationship between categorical variables. The t-test is the most commonly used method to evaluate the differences in means between two groups. Given two data sets, each characterized by its mean, standard deviation and number of data points, we can use the t test to determine whether the means are distinct, provided that the underlying distributions can be assumed to be normal (24). Multiple logistic regression is a statistical technique used to*

*study the relationship between a response variable (outcome, dependent variable) and a set of explanatory variables (covariates, independent variables) (25).*

c. For investigating whether culture conversion at two months is a reliable predictor of relapse survival analysis methods were used - the two groups (two month culture conversion or not) were compared on time to relapse using Kaplan Meier curves and then the relationship was further examined by adjusting for HIV status, smoking, age, BMI, ESR, treatment arm and gender by fitting Cox regression models.

   *Survival analysis is concerned with studying the time between entry to a study and a subsequent event. The Kaplan-Meier method is a nonparametric technique for estimating time-related events (the survivorship function). Cox regression is a particular case of multiple regression where the response variable is a censored survival time (represented by variables time and event) (26).*

d. The association between the six symptoms collected and two-month culture conversion was examined in a similar fashion. For each symptom present at baseline (day 0) the series of values were examined to see whether the symptom has resolved by day 56 (and also to see whether the patient is culture negative) using Kaplan Meier survival estimates of symptom resolution. Symptom resolution was compared to tuberculosis culture conversion. Effect of HIV, smoking and other factors e.g. age, BMI, ESR, treatment arm and gender on time to symptom resolution was investigated using survival analysis methods i.e. by using log rank tests and Cox regression models.

*Log rank test allows for testing for the equality of two or more survival distributions (26 cited previously).*

STATA version 8.2 statistical package was used for all data analysis.

## CHAPTER THREE

*This chapter contains the results of the data analysis carried out and includes both descriptive and inferential analyses.*

### 3.1.0 RESULTS

*3.1.1 Descriptive Analysis results*

*This section gives a summary of the demographic and clinical parameters on entry into the study (table 1a and table 1b). In this section baseline characteristics are also compared by the treatment arm i.e. Mycobacterium vaccae arm and saline placebo arm.*

**Table 1(a): Baseline Characteristics of patients (Continuous variables)**

| Parameter | Mean | Med | Std Dev | iqr | Min | Max |
|---|---|---|---|---|---|---|
| Age in years | 32.3 | 30 | 9.9 | 40 | 17 | 63 |
| BMI | 19.0 | 18.7 | 3.0 | 17.3 | 11.8 | 34.9 |
| ESR in mm/h | 98.3 | 103 | 25.7 | 117 | 5 | 178 |

*Med = median, Std Dev = standard deviation, iqr = inter quartile range, Min = minimum value and Max = maximum value*

Table 1(a) summarizes the status of the patients' age, BMI and ESR on entry to the trial. The average age, BMI, ESR are 32.3 years, 19.0 and 98.3 mm/h respectively

**Table 1(b): Baseline Characteristics of patients (Categorical variables)**

| Parameter | Value | Frequency | Percentage |
|---|---|---|---|
| Gender | Male | 264 | 76.1 |
| | Female | 83 | 23.9 |
| Treatment | *M. vaccae* | 171 | 49.3 |
| | Placebo | 176 | 50.7 |
| HIV Status | Negative | 229 | 66 |
| | Positive | 118 | 34 |
| Smoking status | Ever-smoked | 228 | 65.3 |
| | Never-smoked | 119 | 34.3 |
| Chest pain | Present | 260 | 75 |
| | Absent | 87 | 25 |
| Cough | Present | 347 | 100 |
| | Absent | 0 | 0 |
| Fever | Present | 80 | 23 |
| | Absent | 267 | 73 |
| Haemoptysis | Present | 32 | 9.2 |
| | Absent | 315 | 90.8 |
| Night sweat | Present | 120 | 34.6 |
| | Absent | 227 | 65.4 |
| Weight loss | Present | 237 | 68.3 |
| | Absent | 110 | 31.7 |

Table 1(b) summarizes the status of the patients on a number of parameters on entry to the trial. There were 264 male patients and 83 were female patients. There are roughly equal numbers in the two treatment groups 171 received *M. vaccae* and 176 received placebo. One hundred and eighteen people were HIV positive on entry to the trial while 229 were HIV negative. One hundred and nineteen people smoked at one point or the other in their life while 228 people never smoked. On entry in the trial everyone was coughing while 260, 80, 32 120 and 237 presented with symptoms of chest pain, fever, haemoptysis, night sweat and weight loss respectively.

**Table 2: Baseline characteristics of patients by treatment arm**

| **Parameter** | **Value** | ***M. vaccae* arm** | **Placebo** |
|---|---|---|---|
| Age in years | Mean (sd)* | 32.4 (9.3) | 32.2 (10.4) |
| BMI | Mean (sd) | 19 (3.1) | 19.1 (3.0) |
| ESR in mm/h | Mean (sd) | 98.7 (25.0) | 97.8 (26.3) |
| Gender | Male (%) | 132 (77.2) | 132 (75.0) |
| | Female (%) | 39 (22.8) | 44 (25.0) |
| Smoking status | Ever-smoked | 114 | 114 |
| | Never-smoked | 57 | 62 |
| HIV status | Positive | 60 | 58 |
| | Negative | 111 | 118 |
| Chest pain | Present | 136 | 124 |
| | Absent | 35 | 52 |
| Cough | Present | 171 | 176 |
| | Absent | 0 | 0 |
| Fever | Present | 40 | 40 |
| | Absent | 131 | 136 |
| Haemoptysis | Present | 18 | 14 |
| | Absent | 153 | 162 |
| Night sweat | Present | 66 | 54 |
| | Absent | 105 | 122 |
| Weight loss | Present | 113 | 124 |
| | Absent | 58 | 52 |

*(sd)* standard deviations are given in brackets*

The demographic and clinical characteristics of the patients in the two treatment groups are shown in table 2. The groups are comparable on all parameters

*3.1.2 Inferential Analysis*

*For investigating factors associated with 2-month culture conversion a comparison of the distribution of the potential explanatory variables between those who culture converted and those who did not culture convert was carried out using (a) a chi-square test for categorical explanatory variables notably, treatment group, HIV status, gender and smoking status and (b) t-tests for continuous variables notably age, BMI and ESR and the results are shown in table 3 below. Multiple logistic regression models were fitted to find the most important variables for predicting 2-month culture conversion and the final model is shown in Table 4.*

**Table 3: Distribution of potential explanatory variables by patients' month 2 tuberculosis culture status**

| Parameter | Value | Converted | Unconverted | P-value |
|---|---|---|---|---|
| Age in years | Mean (sd)* | 30.4 (9.7) | 33.5 (9.8) | 0.0035 |
| BMI | Mean (sd) | 19.8 (3.5) | 18.6 (2.6) | 0.0002 |
| ESR in mm/h | Mean (sd) | 98 (25.6) | 98.4 (25.8) | 0.8876 |
| Treatment | *M. vaccae* | 70 (52%) | 101 (48%) | |
| | Placebo | 65 (48%) | 111 (52%) | 0.444 |
| Smoking status | E-smoked | 71 (53%) | 157 (74%) | |
| | N-smoked | 64 (47%) | 55 (26%) | <0.0001 |
| HIV status | Positive | 52 (39%) | 66 (31%) | |
| | Negative | 83 (61%) | 146 (69%) | 0.157 |
| Gender | Male | 87 (64%) | 177 (83%) | |
| | Female | 48 (36%) | 35 (17%) | <0.0001 |

*(sd)* standard deviations are given in brackets*

The percentages given are column percentages e.g. among those patients who converted 39% were HIV positive compared to 31% HIV positive among those who did not convert.

Those who culture converted were on average younger than those who did not culture convert (Mean of 30.4 years for those who culture converted and 33.5 years for those who did not culture convert, P value = 0.0035). On average those who culture converted had a higher BMI than those who did not culture convert at two months (Mean 19.8 for those who culture converted and 18.6 for those who did not culture convert, P value 0.0002). There were statistically significant differences in conversion status between ever smokers (E-smoked) and never smokers (N-smoked) and between males and females. There was no evidence of effect of HIV status and treatment arm on culture conversion (P values of 0.157 and 0.444 respectively).

**Table 4: Multiple Logistic regression model (backward elimination) for 2-month culture conversion**

| Parameter | Odds ratio | z | P-value |
|---|---|---|---|
| Age | 0.976 | -1.75 | 0.079 |
| BMI | 1.114 | 2.66 | 0.008 |
| Ever-smoked | 0.503 | -2.73 | 0.006 |
| HIV positive | 1.147 | 0.55 | 0.704 |

Final model chi-square is 26.60 on 4 degrees of freedom (P<0.0001)

From table 4 above, those who ever smoked were only 50 % as likely to culture convert and for each unit increase in BMI, the odds of culture converting increased by 11 %. For one year increase in age, odds of culture converting decreased by 2 %. After adjusting for age, BMI and smoking status there is no evidence of an association between HIV status and 2-month culture conversion.

*3.1.3 Survival Analysis Results*

*In this section results from Kaplan-Meier curves for investigating whether culture conversion at two months is a reliable predictor of relapse are shown (Fig 1). Log rank tests for the equality of these survival (relapse) functions are also shown (table 6) as well as the Cox regression models for examining these relationships after adjusting for HIV status, smoking, age, BMI, ESR, treatment arm and gender (table 7).*

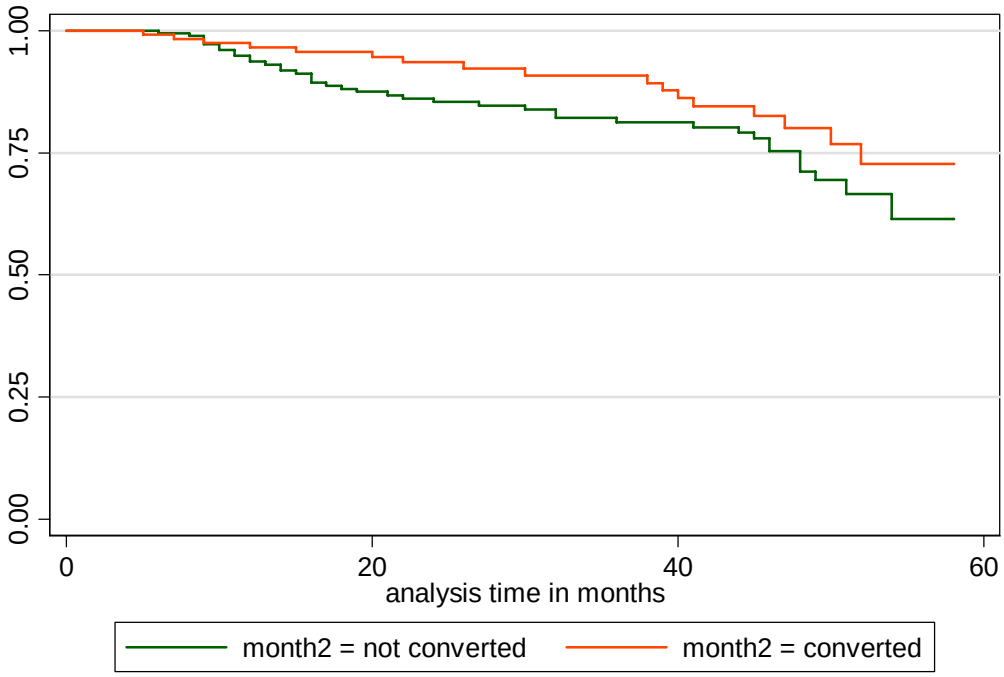



Fig 1. Kaplan-Meier Survival estimates for comparison of relapse between those who had converted at 2 months (top curve) and those who had not converted at two months (bottom curve)

It is striking to report that those who had converted at two months were less likely to relapse than those who had not culture converted.

**Table 5: Log-rank test for equality of survivor (relapse) functions**

| Month 2 Status | Events Observed | Events Expected |
|---|---|---|
| Not Converted | 40 | 34.22 |
| Converted | 19 | 24.78 |

Chi square = 2.46, p value = 0.1165

From the log rank test of equality of relapse rate curves for those who had culture converted at two months and those who had not culture converted we see the same message as shown by the graph above. More people than expected relapsed for those who had not culture converted at two months (40 compared to 34.22) and less people than expected relapsed for those who had culture converted at two months (19 compared to 24.78). The P value of the Log rank test shows that there is an 11 % probability that these differences are due to chance i.e. the difference is not statistically significant even at the 10 % level.

**Table 6: Cox regression model for variables affecting relapse**

| Parameter | Hazard ratio | z | P-value |
|---|---|---|---|
| Converted | 0.598 | -1.63 | 0.104 |
| HIV Positive | 1.650 | 1.70 | 0.090 |
| Age | 0.981 | -1.26 | 0.206 |
| BMI | 0.870 | -2.44 | 0.011 |
| ESR | 1.000 | 1.48 | 0.140 |

Final model chi-square is 15.09 on 5 degrees of freedom ($p<0.01$)

Cox regression models were fitted to determine variables that are most important in affecting relapse and the results are shown in table 6. It is interesting to note that, though it is of borderline statistical significance, those who were HIV positive were 65 % (taken from the hazard ratio of 1.65) more likely to relapse than those who were HIV negative after adjusting for the effect of 2 month culture status, age, BMI and ESR. For each unit increase in BMI the hazard of relapse decreased by 13 % and also those who had culture converted at two months were only 60 % as likely to relapse as those who had not converted at two months after adjusting for HIV status, age, BMI and ESR. Smoking status was dropped from the model as there was no statistical evidence of the effect of smoking on relapse.

*3.1.4 Symptom resolution compared with culture conversion at two months*

*In this section symptom resolution was compared to culture conversion status (table 7). For each symptom present at baseline the status of the symptom (resolved or unresolved) was compared to culture conversion status (converted or unconverted) at two moths.*

**Table 7: Symptom resolution compared with culture conversion**

| Symptom (Baseline total) | Resolution (at month 2) | Converted (at month 2) | Unconverted (at month 2) | P value |
|---|---|---|---|---|
| Coughing (347) | Resolved | 171 (81%) | 116 (86%) | |
| | Unresolved | 41 (19%) | 19 (14%) | 0.206 |
| Chest pain (260) | Resolved | 147 (92%) | 96 (95%) | |
| | Unresolved | 12 (8%) | 5 (5%) | 0.409 |
| Fever (80) | Resolved | 56 (97%) | 21 (95%) | |
| | Unresolved | 2 (3%) | 1 (5%) | 0.818 |
| Haemoptysis (32) | Resolved | 11 (92%) | 20 (100%) | |
| | Unresolved | 1 (8%) | 0 (0%) | 0.190 |
| Night sweat (120) | Resolved | 72 (100%) | 48 (100%) | |
| | Unresolved | 0 | 0 | …..* |
| Weight loss (237) | Resolved | 133 (99%) | 102 (99%) | |
| | Unresolved | 1 (1%) | 1 (1%) | 0.851 |

….* indeterminable

The percentages given are column percentages e.g. among those patients who converted 81% had their coughing symptom resolved compared to 86% with resolved coughing symptom among those who did not culture convert at two months. Symptom resolution is not a reliable predictor of

culture conversion. If we take coughing for example and this is the same for all symptoms we find that some people were no longer coughing yet they were still tuberculosis culture positive and some people were tuberculosis culture negative yet they were still coughing.

*3.1.5 Survival Analysis method for time to symptom resolution*

*For each symptom present at baseline (day 0) the series of values were examined using survival methods to see whether the symptom has resolved by day 56 and the results are shown in Fig 2 through to Fig 7.*

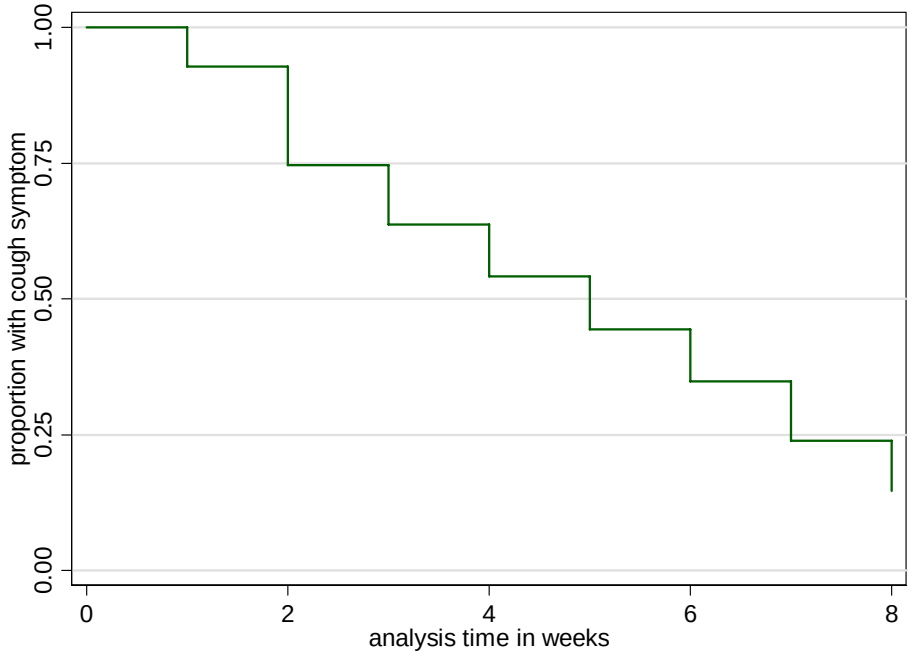



Fig 2. Kaplan-Meier Survival (cough symptom resolution) estimates

Median time to cough symptom resolution is approximately 5 weeks.

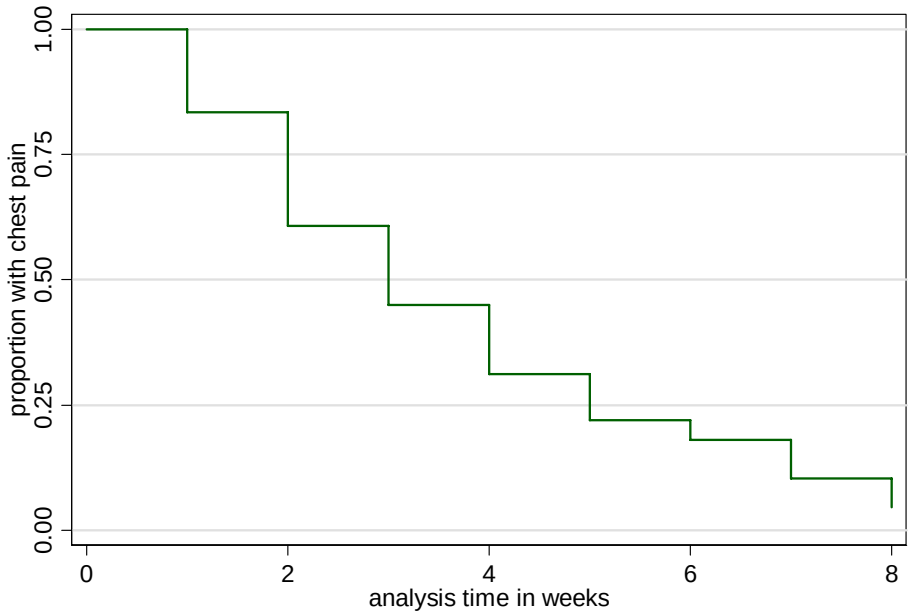



Fig 3. Kaplan-Meier Survival (chest pain symptom resolution) estimates

Median time to chest pain symptom resolution is approximately 3 weeks.

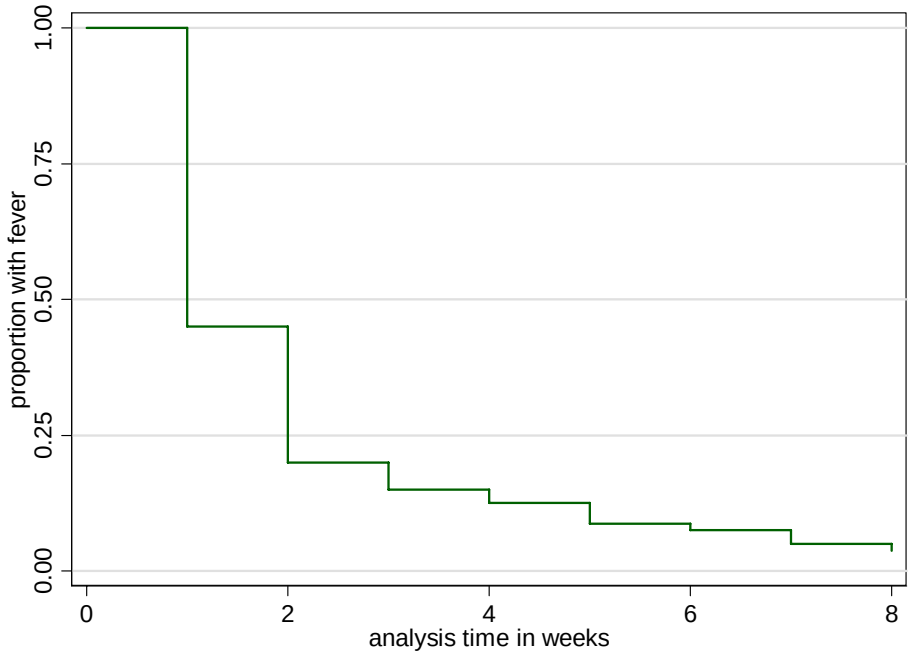



Fig 4. Kaplan-Meier Survival (fever symptom resolution) estimates.

Median time to fever symptom resolution is approximately 1 week.

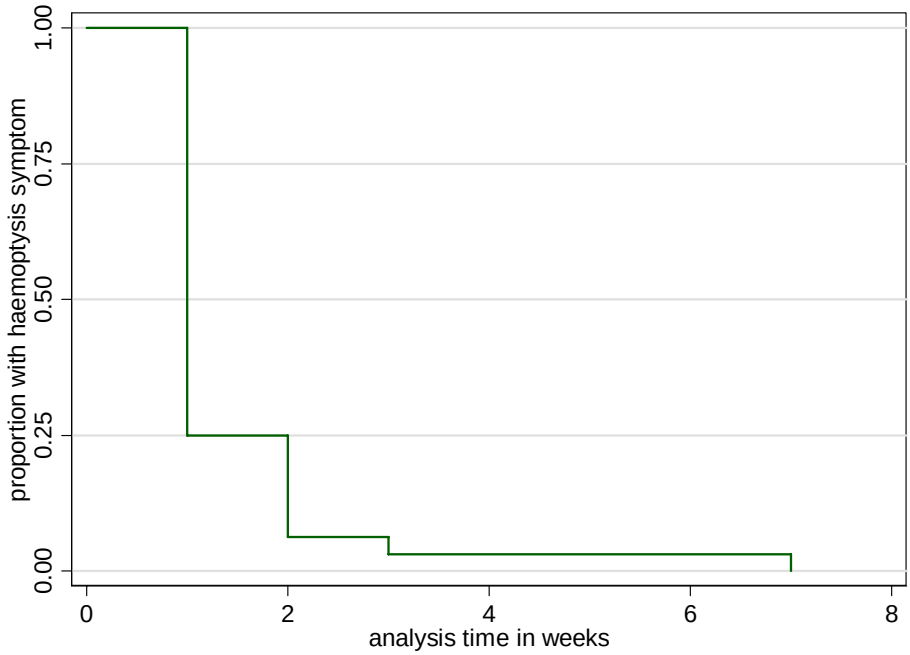



Fig 5. Kaplan-Meier Survival (haemoptysis symptom resolution) estimates

Media time to haemoptysis symptom resolution is approximately 1 week.

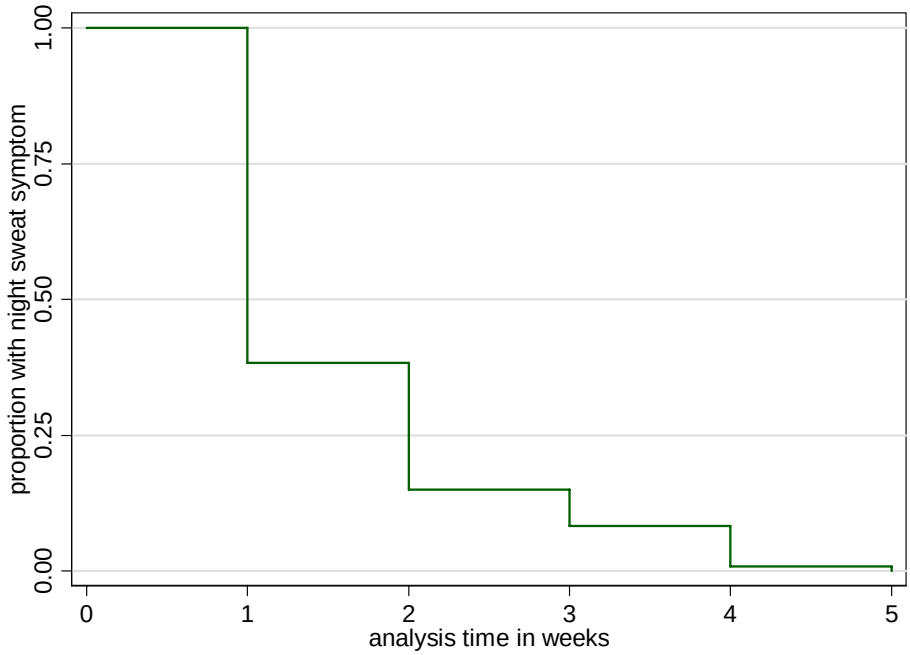



Fig 6. Kaplan-Meier Survival (night sweat symptom resolution) estimates.

Median time to night sweat symptom resolution is approximately 1 week.

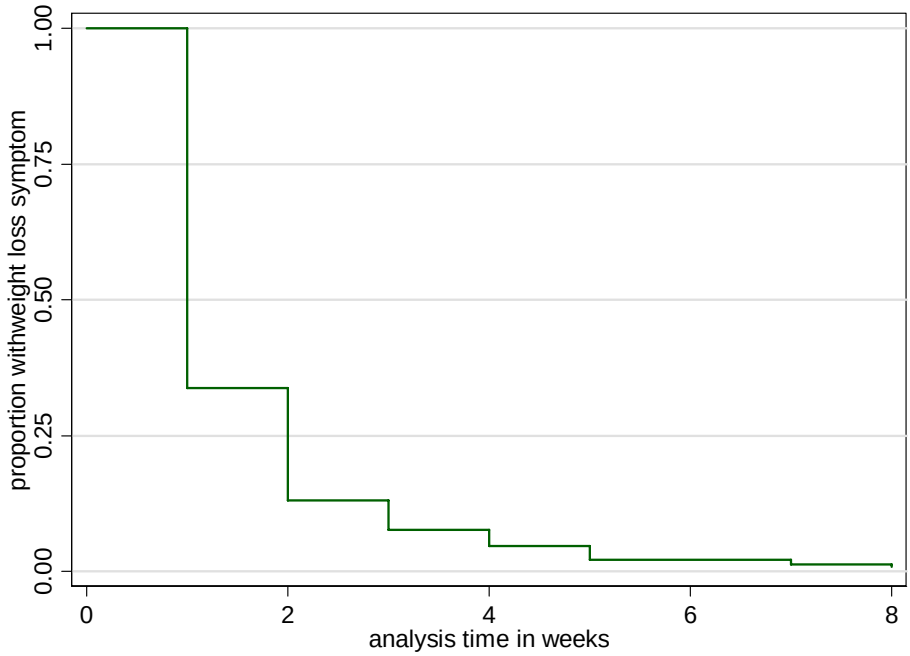



Fig 7. Kaplan-Meier Survival (weight loss symptom resolution) estimates.

Weight loss resolution is anything from at least weight stabilization.

Median time to weight loss symptom resolution is approximately 1 week.

## CHAPTER FOUR

*In this chapter the findings of the study are discussed in the context of available literature. Limitations to the current study are also pointed out.*

### 4.1.0 DISCUSSION

The main objectives of this study were to at investigate factors that are associated with tuberculosis culture conversion at two months and to investigate whether sputum culture conversion at two months is a good predictor of relapse.

Findings suggest that smoking is an important variable in determining culture conversion. Tuberculosis patients who ever smoked are less likely to culture convert than those who never smoked. Long term exposure to cigarette smoking is thought to have an adverse effect on the lung's defense mechanism especially mucocilliary clearance of *Mycobacterium tuberculosis* (27, 28). Age and body mass index also affect response to tuberculosis chemotherapy with elderly and those with lower BMI being less likely to culture convert than the young ones and those with higher BMI. A study carried out in Norway also found out that ageing affects treatment outcome, with the elderly at risk of non-successful treatment (29).

From this study, females were more likely to culture convert than males. A study done in North Carolina found out that old age, male sex, and smoking

prolonged culture conversion time (30). Closely monitoring sputum culture conversion is not only essential in the treatment of TB patients but also important in the control of TB spread (17 cited previously). DOTS, which is the term now used to describe a broader public health strategy to TB control, will be strengthened by further research on the impact of such factors as smoking, HIV on TB treatment outcome.

The goal of tuberculosis treatment is to ensure relapse-free cure while preventing the emergence of drug resistance. Drugs that can result in culture conversion at two months are necessary in the control of tuberculosis. Studies have shown that the time to sputum sterilization is an important determinant of relapse (31, 32, 33). This study suggests that culture conversion at two months results in lower relapse rates. The p value of 0.11 for log rank test for relapse rates between those who had culture converted at two months and those who had not culture converted suggests that a bigger sample size is necessary to detect statistically significant differences in relapse rates. The trend shown by these results is striking to report as it was shown that of the 59 people who relapsed 40 (67.8 %) came from those who had not culture converted at two months and only 19 (32.2 %) from those who had culture converted. Though it is of borderline statistical significance it is also striking to report that HIV infection plays an important role in relapse with those who are HIV positive being 65 % more likely to relapse than those who are HIV negative after adjusting for the effect of 2 month culture status, age, body mass index, and erythrocyte sedimentation rate. TB patients and individuals suspected of having TB

should be tested for HIV infection and offered cotrimoxazole and antiretroviral drugs in appropriate combination with anti-TB drugs (34). Cotrimoxazole prophylaxis reduces the risk of death in TB patients in a setting with high HIV seroprevalence according to the results of a large South African study (35).

It is disturbing to note that even in the early days of the HIV epidemic 34 % of the tuberculosis patients were HIV positive. In an analysis of tuberculosis trends and the impact of HIV infection on the situation in the sub-region, it is estimated that by the year 2006 the smear positive case rate would have increased from an estimated 198 per 100 000 population for the region as a whole in 1996, to 1117 per 100 000 if tuberculosis control efforts are not optimized. Alarmingly, 848 per 100 000 population of these cases would be directly attributable to HIV infection (36, 37).

The difficulties of managing TB in Africa are closely linked to HIV/AIDS and specific solutions will be needed for these problems in Africa. Those working under Stop TB partnerships must adopt a broad strategy which include development of enhanced DOTS strategy based on current technology, and the introduction of new evidence-based technology and innovative procedures arising from new research, including studies on how biological and behavioral factors affects response to tuberculosis chemotherapy (5 cited previously).

One of the factors hampering tuberculosis control measure is the late presentation of cases at health facilities (38). In this study all the study subjects were coughing at base line. Persistent cough can be used as an immediate indicator for the general public to go for TB check up.

### 4.2.0 LIMITATIONS OF THE STUDY

There are other variables such as alcohol consumption, nutritional and socioeconomic status which may affect two month culture status (39, 40); unfortunately these variables were not collected in the *M. vaccae* immunotherapy trial and their effect can not be determined.

The sample size could not show conclusive results on the reliability of the use of two month culture conversion in predicting relapse. A larger sample size leads to more accurate parameter estimates, which leads to a greater ability to find what we were looking for (41). This was secondary data and there is no way the sample size can be increased.

## CHAPTER FIVE

*This chapter contains conclusions that can be drawn from the findings of this study. Recommendations are also made based on the findings.*

### 5.1.0 CONCLUSIONS AND RECOMMENDATIONS

Thirty four percent of the study patient's were HIV seropositive even though the study was carried out in the mid 90's when the HIV epidemic in South Africa was still at an earlier stage. There is a need to exhaust all means of controlling the HIV epidemic and optimizing the management of TB in the face of the growing HIV epidemic.

Age, Body Mass index, Smoking status and Gender affect 2 month culture conversion. HIV status was found not to be associated with 2 month culture conversion. On average the young, those with higher body mass index, never smokers and the females fare better than the elderly, those with lower body mass index, ever smokers and the males. Successful TB management needs to take into account the effect of such factors. Anti-smoking campaigns must be intensified, education on good nutrition is needed to improve body mass index and drugs doses must be sensitive to demographic characteristics. There is need for individualization of drug doses, not for the routine patient, but rather for the patient with behavioral or biological factors that affect response to standard tuberculosis drug regimens (42, 43).

According to this study tuberculosis symptom resolution is not a reliable predictor of tuberculosis culture conversion.

From the figures and trends shown in the results section about the use of two month culture conversion in predicting relapse, we can conclude, with caution, that two months culture conversion can still be used as a predictor of relapse. This study however had too small a sample size to show a conclusive effect and findings from this study can be used together with other findings in a meta-analysis of the use of two months culture conversion as a predictor of relapse.

## 6.0 REFERENCES


1. Revised international definitions of tuberculosis control. *International Journal of Tuberculosis and Lung Disease* 2001; **5**(3): 213-215.
2. Global Alliance for TB Drug Development: Available on <www.tballiance.org/4_2_TBControl.asp> accessed on 7 November 2005.
3. Dye C, Scheeele s, Dolin P, Pathania V and 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): 677-686.
4. Corbett EL, Watt CJ, Walker N, et al., The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med* 2003; **163**(9): 1009-21.
5. Dye C, Watt CJ, Bleed DM, Hosseini SM and Raviglione MC. The evolution of tuberculosis control, and prospects for reaching the millennium development goals. *JAMA* 2005; **293**: 2767-2775.
6. Frieden T.R ed. **Toman's tuberculosis, case detection, treatment and monitoring**, 2nd edition, Geneva, World Health Organisation, 2004, page 234.
7. Global Tuberculosis control. WHO Report 2006. Available on < www.stoptb.org/countries/GlobalReport2006/zaf.pdf > Accessed on 2 August 2006.
8. Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, Zeller K and Andrews J. Extensively drug-resistant tuberculosis as a cause of

death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet 2006*; **368:** 1575 -1580

9. XDR-TB emerges in South Africa. Available on <**http://www.kaisernetwork.org/daily_reports/rep_index.cfm?DR_ID=39212**.> Accessed on 23 March 2007.
10. Fourie B. The burden of Tuberculosis in South Africa. Available on < www.sahealthinfo.org/tb/tbburden.htm> Accessed on 6 June 2006.
11. Tuberculosis Symptoms, Available at , <http://wwww.pulmonologychannel.com/tuberculosis/symptoms> Accessed 17 April 2006.
12. International Standards for TB care [Report for TDR SWF Meeting, Geneva 3-5 October 2005]. Available on <ftp://ftp.who.int > Accessed on 2 August 2006.
13. Mitchinson DA. Understanding the chemotherapy of tuberculosis: current problems, *Journal of Antimicrobial Chemotherapy* 1992; **29**: 477-493
14. Global Tuberculosis control: surveillance, planning, financing. WHO report 2005, World Health Organization.
15. Durban Immunotherapy Trial Group. Immunotherapy with *Mycobacterium vaccae* in patients with newly diagnosed pulmonary tuberculosis: a randomized controlled trial. *The Lancet* 1999; **354**: 116-119.
16. Durban Immunotherapy Group, Randomized controlled trial of immunotherapy with *Mycobacterium vaccae* in newly diagnosed pulmonary tuberculosis patients in South Africa, First Report, 1998.

17. Liu z, Shikret KL and Ellis HM. Predictors of sputum culture conversion among patients with tuberculosis in the era of tuberculosis resurgence. *Arch Intern Med* 1999; **159**: 1110-1116.
18. Burman WJ. The hunt for elusive surrogate marker of sterilizing activity in tuberculosis treatment. Editorial: *American Journal of Respiratory and Critical Care Medicine* 2003; **167**: 1299-1300.
19. Wallis RS and Johnson JL. The role of surrogate Markers in the clinical evaluation of anti-tuberculosis chemotherapy. *Curr Med Chem Anti Infective Agents* 2005; **4**: 287-294.
20. Reider HL. Epidemiologic Basis of Tuberculosis Control. Paris, France: International Union against Tuberculosis and Lung Disease; 1999.
21. Dye C. Epidemiology. In: Davies PDO, ed. **Clinical Tuberculosis**. 3rd Edition. London, Arnold 2003.
22. Draft proposal for a scientific working group meeting on tuberculosis research WHO/TDR 3-5 October 2005: available on <ftp://ftp.who.int> accessed 30 November 2005.
23. Mitchison D.A. The control of tuberculosis: Progress and Prospect. *Indian J Med Res* March 2005; **121**: 137-139.
24. Basic statistics. Stafsoft Electronic textbook. Available on < http://www.statsoft.com/textbook/stbasic.html > Accessed on 28 March 2007.
25. Lee J. An insight on the use of multiple logistic regression analysis to estimate association between risk factor and disease occurrence. *International Journal of Epidemiology* 1986; **(15):**22-29

26. Iachine I. Survival analysis. (2003). Available on <www.biostat.sdu.dk/courses > Accessed on 20 April 2007.
27. den Boon S, van Lill SWP, Borgdorff MW, Verver S, Bateman ED, Lombard CJ, Enarson DA and Beyers N. Association between smoking and tuberculosis infection: a population survey in a high tuberculosis incidence area. *Thorax* 2005; **60**: 555-557.
28. Bothamley GH. Smoking and tuberculosis: a chance or causal association? *Thorax* 2005; **60**: 527-528.
29. Farah MG, Tverdal1 A, Steen TW, Heldal1 E, Brantsaeter AB and Bjune G. Treatment outcome of new culture positive pulmonary tuberculosis in Norway. *BMC Public Health* 2005, **5**(14): 1-7.
30. Salihu HM, Aliyu MH, Ratard R, Pierre-Louis BJ. Characteristics associated with reported sputum culture conversion in the era of re-emergent Mycobacterium tuberculosis in the State of North Carolina, 1993-1998. ; **7**(11): 1070-1076.
31. Vidal, R, Casabana MN, Juan A. Incidence and significance of acid fast bacilli in sputum smears at the end of anti-tuberculosis treatment. *Chest* 1996; **109:** 1562-1565.
32. Singla R, Osman MM, Khan N. Factors predicting persistent sputum smear positivity among pulmonary tuberculosis patients 2 months after treatment. International Journal of Tuberculosis Disease 2003; **7:** 558-564.
33. Mitchison DA. Assessment of new sterilizing drugs for treating pulmonary tuberculsosis by culture at 2 months. *Analytical Review of Respiratory Disease* 1993; **147:** 1062-1063.

34. World health Organization. Interim Policy on Collaborative TB/HIV Activities. Geneva, Switzerland. World Health Organization; 2004. WHO/HTM/TB/2004.330.

35. Grimwade K, Sturm AW, Nunn AJ, Mbatha D, Zungu D and Gilks C. Effectiveness of Cotrimoxazole Prophylaxis on Mortality in Adults with Tuberculosis in Rural South Africa. *AIDS* 2005; **19** (2): 163-168.

36. Mortality due to HIV and TB in South Africa. TAC Electronic Newsletter - 21 June 2006. Available on < www.tac.org.za > Accessed on 2 August 2006.

37. Tuberculosis: The Global burden. Stop TB Partnership, WHO 2005. Available on < www.stoptb.org/ > Accessed on 31 July 2006.

38. Health Action: Current Issues in Tuberculosis control. Published by HealthLink Worldwide., Issue 24 May-December 1999.

39. Guler M, Unsal E, Dursan E, Aydlno O and Capan N. Factor's influencing sputum smear and culture conversion time among patients with new case pulmonary tuberculosis. *International Journal of Clinical Practice* 2007; **61**(2):231-235.

40. Gibson N, Care A and Doering D. Socioeconomic factors affecting tuberculosis treatment and prevention in Aboriginal immigrant populations in Alberta. Available on < www.hecol.ualberta.ca/ngibson.html> Accessed on 26 April 2007.

41. Miles J. Getting the sample size right: A brief introduction to power analysis. Available on < http:www.jeremymiles.co.uk/misc/power/index.html > Accessed on 26 April 2007.

*42.* Vernon AA and Iadernaro MF. In the treatment of tuberculosis, you get what you pay for. *American Journal of Respiratory and Critical Care Medicine* 2004; **170:** 1040-1042.

*43.* Chang KC, Leung CC, Yew WW, Chan SL and Tam CM. Dosing schedules of 6 month regimens and relapse for pulmonary tuberculosis. American Journal for Respiratory and Critical Care 2006; **174**(10): 1153-1158.

## 7.1.0 APPENDICES

### 7.1.1 Letter of permission to use data sets

World Health Organization

20, AVENUE APPIA – CH-1211 GENEVA 27 – SWITZERLAND – TEL CENTRAL +41 22 791 2111 – FAX CENTRAL +41 22 791 3111 – WWW.WHO.INT

Tel. direct: +41 22 791 4478
Fax direct: +41 22 791 4771
E-mail : onyebujohp@who.int

In reply please refer to:

Your reference:

Ms Anisa Keshav
Secretary: Human Research Ethics Committee (Medical)
University of the Witwatersrand
Johannesburg

18th May 2006.

Dear Ms Keshav,

**Re : Protocol M060219**

This is to confirm that as the Principal Investigator on the *Mycobacterium vaccae* tuberculosis trial I have agreed that Mr. MP Mapingure can carry out secondary data analysis on the data from the trial, under the supervision of Dr. Jonathan Levin, who was the trial statistician.

The work will involve no new data being collected and consists of analyses that fall within the ambit of the original ethics approval of this study which was granted by the University of Natal Medical School. Please feel free to contact me if you have any query

Yours faithfully,

Dr. Philip C. Onyebujoh
TB/HIV Research Coordinator

7.1.2 Ethics Clearance certificate from the University of Witwatersrand Committee for Research on Human Subjects.

**UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG**

Division of the Deputy Registrar (Research)

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)**
R14/49 Mapingure

| | |
|---|---|
| **CLEARANCE CERTIFICATE** | **PROTOCOL NUMBER M060219** |
| **PROJECT** | Analysis of Secondary Data from Mycobacterium Vaccae Tuberculosis Clinical Trial |
| **INVESTIGATORS** | Mr MP Mapingure |
| **DEPARTMENT** | School of Public Health |
| **DATE CONSIDERED** | 06.02.24 |
| **DECISION OF THE COMMITTEE*** | Approved unconditionally |

**Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.**

**DATE** 06.06.02 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr JB Levin

---

**DECLARATION OF INVESTIGATOR(S)**

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES