Completion of isoniazid preventive therapy and factors associated with non-completion in an antiretroviral therapy-naive HIV-infected cohort in Cape Town

by

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Thesis

Presented to the School of Public Health

for the Degree of

Masters in Public Health (Epidemiology)

University of Cape Town

February 2012

Declaration

This is to certify that

(i) this thesis comprises only my original work towards this degree,

(ii) any contribution made by others has been duly acknowledged and appropriately referenced.

Acknowledgements

The material in this dissertation is my original work. I was responsible for study design, data analysis and writing of the manuscript and contributed to data collection. A number of people contributed to some aspects of conducting the study. I would like to thank my supervisor David Coetzee for his guidance in the write up of this dissertation. This study would not have been feasible without the support of Relebohile Tsekela, Bekekile Kwaza and Lulama Manjezi, who contributed significantly to data collection and patient follow-up. I would also like to thank Nonzwakazi Bangani and Katalin Wilkinson for their contribution in acquisition of laboratory materials and processing of specimens. I would like to express sincere gratitude to Robert Wilkinson for supporting this thesis through provision of funding, support and guidance making conducting this study possible.

The primary study was funded by a grant from the European Union (Sante/2006/105-061) and Bill and Melinda Gates Foundation GC 6-74 37772.

Abstract

Completion of isoniazid preventive therapy and factors associated with non-completion in an antiretroviral therapy-naive HIV-infected cohort in Cape Town

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TB incidence in South Africa remains high, despite high rates of successful treatment suggesting ongoing transmission and a large reservoir of latently infected persons. Isoniazid preventive therapy (IPT) is recommended as preventive therapy in HIV-infected persons. However, implementation has been slow, impeded by barriers and challenges including the fear of non-adherence.

A protocol was therefore written to conduct a study to measure IPT completion rates and evaluate predictors of non-completion of a six-month IPT course in Khayelitsha, an informal township in Cape Town. Prior to data analysis, a structured literature review was conducted to assess available evidence particularly from high-burden settings on IPT completion rates and factors associated with loss to follow up. Completion rates were similar in low and high-burden settings and varied from 47% to 81%. Treatment-, patient-, and health system provider-related factors identified that were associated with non-adherence included reported isoniazid side effects, male sex, economic limitations and poor relationship with health service providers. The review also

demonstrated a paucity of data from HIV-infected persons in high HIV and TB-burden settings.

The study was conducted between February 2008 and March 2010. One hundred and sixty four antiretroviral therapy (ART)-naïve HIV-infected patients with tuberculin skin test ≥5mm were recruited from Khayelitsha day hospital and followed up monthly. A questionnaire was used to collect demographic information.

The overall completion rate was 69%. In multivariable analysis, self-reported alcohol drinkers (OR 4.05; 95% C.I. 1.89-9.06) had a four-fold higher risk of non-completion, with a strong association between alcohol drinkers and smoking (χ^2 27.08; $p<0.001$). There was a 29% decrease in risk of non-completion for every year after HIV diagnosis (OR 0.81; 95% C.I. 0.68-0.98).

Patients with a recent HIV diagnosis and self-reported drinkers and smokers were identified as being at higher risk of defaulting IPT, suggesting these groups should be identified and targeted for adherence interventions when implementing IPT on a wider scale.

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List of Abbreviations

AIDS Acquired Immunodeficiency Deficiency Syndrome

ART Antiretroviral therapy

BCG Bacille Calmette Guerin

BMI Body mass index

HCT HIV counselling and testing

HCW Health care workers

HIV Human Immunodeficiency Virus-1

ICF Intensified case-finding

INH Isoniazid

IPT Isoniazid preventive therapy

IQR Interquartile range

LTBI Latent tuberculosis infection

M.tb. Mycobacterium tuberculosis

RCT Randomised controlled trial

TB Tuberculosis

TST Tuberculin Skin Test

TU Tuberculin units

VCT Voluntary counselling and testing

Vs Versus

WHO World Health Organisation

PART A: PROTOCOL

A.1 INTRODUCTION

A.1.1 Background

The global burden of tuberculosis (TB) remains high despite widely implemented strategies to control the epidemic. Sub-Saharan Africa bears the brunt of this burden with the World Health Organization (WHO) Africa region having the highest estimated incidence rate (1). This burden is exceptionally high in countries with generalised HIV epidemics, with HIV driving the TB epidemic through an increased risk of infection and reactivation disease. The annual risk of disease progression in HIV co-infected persons is 10% compared to a 10% lifetime risk in HIV negative persons (2). In South Africa, the burden of HIV-associated TB is high with up to two thirds of TB cases occurring in HIV co-infected persons (3). Antiretroviral therapy (ART) reduces the risk of TB in HIV co-infected persons but the risk of TB remains high in this population despite ART (4).

Accurate identification and treatment of latent TB infection (LTBI) is crucial in order to eliminate the reservoir of infections, making prevention of TB in people living with HIV an urgent priority. To this end, the WHO recommends 300mg daily Isoniazid Preventive Therapy (IPT) for at least six months in people living with HIV as one of the key strategies of TB control in addition to intensified case finding and infection control.

A.1.2 Problem statement

IPT has been shown to significantly reduce the risk of incident TB predominantly in those with positive tuberculin skin test (TST) (5). A study compared regimens of shorter duration to 6 months IPT and reported non-superiority compared to the

older 6-month IPT regimen (6). This benefit is dependent on good adherence to the treatment regime.

Testing and treating for LTBI has predominantly been implemented in low burden high-income settings. However, in high TB burden settings, BCG vaccination and passive case-finding with treatment of microscopy/culture positive cases have been the cornerstones of TB control strategies with LTBI treatment being poorly implemented in these settings (7). Furthermore, these strategies could be compromised by inadequate intensified TB disease case-finding and by low adherence. Other barriers to implementation identified include a lack of knowledge and experience on the part of health care workers, concerns regarding isoniazid (INH) resistance, and fear of side effects with hepatitis and peripheral neuropathy most common (8,9). Nonetheless, the potential to interrupt transmission through reducing risk of reactivation and re-infection in HIV-infected persons makes this strategy important.

The benefit of IPT in reducing incident TB has been demonstrated to be predominantly in TST positive people. However, it is often not feasible to perform the tuberculin skin test in settings with generalised HIV epidemics due to resource and operational constraints, further contributing to poor implementation. In response to this, WHO guidelines changed from have TST as a requirement for the diagnosis of LTBI to recommending the use of a simplified clinical screening algorithm to screen for active TB. The new guidelines emphasize that a TST is no longer a requirement for initiating IPT in people living with HIV (10).

This study will aim to evaluate completion rates of IPT within a research context and will also aim to identify predictors of non-completion as a means of identifying those at high risk of non-adherence and targeting retention strategies at these persons.

A.1.3 Research question

What are the completion rates of a 6-month course of IPT and what are the predictors of non-completion in an ART-naive HIV-infected cohort in Khayelitsha, Cape Town, recruited as part of a TB diagnostics study?

A.1.4 Research justification

TB remains a great burden particularly in Sub-Saharan Africa with 1% of the population in South Africa developing the disease annually (11). This epidemic is fuelled by a generalised HIV epidemic with up to two-thirds of TB cases being HIV co-infected (3). It is in response to these co-epidemics that the WHO launched the three "I"s TB control strategy comprising of infection control, intensified case finding and isoniazid preventive therapy.

IPT has been shown to significantly decrease the incidence of TB particularly in tuberculin skin test (TST) positive persons. However, despite strong evidence of the benefit of IPT in HIV-infected adults, this strategy has not been widely implemented in high burden countries such as South Africa. A significant perceived barrier to implementation is fear of non-adherence and an increase in isoniazid mono-resistance and low rates of regimen completion.

Research into the feasibility of completion of a 6-month regimen is therefore essential and few such studies have been conducted in HIV-infected patients in high TB/HIV burden settings. We will report on the completion rates of IPT within a research context. Furthermore, it is important to understand factors that may predispose to non-adherence to treatment in order to develop strategies targeting those identified as being at high risk of defaulting treatment. We will therefore also examine predictors of non-completion in our study population.

A.1.5 Objectives

- To describe completion rates of IPT in a cohort of antiretroviral therapy naive HIV-infected persons identified as part of a larger cross-sectional TB diagnostics study between 01 February 2008 and 30 November 2010.
- To evaluate predictors of non-completion of IPT in this cohort.

A.1.6 Summary of literature review

We conducted a literature review using PubMed to summarise available evidence on completion rates and predictors of non-completion of IPT in low and high TB burden settings. We included all relevant studies in the English language that reported data on IPT completion rates and predictors of loss to follow up, non-adherence or non-completion. Thirteen studies met our inclusion criteria, five of which were conducted in high TB-burden settings (12-16). IPT completion rates were similar in high- and low-burden settings and varied from 47%-78% to 45%-81% in respectively. This demonstrates that although some studies were conducted within research contexts, it is possible to provide IPT in these settings.

Predictors of non-adherence can be divided into treatment-, patient- and provider-related barriers.

A.1.6.1 Treatment related barriers

Potential treatment barriers were identified in both low- and high-burden settings. Some studies in high-burden settings identified reported INH side effects and reported difficulty taking pills as being predictive of non-adherence (12,16). INH is known to have side effects, most common of which are peripheral neuropathy and drug-induced hepatitis, although the former is more common.

Studies reviewed that explored rifamycin-based regimens, which are shorter, were all conducted in low-burden settings. These studies reported that shorter regimens were more acceptable to patients and were associated with better adherence (17,18). As no high-burden settings conducted studies on other regimens, a comparison could not be made. However, a recent study evaluated three new regimens for LTBI treatment in South Africa in TST positive, HIV-infected adults (6). They reported that neither a 3-month course of intermittent rifapentine or rifampicin with isoniazid was superior to 6 months of isoniazid at prevention of incident TB. In keeping with other studies, they reported lower adherence in the 6-month INH group compared to the rifapentine-isoniazid and rifampicin-isoniazid groups (84% vs 95.7% and 94.8% respectively). These findings suggest that new, shorter regimens could potentially improve completion rates and should be explored, especially in high burden countries.

A.1.6.2 Patient related barriers

Across the studies and settings, males were identified consistently as being at higher risk of non-adherence. In low-income, high-burden settings, economic limitations such as commuting difficulty and lack of control over resources were identified as significant barriers to treatment completion (14). In addition, education and beliefs appear to play a role in adherence. Patients who believed that the drugs were safe were more likely to be adherent while those who perceived competition between western and traditional medicines as well as those who struggled to take the medication due to lack of symptoms were more likely to be non-adherent (13,14). These factors suggest a lack of understanding of the purpose and potential benefit of LTBI treatment. In these cases, careful counselling and explanation about latent TB infection and the need for treatment despite lack of symptoms could potentially improve completion rates. Some studies also identified a lack of good support networks, HIV status non-disclosure and perceived stigma as barriers to treatment completion (15). The

ART model of repeated counselling sessions prior to initiation of treatment and the use of treatment supporters or “buddies” could be explored in these settings as a strategy to tackle these barriers.

In high-income settings, barriers were more related to social risk factors, with marginalized groups such as recent migrants, those without medical insurance and the homeless at higher risk of non-adherence (19). Smokers, who are at higher risk of TB disease, were also identified by one study as being less adherent that non-smokers (20).

A.1.6.3 Health service provider related barriers

As attendance of health facilities is necessary to access LTBI treatment, the relationship between patients and health care providers could influence completion rates. Two studies reported that outpatients and patients who regularly attended the clinic were less likely to be non-adherent (15,21). A poor relationship with the health service provider, described in focus group discussions as believing in the healthcare providers, could result in patients not attending clinic appointments resulting in non-adherence. One study specifically mentions a good patient/provider relationship as being predictive of completion of treatment (15).

Moreover, we also highlight a paucity of much needed data on predictors of non-adherence to LTBI treatment in ART-naive HIV co-infected persons attending HIV clinics in high burden settings.

A.2 METHODS

A.2.1 Study design

This study is a longitudinal study using participants that will be identified in a larger cross-sectional TB diagnostics study to develop biomarkers that distinguish active from latent TB (REC 012/2007). As part of this larger study, participants with active and latent TB as well as those with other infections resembling TB will be recruited in both HIV-infected and uninfected cohorts. Latent TB (LTBI) will be diagnosed by the Mantoux test and a transverse induration diameter ≥5mm is considered positive in HIV-infected persons. As per South African national guidelines, HIV-infected persons diagnosed with LTBI will be initiated on Isoniazid Preventive Therapy for six months, with monthly visits to monitor adherence and side effects. Although this is national policy, it is not implemented at the health facility in Khayelitsha; therefore the author will be responsible for identifying eligible individuals and initiating IPT. Adherence will be measured by self-reported adherence, and attendance of follow-up appointments over the six-month period. Patients late for their appointments will receive a telephonic reminder and failing that, a home visit by a clinical research worker to encourage clinic attendance. IPT will therefore be implemented more within a research context than an operational clinic setting, as we will have more resources available to follow-up participants than might otherwise be the case in a regular health facility.

A.2.2 Study setting

The study will be conducted at site B Day Hospital, a primary care hospital in the informal township of Khayelitsha. Located on the outskirts of Cape Town, Khayelitsha has a population of over 400,000 and a TB case notification rate of over 1600/100,000.

A.2.3 Population and sampling strategy

HIV-infected persons with no signs or symptoms of active TB and with evidence of LTBI (TST≥5 mm) will be eligible for this study. The study population will be drawn from HIV-1 infected adults attending the wellness clinic at site B Day Hospital, between 01 February 2008 and 30 November 2010. They will either be newly diagnosed or attending for minor ailments, and will all be antiretroviral therapy (ART)- naive on account of not being eligible for ART as per the national guidelines at the time (CD4≥200 cells/mm^3). These participants will be identified as the LTBI cohort of the larger TB diagnostics study. Written informed consent will be obtained from all participants and the University of Cape Town research ethics committee approved the TB diagnostics study (REC 012/2007) and the amendment for this study. Participants that give written consent will have a baseline CD4 count and induced sputum microscopy and culture performed to exclude active TB. A tuberculin skin test will be performed on all participants using 2 tuberculin units of Purified Protein Derivative (PPD) RT23 injected intradermally into the volar aspect of the forearm. All those with a skin induration diameter of ≥5 mm will have a chest x-ray performed and will be started on isoniazid preventive therapy for 6 months if the x-ray is normal. Non-completion of IPT will be defined as a failure to complete a six-month IPT course within nine months. As the provision of IPT in TST positive HIV-infected persons is compliant with national guidelines, IPT will form part of gold standard health care and not a research intervention.

Attendance of IPT appointments will be noted, with failure to attend resulting in a telephonic reminder or home-visit to optimize retention.

A.2.4 Sample size calculation

The sample size calculation was performed for the primary TB diagnostics cross sectional study that aimed to develop biomarkers for active TB and was based on the minimum sample size required to identify significantly differentially

expressed genes in active and latently infected TB patients. However, with a sample size of 164, assuming a background non-completion rate of 30%, we will detect a two-fold increase in the proportion of non-completers due to risk factors such as drinking alcohol with a power of 97%.

Our study represents an observational study with longitudinal follow-up of participants.

A.2.5 Data collection

Data collection instruments in Appendices 3 and 4. The data collection tool was designed for the TB diagnostics study. The variables for this study will be extracted from this tool.

A.2.5.1 Study variables

We will collect information on the measured variables for the study listed below in Table 1. In addition to the variables identified in the literature review, we have also identified variables that could potentially influence IPT completion. Examples include TB contact history (might be more likely to complete IPT if close contact with known TB case due to fear of getting sick), BMI (low BMI could be associated with ill-health a fear of being sick) and CD4 count (lower CD4 count could lead to fear of ill-health). We will compare the characteristics of participants with respect to the outcome (non-completion of IPT). Non-completion (or loss to follow up) is defined as more than 3 months non-attendance within a 9-month period from initiation of IPT.

Variables	Type	Definition	Categories
Age	Continuous	Independent	>18

Variables	Type	Definition	Categories
Sex	Categorical	Independent	Male Female
Employment	Categorical	Independent	Employed (Employed, full-time, Employed, part-time, Employed, casual Unemployed (Student, Homemaker, Unemployed or seeking work, Government grant or pension)
Marital status	Categorical	Independent	Single Married
Educational level	Ordinal	Independent	Grades 0-12
Smoking	Categorical	Independent	Smoker (Current smoker) Non smoker (Never smoked, Ex-smoker)
Alcohol consumption	Categorical	Independent	Drinker (Current drinker) Non-drinker (Non-drinker, Ex-drinker ≥ 1 year, Ex-drinker < 1 year
Time lived in Khayelitsha	Continuous	Independent	Months
Type of dwelling	Categorical	Independent	Brick building (House/permanent building) Shack (Shack on serviced site, Shack on unserviced site or other open land)
Number of persons sleeping/room	Ordinal	Independent	1+
Time since HIV diagnosis	Continuous	Independent	>0 +

Variables	Type	Definition	Categories
CD4	Continuous	Independent	>20 +
Previous TB	Categorical	Independent	Yes No
Recent TB contact	Categorical	Independent	Yes No
BCG vaccination	Categorical	Independent	Yes No
BMI	Continuous	Independent	16+
Mantoux induration	Continuous	Independent	5+
Number of months IPT completed	Continuous	Dependent	Any number
Non-completion	Categorical	Dependent	Yes No

Table A.1 Study variables to be measured

A.2.6 Statistical analysis plan

Patient baseline characteristics will be tabulated stratified by the outcome (completion/non-completion of IPT) and summarised using simple proportions. Overall completion rate of IPT will be presented as a proportion. Predictors of non-completion will be analysed by logistic regression. Nested models will be compared using the likelihood ratio test, which assesses the extent to which the additional terms improve the fit of the model by measuring the difference in the deviances of the 2 nested models. The Akaike's Information Criterion (AIC) will be used to compare non-nested models with a lower AIC indicating a better model. The interactions between confounding and exposure variables will also be examined. The fit of the model will be assessed using Pearson's goodness-

of-fit test with a p-value >0.05 indicating a good fit of the model to the data. Model diagnostics will be performed on final models by checking the form of the linear predictors. Scatterplot diagrams of the observation number versus the Pearson and deviance residuals will be used to identify a systematic pattern which would indicate an incorrect model. Similarly, a scatterplot diagram of residuals versus linear predictors will be used to demonstrate good fit. In a good model, residuals of cases should be closer to the horizontal line as linear predictors get larger and vice versa for smaller linear predictors. As the dependent variable (non-completion) is a binary variable, the logit transformation is deemed appropriate and the adequacy of the link function does not require checking. Outlying observations will be identified as standardised residuals greater than +2 or smaller than -2 and scatterplot diagrams of standardised residuals versus the observation numbers will be used to visually identify outlying observations. Influential and large leverage observations will be identified respectively using the Hosmer and Lemeshow test of influence (dx2) and hat matrix (hat), a measure of the leverage of covariate pattern i.e. how far the covariate pattern lies from the average covariate pattern and thus how much of an effect it has on the estimated model. A value greater than $^{2p}/_{n}$, where p=number of variables in the model and n=number of covariate patterns, is indicative of large leverage. Scatterplot diagrams of dx2 and hat versus the observation numbers will be used to visually identify influential and high leverage observations. The variance inflation factor (uncentered) will be used to detect collinearity with a VIF>>>1 suggesting multi-collinearity could be seriously influencing the model.

Significance testing will be done using a combination of two-sided p-values and 95% confidence intervals. All data will be analysed using STATA 10.0 (StataCorp, College Station, TX, USA).

A.3 ETHICAL CONSIDERATIONS

A.3.1 Harm

A physician and nurse-counsellor will oversee informed consent procedures. The consent form will be provided in the language that the participants are most comfortable with. The informed consent document describes the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to participants. Although not a drug trial, this study will be conducted according to Good Clinical Practice (GCP). The intervention above normal care is phlebotomy for a baseline CD4 count. The risks of blood withdrawal are slight. Very seldom needle injury to soft tissue or haematoma can occur. Participants will receive a ZAR30 compensation for travel expenses. Study staff will receive training on the use of preventive measures to avoid accidental punctures with contaminated material. Counselling and, if indicated, emergency antiretroviral treatment will be made available to study staff who are accidentally exposed.

Due to the sensitive nature of the data to be collected, maintaining the confidentiality of clinical information (including, but not limited to HIV results) is of high priority. Measures to ensure confidentiality will include:

a) Recruitment and personal interviews will be done in complete privacy.

b) Questionnaires that have a detachable section containing the person's identification data.

c) Storage of questionnaires in a locked room accessible only to select personnel.

d) Computerized data will identify individuals only by code.

e) All reports and publications will refer only to anonymous or pooled data.

A.3.2 Autonomy

Participant autonomy will be emphasized at all times with the decision to participate entirely at the patient's discretion. It will be explained to potential participants that a decision not to participate would not disadvantage their treatment in any way and that they will be free to withdraw from the study at any point without disadvantage.

A.3.3 Benefit

Participants will benefit by having a TST performed and provision of IPT in those diagnosed with LTBI with normal chest X-ray. Although this service is as per national guidelines, it is not being implemented in most government health facilities including the one in which the study will be conducted. Therefore participation in the study will result in receipt of care above the normal standard of care. Moreover, resources from the parent TB diagnostics study will be used to encourage completion of IPT through visit reminders and home visits.

A.3.4 Confidentiality

Due to the sensitive nature of the data collected, maintaining the confidentiality of clinical information (including, but not limited to HIV results) was of high priority. Measures to ensure confidentiality included:

a) Recruitment and personal interviews were done in complete privacy.

b) Questionnaires have a detachable section containing the person's identification data.

c) Storage of questionnaires in a locked room accessible only to select personnel.

d) Computerized data identifies individuals only by code

e) All reports and publications refer only to anonymous or pooled data.

A.4 WRITE UP AND DISSEMINATION

Implementation of IPT is poor in the Western Cape. Albeit within a research setting, our data will provide useful information of completion rates of IPT and assist in early identification of potential defaulters and development of targeted strategies to improve retention in care. The potential users of results generated from this study are other researchers, governmental and non-governmental organizations and the communities who are researched themselves. We will aim to disseminate findings at internal, national and international meetings, and in an open access journal.

A.5 TIMESCALES

Participant recruitment for this study will be conducted as part of the parent TB diagnostics study between February 2008 and 30 November 2010 with the last participant completing IPT in June 2011.

A.6 BUDGET

The parent TB diagnostics study is funded by the European Union with a budget of €4532158 (see appendix 8). Participant follow up for this study will not require any additional funding.

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PART B: STRUCTURED LITERATURE REVIEW

B.1 INTRODUCTION

Isoniazid preventive therapy (IPT) has been shown to significantly reduce TB incidence, predominantly in tuberculin skin test positive persons (1,2) and at least six months of IPT is recommended by the World Health Organization (WHO) as part of the three "I"s for TB/HIV campaign (3). This strategy has predominantly been implemented in low-burden, high-income settings. However, in high TB burden settings, BCG vaccination and passive case-finding remain the cornerstones of TB control strategies. This slow implementation is in part due to fear of low adherence and non-completion of the IPT course. A better understanding of predictors of non-adherence would therefore facilitate targeted interventions to improve adherence and increase implementation of IPT in high burden settings.

B.2 OBJECTIVES

To summarise available evidence on completion rates and predictors of non-completion of IPT in low and high TB burden settings.

B.3 METHODOLOGY

B.3.1 Search strategy

We searched for primary studies and reviews from PubMed (1950 to September 2011). Our search strategy included the following terms: "tuberculosis", "latent infection", "isoniazid", prevent* therapy, "chemoprophylaxis", "risk factor*", "predictor*", "adherence", "compliance", "complet*". This search yielded 426 citations. The titles and abstracts were screened and relevant studies identified.

B.3.2 Study selection and data extraction

The search strategy aimed to identify all relevant studies in the English language that reported data on the population sampled, completion rates of IPT and predictors of loss to follow-up (LTFU) or non-adherence in the absence of specific interventions to improve adherence. We excluded non-English studies, paediatric studies, primary adherence intervention studies, primary studies that were included in the systematic review (n=2) and studies that did not report risk factors for non-adherence. We included both quantitative and qualitative studies. We initially aimed to restrict the studies to those from low and middle income countries with intermediate to high burden of disease but these criteria were extended due to paucity of data. Data were extracted from a subset of eligible studies. These include: author, country, year, study population sampled, proportion HIV-infected, IPT completion rates, and reported predictors of LTFU.

B.4 RESULTS

Of the 426 citations identified using the search strategy, 13 met our inclusion criteria (12 primary and 1 systematic review) and were reviewed in detail. Five out of 13 studies (38%) were conducted in high TB burden settings. The majority of studies were conducted in high income, low TB/HIV burden countries. Four studies were conducted solely in HIV-infected persons (4-7), all of which were in high disease burden settings. Completion of IPT was measured using a variety of methods including pharmacy refill rates, detection of INH urine metabolites, pill count, self-report and completion of 6-9 months IPT within a 9-12 month period. Two studies did not report IPT completion rates (5,8) but analysed data for predictors of LTFU and were thus included in our review. To allow for greater generalisability of results, results were stratified by low and high disease burden settings.

B.4.1 High-burden settings

Reported IPT completion rates varied from 47.1% in rural South Africa (6) to 78% in HIV-infected patients participating in a randomised controlled trial (RCT) in Botswana (4). In the latter study, the regulated trial conditions are likely to have contributed to higher IPT completion rates. Two studies reported male sex as being a risk factor for non-adherence (4,7) with men being twice as likely to be non-adherence and women being 3 times as likely as men to be adherent, respectively. Several studies reported perceived isoniazid (INH) side effects as being a barrier to adherence (Table B.1). Two studies explored barriers using qualitative techniques. Two running themes identified were stigma and economic barriers. Stigma was often cited as the reason for HIV status non-disclosure resulting in a lack of social and family support and leading to a non-acceptance of HIV status. Szakacs *et al* reported that a belief in a high chance of getting sick without IPT was associated with non-adherence (5). It was suggested that this counter-intuitive finding might be related to a perceived incurability of HIV and associated stigma preventing patients from taking their medication. This highlights a need to encourage and support patients with HIV status disclosure prior to IPT initiation in a similar manner to counselling offered prior to initiating antiretroviral therapy as an adherence improvement strategy. Economic factors were also cited with economic limitations, lack of transport, hunger and a lack of control over resources identified as barriers to adherence.

It was also reported that patients who report difficulty taking meds or taking pills in the absence of symptoms as well as those with whom there was conflict between Western and traditional medicine, were more likely to be non-adherent. This suggests that a lack of education about the importance of IPT and explanation about prevention of TB might contribute to non-adherence.

Reference	Country	Study population	IPT completion	Risk Factors OR (95% CI) unless otherwise stated
(9)	Brazil	TB household contacts	53.5%	*Non-adherence factors* Side effects RR* 2.69 (1.3-5.8) Commuting difficult RR* 1.8 (1.01-3.3)
(4)	Botswana	HIV patients in RCT*	78% adherence to ≥80% meds	*Non-adherence factors* Male 2.24 (1.24-4.04) Difficulty taking meds 3.4 (1.75-6.60) Younger age 0.95 (0.91-0.98)
(5)	Pietermaritzburg hospital South Africa	HIV patients on IPT*; Adherence by INH* urine levels	72% had positive INH* urine tests	*Non-adherence factors* Time since last reported INH* dose 1.34 (1.13-1.59) Belief that INH* is safe 0.34 (0.15-0.77) Belief in high chance of getting sick without INH* 3.00 (1.32-6.79)
(6)	Limpopo, rural South Africa	HIV patients on IPT*	47.1%	*Qualitative assessment of barriers* Economic limitations Transport Hunger Lack of control over resources HIV non-disclosure and lack of social and family support Reluctance to take meds in absence of symptoms Competition between western and traditional medicine

Reference	Country	Study population	IPT completion	Risk Factors OR (95% CI) unless otherwise stated
(7)	Thailand	HIV patients on IPT*	67.5% completed ≥80% of meds	*Quantitative factors for adherence* Female 3.00 (1.56-5.88) History of physical symptoms 0.50 (0.32-0.79) Outpatients 1.56 (1.49-2.63) Sex workers 0.13 (0.05-0.36) *Qualitative factors for non-adherence* HIV non-disclosure Out-migration Perceived INH* side effects *Qualitative factors for adherence* Acceptance if HIV status Concern about children and family Good health provider relationship

Table B.1 Summary of studies conducted in high-burden settings

*RR relative risk, RCT randomised controlled trial, INH isoniazid, IPT isoniazid preventive therapy.

B.4.1 Low-burden settings

In low disease burden settings, IPT completion rates varied from 44.6% in participants in an RCT to 80.8% in HIV negative patients attending TB clinics for IPT. Of note, the former study, an RCT conducted in the United States of America was based in an inner city facility with rates of unemployment and homelessness relatively comparable to low income settings (10). This range is similar to that reported in studies in high disease burden settings. Three of these studies offered shorter preventive therapy regimens and all reported shorter

LTBI treatment regimens to be associated with better adherence, highlighting the required duration of treatment as a potential barrier to treatment completion. This was not explored in low-income settings, therefore a comparison was not possible. Younger age and male sex were also identified as predictors of non-adherence, although the systematic review of studies in USA and Canada reported inconsistency with respect to age as a non-adherence factor with studies reporting positive adherence associated with age >65, age <35 and increasing age (11). Smoking is a known risk factor for TB and one study explored smoking as a predictor of non-adherence and reported non-smokers were almost twice as likely as smokers to be adherent to LTBI treatment (12). This study suggested that non-adherence in smokers could contribute to a higher risk of TB infection and disease.

A running theme across a few of the studies was that social circumstances and marginalisation from society played a significant role in non-adherence to therapy. Predictors of non-adherence identified include alcohol use (especially in the homeless), recent immigrants, lower education, poor social support and lack of medical insurance (Table B.2).

Reference	Country	Study population	IPT completion	Risk Factors OR (95% CI) unless otherwise stated
(8)	Canada, Saudi Arabia, Brazil	Participants in LTBI* RCT*	Not reported	*Adherence factors* Shorter LTBI* treatment regimen 4.3 (2.7-6.8) *Non-adherence factors* Late first visit attendance RR* 0.90 (0.8-0.98) >20%missed doses RR* 0.4 (0.3-0.6)

Reference	Country	Study population	IPT completion	Risk Factors OR (95% CI) unless otherwise stated
(10)	USA	Participants in LTBI* RCT*	44.6%	*Adherence factors* Marriage 2.153 (1.301-3.5620) (+foreign birth) *Non-adherence factors* Alcohol use 0.53 (0.32-0.877) (+homeless)
(13)	Spain	HIV negative persons at TB clinics	80.8%	*Non-adherence factors* Age<36 0.33 (0.3-0.76) Male sex 0.58 (0.37-0.92) Immigrant<5years 0.21 (0.12-0.37) Presence of social risk factors 0.21 (0.11-0.39)
(14)	USA	Chest clinic patients (55% HIV infected)	45.2%	*Adherence factors* Age≥35 RR* 1.2 (1.1-1.2) TB contacts RR* 1.5 (1.4-1.7) DOPT* RR* 1.3 (1.2-1.3) Shorter regimen RR* 1.2 (1.1-1.3)
(11)	USA/Canada	Systematic review of USA/ Canada studies	TB contacts 35-64% Prisoners 32-61% Migrants 22-90% Drug users 39-70% HIV, homeless, HCW* 27-82%	*Adherence factors* Inconsistent for age with adherence assoc. with >65, <35 and increasing age TB contact Good social support Higher education
(15)	USA	TB clinic attendants	61.7%	*Non-adherence factors* Lack of medical insurance 1.7 (1.1-2.7) Reported side effects 3.6 (2.2-6.2) Post partum 3.4 (0.9-12.6) Age<20 2.3 (0.8-6.5)

Reference	Country	Study population	IPT completion	Risk Factors OR (95% CI) unless otherwise stated
(12)	Canada	LTBI* patients convenience sample	72%	Adherence factors Women 2.0 (1.2-3.3) Non-smokers 1.8 (1.0-1.33)
(16)	UK	LTBI regimen study participants	53.5%	*Adherence factors* Choice of LTBI* treatment regimen 0.43 (0.30-0.60) Clinic attendance before IPT* 0.54 (0.37-0.78) Age 1.04 (1.02-1.06)

Table B.2 Summary of studies conducted in low-burden settings

* LTBI latent tuberculosis infection, RCT randomized controlled trial, DOPT direct observed preventive therapy, HCW health care worker, IPT isoniazid preventive therapy.

B.5 DISCUSSION

This review presents 13 studies evaluating LTBI treatment completion rates and factors associated with adherence and non-adherence. The majority of studies assessed isoniazed based regimens as LTBI treatment with a few, in high-income settings exploring other shorter rifamycin-based regimens. We reported findings stratified by disease burden of the study setting. Completion rates did not significantly vary between high and low burden settings. This demonstrates that, albeit within mainly research contexts, it is possible to provide IPT in high-burden, low-income settings.

Predictors of non-adherence can be divided into treatment-, patient- and provider-related barriers.

B.5.1 Treatment-related barriers

Potential treatment barriers were identified in both low and high burden settings. Some studies in high burden settings identified reported INH side effects and reported difficulty taking pills as being predictive of non-adherence. Most studies in high burden settings involved HIV-infected patients. INH is known to have side effects most common of which are peripheral neuropathy and drug-induced hepatitis, although the former is more common.

Studies that explored rifamycin-based regimens, which are shorter, were all conducted in low burden settings. These studies reported that shorter regimens were more acceptable to patients and were associated with better adherence. As no high-burden settings conducted studies on other regimens, a comparison could not be made. However, a recent study evaluated three new regimens for LTBI treatment in South Africa in TST positive, HIV-infected adults (17). They reported that neither a 3-month course of intermittent rifapentine or rifampicin with isoniazid was superior to 6 months of isoniazid at prevention of incident TB. In keeping with other studies, they reported lower adherence in the 6-month INH group compared to the rifapentine-isoniazid and rifampicin-isoniazid groups (84% vs 95.7% and 94.8% respectively). These findings suggest that new, shorter regimens could potentially improve completion rates and should be explored, especially in high burden countries.

B.5.2 Patient-related barriers

Across the studies and settings, males were identified as being at higher risk of non-adherence. This may relate to gender-related differences in health-seeking behavior and health facility attendance patterns. In low income, high burden settings, economic limitations such as commuting difficulty and lack of control over resources were identified as significant barriers to treatment completion. In addition, education and beliefs appear to play a role in adherence. Patients who

believed that the drugs were safe were more likely to be adherent while those who perceived competition between western and traditional medicines as well as those who struggled to take the medication due to lack of symptoms were more likely to be non-adherent. These factors suggest a lack of understanding of the purpose and potential benefit of LTBI treatment. Some studies also identified a lack of good support networks, HIV status non-disclosure and perceived stigma as barriers to treatment completion. The ART model of repeated counselling sessions prior to initiation of treatment and the use of treatment supporters or "buddies" could be explored in these settings as a strategy to tackle these barriers.

In high-income settings, barriers were more related to social risk factors, with marginalized groups such as recent migrants, those without medical insurance and the homeless at higher risk of non-adherence. Smokers, who are at higher risk of TB disease, were also identified by one study as being less adherent that non-smokers. Therefore in these settings, targeting these at-risk groups for adherence interventions could contribute to better completion rates.

B.5.3 Health service provider-related barriers

Attendance of health facilities is required to receive IPT. Therefore the relationship between patients and health care providers could serve as a factor for adherence or non-adherence. Two studies reported that outpatients were less likely to be non-adherent. A poor relationship with the health service provider could result in patients not attending clinic appointments and non-adherence. One study specifically mentions a good patient/provider relationship as being predictive of completion of treatment.

B.5.4 Strengths and limitations

The treatment of LTBI is crucial to TB elimination, particularly in high HIV/TB burden settings. However implementation of this TB control strategy is poor in these settings. A strength of this review is its relevance to TB control in high burden and high transmission settings.

Risk factors for non-adherence could potentially vary in different settings. Therefore another strength of this review is the stratification of risk factors by burden of disease in the study setting. This strategy highlighted key differences in significant predictors of treatment completion in high- and low-burden settings, crucial in translation of research findings into interventions to improve completion rates.

The majority of studies included in the review provided IPT within a research setting with potentially more available resources compared to the health care system.

Only English language studies were included in this review and only one database was searched for studies. It is therefore likely that other published studies that would otherwise fit our inclusion criteria were missed. However, the studies that were reviewed reported similar results and we are therefore confident that the majority of significant predictors have been identified.

B.6 CONCLUSION

This review highlights a need to implement interventions to improve adherence alongside roll-out of LTBI treatment. We highlight patient, treatment and provider factors as well as specific patient groups that could be targeted in implementing strategies to improve IPT uptake and completion. The differences between predictors in different settings highlight the need to contextualise any adherence

strategies to the local setting in order to maximise the impact of these interventions.

The majority of these studies were conducted within research contexts and this could impact on generalizability of findings. A qualitative study conducted in South Africa, a setting where IPT is recommended but implementation is variable reported a lack of provider knowledge and experience with IPT as a primary barrier to delivery and found that none of the patients interviewed had ever heard of IPT (18). This highlights the differences between research and non-research health care contexts and the importance of appreciating these differences in exploring barriers to IPT use and adherence.

Moreover, we also highlight a paucity of much needed data on predictors of non-adherence to LTBI treatment in ART-naive HIV co-infected persons attending HIV clinics (not within a trial context) in high-burden settings. The burden of TB is greatest in developing countries with generalised HIV epidemics, with a high rate of HIV-associated TB. This reservoir of infections further fuels the TB epidemic resulting in greater morbidity and mortality. Eliminating TB will therefore necessarily involve identifying and effectively treating HIV-infected persons infected with *Mycobacterium tuberculosis* and further research is required to evaluate predictors of non-completion of treatment and interventions to improve adherence.

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PART C: JOURNAL MANUSCRIPT

Completion of isoniazid preventive therapy and factors associated with non-completion in an antiretroviral therapy-naive HIV-infected cohort in Cape Town

Running head: IPT completion rate and predictors of non-completion

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Formatted for the International Journal of Tuberculosis and Lung Disease[1]

Word count (excluding footnote, tables, and figures): 2308

Key words IPT, predictors, loss to follow up

[1] For readability, figures and tables are inserted in the text rather than appended at the end of the article. Text, tables and figure numbering are formatted to align with dissertation.

C.1 ABSTRACT

C.1.1 Introduction

TB incidence in South Africa remains high, despite high rates of successful treatment suggesting ongoing transmission and a large reservoir of latently infected persons. Isoniazid preventive therapy (IPT) is recommended as preventive therapy in HIV-infected persons. However, implementation has been slow, impeded by barriers and challenges including the fear of non-adherence.

C.1.2 Objective and Methods

The aim was to measure IPT completion rates within a research setting and to evaluate predictors of IPT non-completion. One hundred and sixty four antiretroviral therapy (ART)-naïve HIV-infected patients with tuberculin skin test ≥5mm were recruited from Khayelitsha day hospital and followed up monthly. A questionnaire was used to collect demographic information.

C.1.3 Results

The overall completion rate was 69%. In multivariable analysis, self-reported alcohol drinkers (OR 4.05; 95% C.I. 1.89-9.06) had a four-fold higher risk of non-completion, with a strong association between alcohol drinkers and smoking (χ^2 27.08; $p<0.001$). There was a 29% decrease in risk of non-completion for every year after HIV diagnosis (OR 0.81; 95% C.I. 0.68-0.98).

C.1.4 Conclusion

We identify patients with a recent HIV diagnosis and self-reported drinkers and smokers as being at higher risk of defaulting IPT, suggesting these groups should be identified and targeted for adherence interventions when implementing IPT on a wider scale.

C.2 INTRODUCTION

Tuberculosis (TB) is an ever-expanding threat to global public health, despite the availability of efficacious treatment, with a third of people estimated to be latently infected worldwide, 33.2 million with HIV infection (1). HIV-1 (HIV) is the strongest known risk factor for TB and where these pandemics intersect, they are the most common cause of death among young adults in many countries. Sub-Saharan Africa bears the brunt of this burden with the World Health Organization (WHO) Africa region having the highest estimated incidence rate (2). This burden is exceptionally high in countries with generalised HIV epidemics with HIV driving the TB epidemic through an increased risk of infection and reactivation disease.

In response to these dual epidemics, the WHO's STOP TB strategy to dramatically reduce the global burden of TB by 2015, recommends collaborative HIV/TB activities including the three "I"s for HIV/TB: Intensified Case-Finding (ICF), Isoniazid Preventive Therapy (IPT), and Infection Control in addition to antiretroviral therapy (ART). IPT has been shown to significantly reduce the risk of incident TB (3) predominantly in those with positive tuberculin skin test. This benefit is dependent on good adherence to the treatment regime. While ART roll-out has been expanding, implementation of ICF and IPT have been slow, impeded by barriers and challenges. This lack of implementation is in part due to fear of non-adherence.

South Africa is a high TB burden setting with an estimated incidence rate in 2010 of 981/100,000, and with 60% of TB cases occurring in HIV-infected persons (4). This makes IPT crucial to TB control in this high-burden setting. IPT is slowly being implemented in the public health sector. WHO estimates of the number of people on IPT in South Africa show an increase from 7359 in 2008 to 23,589 persons in 2009 (5). However, this represents a small fraction of people eligible

to receive IPT. Furthermore, this does not elaborate on the proportion of persons initiating IPT that complete the course. This highlights a need for data on completion rates and research evaluating barriers to implementation in the public health sector as well as identifying possible predictors of non-completion of IPT amongst eligible persons.

We present the findings of a study examining completion rates of IPT within a research context and evaluated patient-related predictors of non-completion as a means of identifying those at high risk of non-adherence and targeting retention strategies at these persons.

C.3 METHODS

C.3.1 Study design, setting and population

Participants in this study were identified from a larger cross-sectional TB diagnostics study to develop biomarkers that distinguish active from latent TB and other infections. The study was conducted at site B Day Hospital, a primary care health facility in the informal township of Khayelitsha. Located 30km from Cape Town, Khayelitsha has a population of over 400,000 and a TB case notification rate of over 1600/100 000, 70% of which occur in HIV co-infected persons (6).

C.3.2 Sampling and recruitment strategy

Study participants were recruited from the HIV wellness clinic, comprised of patients newly diagnosed with HIV and those not eligible for antiretroviral therapy (ART) at Khayelitsha day hospital, between February 2008 and March 2010. Written informed consent was obtained from all participants recruited as part of a larger TB diagnostics study. This study was approved by the University of Cape Town health research ethics committee (REC 012/2007). Participants were either newly diagnosed or ART-naive patients with CD4 ≥200cells/mm^3 attending

for minor ailments. Consecutive patients attending the clinic without any symptoms of TB were invited to participate and tested for evidence of latent TB. One hundred and sixty four persons were identified with a skin induration diameter of ≥5mm and no evidence of active TB disease. With a sample size of 164, assuming a background non-completion rate of 30% based on available data from Southern Africa, we could detect a two-fold increase in the proportion of non-completers due to risk factors such as drinking alcohol with a power of 97%.

C.3.3 Measures

Latent TB (LTBI) was diagnosed in these HIV-infected persons by the tuberculin skin test (TST) using 2 TU of tuberculin PPD RT23 injected intradermally into the volar aspect of the forearm. A transverse induration diameter ≥5mm was considered as evidence of LTBI. At recruitment, all participants were also screened for TB with sputum microscopy and culture to exclude active TB disease and a baseline CD4 count performed.

All participants were commenced on IPT for six months, as per South African national guidelines, with monthly visits to monitor adherence and side effects. Adherence was measured by self-reported adherence, and attendance of follow-up appointments. Self-reported adherence was measured by asking patients if they took the pills as prescribed everyday in the preceding month, and was ascertained at monthly visits by the same investigator. Patients who missed an appointment received daily telephonic reminder and failing that after a week, a home visit by the trained clinical research assistant to encourage clinic attendance. Non-completion of IPT (outcome variable) was defined as a failure to complete a six-month IPT course within a nine-month period.

In addition, a questionnaire, applied by trained study personnel, was used to collect baseline demographic information including age, employment status, alcohol consumption, smoking status, past TB history, recent TB contact, type of accommodation lived in, and date of HIV diagnosis. This questionnaire was designed for the larger TB diagnostics study and data for this study extracted from the database.

C.3.4 Hypothesis

We hypothesized, based on existing literature, that being male, employed, consuming alcohol and smoking would be predictors of non-completion of IPT.

C.3.5 Statistical analysis

Patient baseline characteristics were stratified by outcome and summarised using simple proportions. Predictors of non-completion were analysed by logistic regression and nested models compared using the likelihood ratio test. The Chi squared test was used to compare proportions. The Akaike's Information Criterion (AIC) was used to compare non-nested models with a lower AIC indicating a better model. The interactions between confounding and exposure variables were then examined. The fit of the model was assessed using Pearson's goodness-of-fit test with a p-value >0.05 indicating a good fit. Model diagnostics were performed on final models by checking the form of the linear predictors.

Significance testing was done using two-sided p-values and 95% confidence intervals. All data were analysed using STATA 10.0 (StataCorp, College Station, TX, USA).

C.4 RESULTS

C.4.1 Baseline characteristics and IPT completion rate

Of the one hundred and sixty four participants identified with LTBI, all accepted, and were initiated on, IPT. Overall, one hundred and thirteen persons (69%) completed the six-month IPT course. For the first thirty-three patients initiated on IPT, adherence was encouraged by explaining that the benefits of IPT could only be achieved if the course was completed. This message was delivered by the same study doctor in a standardized manner at monthly follow-up visits. In this group, the IPT completion rate was 42% with eighteen out of thirty-three (58%) not completing the full IPT course. The subsequent use of a research assistant resulted in an improvement in completion rates; of the one hundred and thirty one patients recruited thereafter, 75% completed IPT.

Baseline characteristics were compared, stratified by completion or non-completion of a six-month course of IPT, and summarized in Table C.1. Amongst those that did not complete the IPT course, a median of two months IPT (IQR 0-3 months) was completed. Compared to those who completed IPT, patients who did not complete the course had a higher proportion of smokers (21.6% versus 9.7%; p=0.04) and alcohol drinkers (37.2% versus 14.2%; p=0.001). They were more likely to have been recently diagnosed with HIV (median time since HIV diagnosis 54 days (IQR 6-910) versus 421 days (IQR 43-1479); p=0.002). There were also a higher proportion of men versus women (22.4% versus 15%; p=0.252) in the non-completers group. Similarly, a greater proportion of non-completers were recent migrants to Khayelitsha (11.9% versus 5.3%; p=0.155) although numbers were small. The distribution of all other measured baseline characteristics were similar between the two groups.

Characteristics		Completers N (%)	Non-completers N (%)	Total N (%)
Gender	Female	96 (85.0)	38 (77.6)	134 (82.7)
	Male	17 (15.0)	11 (22.4)	28 (17.3)
Marital status	Single	80 (70.8)	41 (80.4)	121 (73.8)
	Married	33 (29.2)	10 (19.6)	43 (26.2)
Smoker	No	102 (90.3)	40 (78.4)	142 (86.6)
	Yes	11 (9.7)	11 (21.6)	22 (13.4)
Alcohol	No	97 (85.8)	32 (62.8)	129 (78.7)
	Yes	16 (14.2)	19 (37.2)	35 (21.3)
TB contact	No	78 (69.0)	41 (80.4)	119 (72.6)
	Yes	30 (26.6)	10 (19.6)	40 (24.4)
	Don't know	5 (4.4)	0 (0.0)	5 (3.0)
Previous TB	No	98 (86.7)	45 (88.2)	143 (87.2)
	Yes	15 (13.3)	6 (11.8)	21 (12.8)
BCG Scar	No	51 (45.1)	28 (54.9)	79 (48.2)
	Yes	62 (54.9)	23 (45.1)	85 (51.8)
Self-reported BCG	No	19 (16.8)	11 (21.6)	30 (18.3)
	Yes	80 (70.8)	33 (64.7)	113 (68.9)
	Don't know	14 (12.4)	7 (13.7)	21 (12.8)
Employed	No	71 (63.4)	34 (66.7)	105 (64.4)
	Yes	41 (36.6)	17 (33.3)	58 (35.6)
Accommodation	Shack	66 (58.4)	21 (50.0)	87 (56.1) 68
	House	47 (41.6)	21 (50.0)	(43.9)
Time in Khayelitsha	<1 year	6 (5.3)	5 (11.9)	11 (7.1)
	>=1 year	107 (94.7)	37 (88.1)	144 (92.9)
Characteristics		Median (IQR)	Median (IQR)	Median (IQR)
Age (years)		32.7 (27.4-37.8)	29.8 (24.5-35.9)	31.4 (26.7-36.9)
BMI		27.2 (22.8-31.6)	24.2 (21.3-30.5)	26.6 (22.3-31.5)
Education	Highest school	11 (9-12)	11 (10-12)	11 (9-12)

grade achieved			
Persons/bedroom	2.33 (1.67-3)	2 (2-3)	2 (1.67-3)
CD4 count (cells/mm3)	360 (269-508)	363 (261-526)	361 (266-515)
Years since HIV diagnosis	1.15 (0.12-4.05)	0.15 (0.02-2.49)	0.91 (0.05-3.61)
Mantoux diameter (mm)	20 (15-24)	20 (12-24)	20 (15-24)
Total number of months IPT completed	6 (6-6)	2 (0-3)	6 (3.5-6)

Table C.1 Baseline characteristics stratified by completion and non-completion of IPT

C.4.2 Predictors of non-completion of IPT

On bivariable analysis, being male, smoking, alcohol, and a recent HIV diagnosis were associated with an increased risk of IPT non-completion (Table C.2). There was a significant association between being male and drinking alcohol (χ^2 9.76; p=0.002) and smoking (χ^2 31.12; p<0.001). There was also a strong association between smoking and drinking (χ^2 27.08; p<0.001).

	Univariable analysis		Multivariable analysis	
	OR (95% C.I.)	P-value	OR (95% C.I.)	P-value
Alcohol	4.66 (2.03-10.69)	<0.001	**4.05 (1.89-9.06)**	**0.001**
Years since HIV diagnosis	0.82 (0.67-0.99)	0.038	**0.81 (0.68-0.98)**	**0.030**
Smoker	3.59 (1.41-9.18)	0.008		
Gender	2.34 (0.97-5.64)	0.058		
TB contact	0.54 (0.23-1.26)	0.152		
Previous TB	0.94 (0.32-2.79)	0.910		
BCG Scar	0.92 (0.44-1.94)	0.832		
Self-reported BCG	1.13 (0.58-2.22)	0.720		
Employed	0.85 (0.39-1.84)	0.680		
Accommodation	1.46 (0.69-3.06)	0.323		
Time in Khayelitsha	0.39 (0.11-1.36)	0.138		
Marital status	0.93 (0.40-2.14)	0.862		
Age (years)	0.99 (0.94-1.04)	0.597		
BMI	0.96 (0.90-1.02)	0.186		
Education	1.07 (0.90-1.29)	0.440		
Persons/bedroom	0.91 (0.68-1.20)	0.497		
CD4 count (cells/mm3)	1.00 (1.00-1.00)	0.617		
Mantoux diameter (mm)	1.03 (0.99-1.07)	0.132		

Table C.2 Bivariable and multivariable analysis using non-completion of IPT as the outcome variable

On multivariable analysis the period of time since HIV diagnosis and drinking alcohol remained significant with a four fold increase in odds of non-completion in drinkers compared to non-drinkers and a 19% decrease in odds of non-completion with every year after HIV diagnosis.

Due to the strong association between smoking and drinking we chose to include the alcohol variable, as its inclusion in the model resulted in a marginally better fit of the model to the data using the Pearson goodness of fit test compared to a model that included smoking. However, given the association between these two variables, both should be considered in the interpretation of these results. There was no significant effect modification between sex and drinking or smoking although it should be noted that a greater proportion of male versus female participants reported being smokers (46% versus 7%) and drinkers (43% versus 16%).

We further explored the relationship between the period of time since HIV diagnosis and non-completion of IPT by examining the proportion of non-completers stratified into four time periods since HIV diagnosis (<6 months, 6-12 months, 1-5 years, and >5 years since HIV diagnosis). Figure C.1 shows that non-completers were most likely to default IPT if initiated within six months of HIV diagnosis compared to persons initiated after >six months post-HIV diagnosis (Pearson Chi^2 7.60; p=0.006).

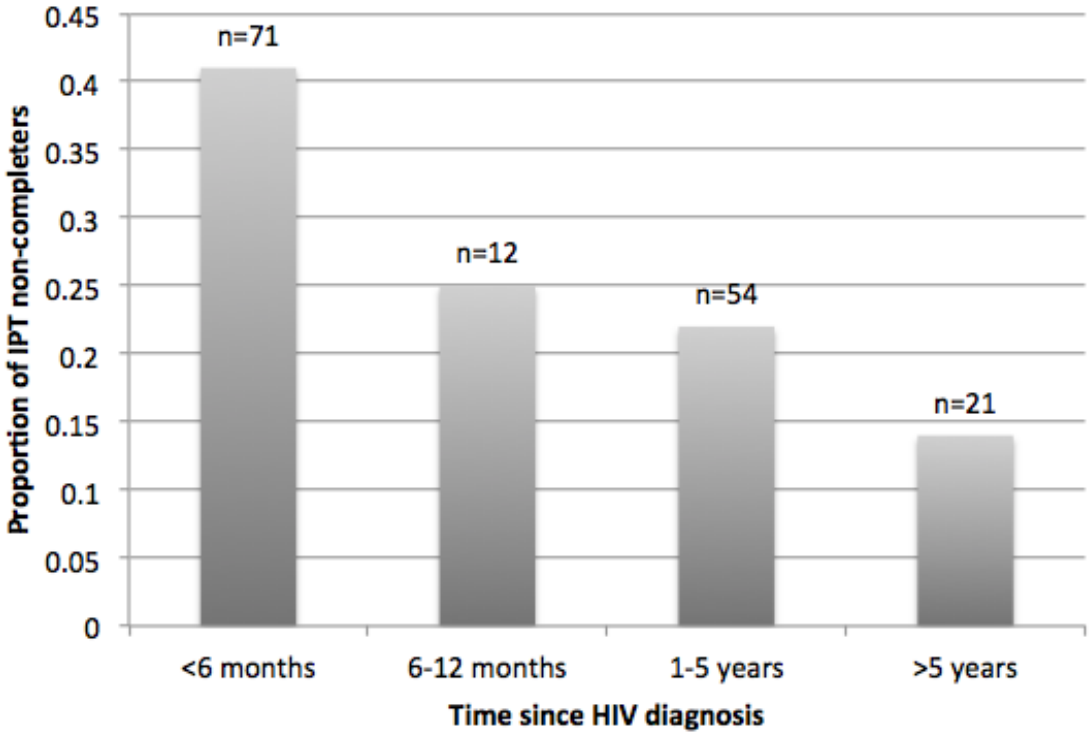

Figure C.1 Histogram showing the proportion of IPT non-completers stratified by time period since HIV diagnosis. N represents the total sample size of participants in each time period

C.5 DISCUSSION

We report a 69% IPT completion rate in this ART-naïve HIV-infected population in Cape Town. Studies conducted in similar high burden settings have reported IPT completion rates from 47% in HIV-infected adults attending clinics in Limpopo, rural South Africa to 78% in participants involved in a randomized controlled trial in Botswana (7,8). In the latter study, the regulated trial conditions are likely to have contributed to higher IPT completion rates.

We also explored quantitative patient predictors of non-completion of IPT and found a recent HIV diagnosis and self-reported alcohol drinking and smoking to significantly increase the risk of non-completion. Studies examining risk factors

for non-completion have reported male patients as being at risk of non-completion (8,9) with men being twice as likely to be non-completers and women being three times as likely as men to complete the course, respectively. Studies have also reported perceived INH side effects as being a barrier to adherence (9,10). One study examining qualitative barriers to IPT completion reported that patients who reported difficulty taking meds or taking pills in the absence of symptoms as well as those in whom there was conflict between Western and traditional medicine, were more likely to be non-adherent (7). This suggests that a lack of education about the importance of IPT and explanation about prevention of TB might contribute to non-adherence. In our study, prior to the use of telephonic reminders, our completion rate was 42%, increasing to 75% with the use of reminders. These reminders could therefore serve as an opportunity to educate patients on the importance of completing IPT, despite the absence of symptoms.

We found patients with a more recent HIV diagnosis had a higher risk of non-completion. This could relate to non-acceptance of HIV status and a resulting reluctance to attend follow-up appointments. It could also relate to non-disclosure of HIV status to friends and family and wanting to keep their HIV status confidential, resulting in an unwillingness to attend the health facility. A study conducted in Thailand reported acceptance of personal HIV status as a predictor of adherence (9). Patients established in HIV clinics are more likely to have accepted their HIV status and developed a habit of attending the health facility for routine appointments and for minor ailments, which could facilitate IPT adherence. In addition, this could be related to their perceived risk and lack of education on HIV opportunistic infections such as TB.

We also reported alcohol drinking and smoking as risk factors for non-completion of IPT. These factors have been reported in other studies conducted in low

burden settings (11,12). In this high-burden setting, this association possibly relates to marginalization representing a social risk factor. This was demonstrated by a study in Spain that reported an association between non-adherence and patients with unfavourable social circumstances such as alcohol and drug abuse and recent immigrants (13).

Based on existing literature, we hypothesized that male and unemployed patients would also be at higher risk of non-completion. Unemployment rates are high in Khayelitsha and this could explain why employment was not associated with adherence in our study. Sixty-five percent of drinkers and 59% of smokers were male suggesting that the expected association between male sex and non-completion of IPT could relate to a greater proportion of alcohol drinkers and smokers within this group and could explain why being male was not a significant risk factor in this study.

C.5.1 Limitations

Although IPT follow-up was conducted within the health facility, it was conducted within a research context and this could limit the generalizability of our findings in under-resourced settings. Furthermore, completion of IPT was based on self-reported adherence. This could result in an over-estimation of completion rates. Several studies have examined barriers to IPT roll-out in high burden settings. Healthcare worker related barriers were identified by Getahun *et al*, with lack of experience and knowledge of current guidelines cited as an important barrier (5). Other barriers to implementation identified include concerns about INH resistance, and fear of side effects with hepatitis and peripheral neuropathy most common (9,14). Our study did not examine health system factors, such as a good patient-health provider relationship, which could play a significant role in impeding completion of IPT.

C.6 CONCLUSION AND RECOMMENDATIONS

The WHO has identified IPT as one of the main interventions to reduce morbidity and mortality from TB in people living with HIV. A better understanding of predictors on non-adherence to IPT is therefore crucial to optimize adherence in high HIV/TB burden settings. Implementation of IPT has been slow in South Africa. The findings of this study are therefore important as the completion rate reported demonstrates the potential feasibility of providing IPT in this setting. The identification of a recent HIV diagnosis as a significant predictor of non-completion suggests that in this setting, IPT should not be initiated in HIV-infected persons too soon after diagnosis. Our results also suggest that when initiating IPT, the smoking and alcohol history of patients should be ascertained, with targeted adherence interventions implemented aimed at those who smoke or drink and those with a more recent HIV diagnosis. We found IPT completion improved with the use of a research worker suggesting that in the implantation of IPT in this setting, adherence interventions using lay community workers to give reminders of appointments and continuous education could improve IPT completion.

Key areas for future research include into health systems barriers and the impact of adherence interventions, such as community supporters as part of an integrated chronic disease management package, in improving rates of IPT completion.

C.7 ACKNOWLEDGEMENTS

The primary study was funded by a grant from the European Union (Sante/2006/105-061) and Bill and Melinda Gates Foundation GC 6-74 37772. The funders did not play a role in the design of the study or preparation of the manuscript. Relebohile Tsekela, Bekekile Kwaza and Lulama Manjezi contributed to data collection. Nonzwakazi Bangani and Katalin A Wilkinson

were responsible for acquisition of laboratory material and processing of specimens.

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PART D: APPENDICES

APPENDIX 1- DETAILED STATISTICAL ANALYSIS

Patient baseline characteristics were tabulated stratified by the outcome (whether or not they were lost to follow up) and summarised using simple proportions. Overall completion rate of IPT was presented as a proportion. Predictors of loss-to-follow-up were analysed by logistic regression. Nested models were compared using the likelihood ratio test, which assesses the extent to which the additional terms improve the fit of the model by measuring the difference in the deviances of the 2 nested models. The Akaike's Information Criterion (AIC) was used to compare non-nested models with a lower AIC indicating a better model. The interactions between confounding and exposure variables were then examined. The fit of the model was assessed using Pearson's goodness-of-fit test with a p-value >0.05 indicating a good fit of the model to the data. Model diagnostics were performed on final models by checking the form of the linear predictors. Scatterplot diagrams of the observation number versus the Pearson and deviance residuals, were used to identify a systematic pattern which would indicate an incorrect model. Similarly, a scatterplot diagram of residuals versus linear predictors was used to demonstrate good fit. In a good model, residuals of cases should be closer to the horizontal line as linear predictors get larger and vice versa for smaller linear predictors. As the dependent variable (loss to follow up) is a binary variable, the logit transformation was deemed appropriate and the adequacy of the link function did not require checking. Outlying observations were identified as standardised residuals greater than +2 or smaller than -2 and scatterplot diagrams of standardised residuals versus the observation numbers were used to visually identify outlying observations. Influential and large leverage observations were identified respectively using the Hosmer and Lemeshow test of influence (dx2) and hat matrix (hat), a measure of the leverage of covariate

pattern i.e. how far the covariate pattern lies from the average covariate pattern and thus how much of an effect it has on the estimated model. A value greater than $^{2p}/_{n}$, where p=number of variables in the model and n=number of covariate patterns, was indicative of large leverage. Scatterplot diagrams of dx2 and hat versus the observation numbers were used to visually identify influential and high leverage observations. The variance inflation factor (uncentered) was used to detect collinearity with a VIF>>>1 suggesting multi-collinearity could be seriously influencing the model.

Significance testing was done using a combination of two-sided p-values and 95% confidence intervals. All data were analysed using STATA 10.0 (StataCorp, College Station, TX, USA).

APPENDIX 2- MODEL-BUILDING TABLE

Model	Log Likelihood	LR χ^2	Likelihood ratio test χ^2	p-value	Vs.	AIC
A LTFU	-83.71	0.00	.	.	.	169.416
B LTFU + Age	-83.57	0.28	0.28	0.595	A	171.101
C LTFU + Sex	-81.97	3.47	3.47	0.063	A	167.946
D LTFU + BMI	-82.79	1.83	1.83	0.176	A	169.588
E LTFU + Smoker	-80.23	6.96	6.96	0.008	A	164.459
F LTFU + Previous TB	-83.70	0.01	0.01	0.910	A	171.403
G LTFU + BCG Scar	-83.69	0.05	0.05	0.832	A	171.371
H LTFU + TB contact	-82.55	2.31	2.31	0.129	A	169.107
I LTFU + Education	-83.39	0.63	0.63	0.427	A	170.784
J LTFU + Employment	-83.62	0.17	0.17	0.679	A	171.245
K LTFU +Informal housing	-83.22	0.98	0.98	0.323	A	170.439
L LTFU+ Overcrowding	-83.47	0.49	0.49	0.486	A	170.930
M LTFU+ Time in Khayelitsha	-82.66	2.10	2.10	0.148	A	169.318
N LTFU + CD4 count	-83.58	0.26	0.26	0.613	A	171.161
O LTFU+ Days with HIV	-81.18	5.05	5.05	0.025	A	166.365
P LTFU+ Years with HIV	-81.19	5.04	5.04	0.025	A	166.371
Q LTFU+ Self reported BCG	-83.64	0.13	0.13	0.720	A	171.288
R LTFU+ Alcohol drinker	**-77.11**	**13.19**	**13.19**	**<0.001**	**A**	**158.228**
S LTFU+ Mantoux diameter	-82.59	2.24	2.24	0.134	A	169.173
T LTFU+ Marital status DROP HIV YEARS	-83.69	0.03	0.03	0.862	A	171.386
R LTFU+ Alcohol drinker	-77.11	13.19	13.19	<0.001	A	158.228
U LTFU+ Alcohol drinker+ Smoker	-76.30	14.81	1.62	0.203	R	158.605
V LTFU+ Alcohol drinker+ Days with HIV	**-74.63**	**18.16**	**4.97**	**0.026**	**R**	**155.255**
W LTFU+ Alcohol drinker+ Sex	-76.60	14.22	1.03	0.311	R	159.201
X LTFU+ Alcohol drinker+ TB contact	-76.31	14.79	1.60	0.206	R	158.627
Y LTFU+ Alcohol drinker+ Mantoux diameter	-75.58	16.25	3.06	0.080	R	157.165
Z LTFU+ Alcohol drinker+ Time in Khayelitsha	-75.75	15.91	2.72	0.099	R	157.505
AA LTFU+ Alcohol drinker+ BMI	-76.79	13.83	0.64	0.423	R	159.587
AB LTFU+ Alcohol drinker+ Informal housing	-76.38	14.65	1.46	0.227	R	158.767
AC LTFU+ Alcohol drinker+ Education	-76.74	13.93	0.74	0.389	R	159.485
AD LTFU+ Alcohol drinker+ Overcrowding	-76.93	13.55	0.37	0.545	R	159.862
AE LTFU+ Alcohol drinker+ Age	-76.99	13.45	0.27	0.606	R	159.962
AF LTFU+ Alcohol drinker+ CD4 count	-77.11	13.20	0.01	0.905	R	160.214

AG LTFU+ Alcohol drinker+ Employment	-76.97	13.48	0.30	0.587	R	159.932
AH LTFU+ Alcohol drinker+ Self reported BCG	-77.08	13.26	0.07	0.785	R	160.154
AI LTFU+ Alcohol drinker+ BCG scar	-77.10	13.21	0.02	0.879	R	160.205
AJ LTFU+ Alcohol drinker+ Marital status	-77.11	13.19	0.00	0.976	R	160.227
AK LTFU+ Alcohol drinker+ Previous TB	-77.11	13.19	0.00	0.984	R	160.228
V LTFU+ Alcohol drinker+ Days with HIV	**-74.63**	**18.16**	**4.97**	**0.026**	**R**	**155.255**
AL LTFU+ Alcohol drinker+ Days with HIV+ Mantoux diameter	-73.34	20.73	2.57	0.109	V	154.685
AM LTFU+ Alcohol drinker+ Days with HIV+ Time in Khayelitsha	-73.69	20.03	1.87	0.171	V	155.384
AN LTFU+ Alcohol drinker+ Days with HIV+ Smoker	-74.11	19.19	1.03	0.310	V	156.223
AO LTFU+ Alcohol drinker+ Days with HIV+ TB contact	-74.11	19.19	1.03	0.311	V	156.227
AP LTFU+ Alcohol drinker+ Days with HIV+ Informal housing	-74.18	19.05	0.89	0.347	V	156.369
AQ LTFU+ Alcohol drinker+ Days with HIV+ Sex	-74.51	18.39	0.23	0.629	V	157.021
AR LTFU+ Alcohol drinker+ Days with HIV+ Education	-74.18	19.06	0.90	0.342	V	156.352
AS LTFU+ Alcohol drinker+ Days with HIV+ BMI	-74.504	18.41	0.25	0.619	V	157.008
AT LTFU+ Alcohol drinker+ Days with HIV+ Overcrowding	-74.49	18.44	0.28	0.595	V	156.972
AU LTFU+ Alcohol drinker+ Days with HIV+ Employment	-74.39	18.64	0.48	0.489	V	156.777
AV LTFU+ Alcohol drinker+ Days with HIV+ Age	-74.37	18.67	0.51	0.474	V	156.743
AW LTFU+ Alcohol drinker+ Days with HIV+ Self reported BCG	-74.61	18.19	0.03	0.857	V	157.223
AX LTFU+ Alcohol drinker+ Days with HIV+ BCG scar	-74.60	18.21	0.05	0.825	V	157.206
AY LTFU+ Alcohol drinker+ Days with HIV+ CD4 count	-74.62	18.18	0.01	0.906	V	157.241
AZ LTFU+ Alcohol drinker+ Days with HIV+ Marital status	-74.63	18.16	0.00	0.978	V	157.254

BA drinker+ Previous TB	LTFU+ Alcohol Days with HIV+	-74.63	18.16	0.00	0.952	V	157.251

```
. /*FINAL MODEL*/
. logistic ltfu drinker hivdays

Logistic regression                                     Number of obs   =        158
                                                        LR chi2(2)      =      17.54
                                                        Prob > chi2     =     0.0002
Log likelihood = -87.405166                             Pseudo R2       =     0.0912

------------------------------------------------------------------------------
        ltfu | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     drinker |    4.04804   1.663666     3.40   0.001     1.808912     9.05883
     hivdays |   .9994392   .0002582    -2.17   0.030     .9989334    .9999453
------------------------------------------------------------------------------

. logistic ltfu drinker hivyears

Logistic regression                                     Number of obs   =        158
                                                        LR chi2(2)      =      17.54
                                                        Prob > chi2     =     0.0002
Log likelihood = -87.405588                             Pseudo R2       =     0.0912

------------------------------------------------------------------------------
        ltfu | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     drinker |    4.04913   1.664135     3.40   0.001     1.809381    9.061361
    hivyears |   .8148654   .0768247    -2.17   0.030     .6773848    .9802487
------------------------------------------------------------------------------

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APPENDIX 3- DATA COLLECTION TOOL FOR THE TB DIAGNOSTICS, CROSS-SECTIONAL PARENT STUDY

QID	Question	Code of Response	Coded Response
Demographics, Socio-Economic Status and Social Habits			
1	Sex?	1= Male 2= Female	
2	Date of birth?	***(dd/mmm/yyyy)*** 09/SEPT/1999=Unknown	
3	Relationship status:	1=single 2= not married, but in a long-term relationship 3=married 4=separated 5=divorced	
4	Who do you live with?	1= Partner 2= Family 3= Friend(s) 4= Alone 5= Other (specify)….	
5a	What is your current occupation?	1= Student 2= Homemaker 3= Unemployed or seeking work 4= Employed, full-time 5= Employed, part-time 6= Employed, casual 7= Self-employed 8= Government grant/pension	
5b	If employed, what kind of work do you do?		
5c	Highest grade achieved	……..	
6	What is your smoking habit?	1= Current smoker 2= Ex-smoker 3= Never smoked if ex-smoker, when quit Quantity / day…… No of years smoked	

7	Do you use recreational drugs?	1= No 2= Dagga 3= Tic 4= Other (Specify)	

8a	Do you drink beer, wine or other alcoholic beverages, either now or in the past? (Alcohol History)	1=Current drinker 2=Non-drinker 3=Ex drinker ≥ 1 year 4=Ex Drinker < 1 year ***If Non-drinker, Go to Q11***	
8b	Have you ever felt the need to **Cut** down on your drinking?	1=Yes 2=No	
8c	Have people **Annoyed** you by criticizing your drinking?	1=Yes 2=No	
8d	Have you ever felt bad or **Guilty** about your drinking?	1=Yes 2=No	
8e	Have you ever had a drink first thing in the morning (**Eye opener**) to steady your nerves or get rid of a hangover?	1=Yes 2=No	
9	If subject answered yes to any TWO of Q8 a,b,c, or d, subject may be considered to have an alcohol problem.	1=Yes 2=No	
10	During the last month, average number of units of alcohol per day during the week? (Monday – Thursday)	00-50 99=Don't know	
11	How long have you lived in Khayelitsha?	1= 0-3mo 2=4-6mo 3=7-12mo 4=1-5 years 5= More than 5 years 9=Don't Know	

12	What type of accomodation do you live in?	1= House/permanent building 2= Shack on serviced site (e.g water on site) 3= Shack on unserviced site or other open land (ie nothing available) 4= Other (specify)……. 9= Don't Know	
13	How many rooms are there in the house?	01-20	
14	How many rooms are used for sleeping?	01-20	
15	How many sleep in the house?	01-20	
Pregnancy			
16	Are you currently pregnant?	1=Yes 2=No 7=N/A (Male) 9= Don't know Date of last menstrual period: specify dd/mmm/yyyy	
HIV and TB History			
17	Did you receive BCG as a baby?	1=Yes 2=No 9= Don't know	
18	Previous TB?	1=Yes 2=No 9= Don't know ***If NO, skip to Q24***	
19	How many times have you had TB?	00-10 99=Don't know	
20	When did you last have TB?	1= Past 6 months 2 = Past 6-12 months 3 = Past 12-24 months 4 = >24 months ago If known please specifiy: mmm/yyyy	

21	Have you ever received treatment for TB?	1=Yes 2=No 9= Don't know	
22	The last time you had TB; did you complete your TB treatment?	1=Yes 2=No 9= Don't know	
23	The last time you had TB, how many months of TB treatment did you have?	00-98 99=Don't know	
24	Has anyone close to you had TB in the past 1 year	1=Yes 2= No if yes Nature of contact …… Date of contact ……..	
25	Have you ever received isoniazid prophylaxis?	1=Yes 2=No 9=Don't know If known, specify date: mmm/yyyy	
26	HIV status?	1= Negative 2= Positive 9= Unknown	
27	HIV results seen?	1=Yes 2=No	
28	If HIV positive, date of HIV diagnosis?	MMM / YYYY	
29	Are you taking ARVs?	1=Yes 2=No If yes Which ARVs? for how long? ……..	
Past Medical History			
30	Do you have Hypertension?	1=Yes 2=No 9= Don't know	

31	Do you have Diabetes?	1=Yes 2=No 9= Don't know
32	Do you have epilepsy?	1=Yes 2=No 9= Don't know
	Drug History	
33	Are you currently taking any medications from the doctor, pharmacist or traditional healers?	1=None 2=Steroids 3=Anti-TB meds (including recent fluoroquinolone /amikacin) 4=Cotrimoxazole 99=Don't know 11=Other (specifiy)

34.Temp	35. PR	36.BP	37. Weight	38. Height

Signature of person completing form and Date____ ____/ ____ /_____
DD MMM YYYY

APPENDIX 4- DATA COLLECTION TOOL FOR IPT FOLLOW UP

INH FOLLOW-UP VISIT NO:

OBS

Temp	PR	Weight	BP

Date last INH received	
Months completed on INH	

CURRENT SYMPTOMS

Results (or pending results) from Last Visit:

SYMPTOMS	Y/N	Duration
Cough		
Blood in sputum		
SOB		
Pleuritic pain		
Night sweats		
INH SIDE-EFFECTS		
P. neuropathy		
Jaundice		

ON EXAMINATION

Chest:

Abdo:

Other:

SYMPTOMS BETWEEN VISITS

Any symptoms since last visit? (NOT current symptoms): Y /N

RESPIRATORY SYMPTOMS	Y/N	Duration	Resolved? Y/N
Cough			
Blood in sputum			
Pleuritic pain			
SOB			
Did you get antibiotics?			
Were you screened for TB D/C/S?			
Did you need hospital admission?			

OTHER SYMPTOMS	Duration	Resolved Y/N	Meds given Y/N
Were you screened for TB D/C/S?			
Did you need hospital admission?			

If yes, complete table below:

List investigations required, if any ….....

TB D/C/S required? Y / N

CXR required? Y / N

INH month …… / 6 months; prescribed / withheld (circle as appropriate). Pyridoxine 25mg given ✓/ ✗

Other medication prescribed…… ……

Next appointment date: __ __/ __ __ __/__ __ __ __ If INH course complete, CD4 @discharge …....

APPENDIX 5- CONSENT FORM FOR TB DIAGNOSTICS PRIMARY STUDY

UNIVERSITY OF CAPE TOWN

Room S3.03.05
Institute of Infectious Diseases and Molecular Medicine
Faculty of Health Sciences
Observatory 7925
South Africa
Tel: +27 (0)21 406 6084

Robert J Wilkinson MA PhD BM BCh DTM&H FRCP
Wellcome Trust Senior Fellow in Clinical Tropical Medicine
Honorary Associate Professor (IIDMM)
rwilkin@curie.uct.ac.za
r.j.wilkinson@imperial.ac.uk

Increasing prevention and treatment of TB through development of a rapid, sensitive, and affordable biological marker (genomic or proteomic) for diagnosis of TB in HIV positive and negative populations.

INFORMED CONSENT and INFORMATION FORM

My name is _____

I wish to invite you to participate in a study that is trying to produce new blood tests to diagnose tuberculosis (TB). This study is being run by the Institute of Infectious Diseases and Molecular Medicine at the University of Cape Town. Dr Robert J Wilkinson is the Principal Investigator.

Firstly, I wish to explain some of these terms to you:

TB is a disease that is seen very commonly in Cape Town and, indeed, in most of South Africa. It is caused by bacteria that people breathe in and the infection results in cough, fevers and weight loss. The disease can be more severe in patients with HIV infection.

Early diagnosis and treatment of TB can save lives. The current methods of diagnosing TB can take up to two months and are not always successful in diagnosing TB in patients with HIV, although these patients are the ones who need most urgent treatment.

A skin test is sometimes used although its reliability is not very good especially in HIV infected people. We are therefore looking at new blood tests which may help us to diagnose TB very quickly (within 2-3 days). We want to compare these tests with the skin test and the other usual methods to diagnose TB. We hope these tests will help us identify patients in urgent need of TB medicines in the future without having to wait several weeks for their culture reports.

We request your participation in this study because you are being investigated for TB as a possible cause for your illness. You have also undergone voluntary counselling and testing for HIV infection (VCT) as part of this process. We need to know whether you are HIV infected because this can alter the way the test performs. The decision regarding your TB treatment will be made by your doctor and being a part of this study will not affect your doctor's decision whether to start TB treatment. One of the tests that we would like to do on your sputum (spit) is a new test which, if you have TB, we hope will tell us whether this TB is resistant to one of the drugs for treatment. If this test shows that this drug might not work against your TB, we will use another test to check that this is correct. If the second test shows the same result, then we will tell your doctor so that

she/he can change your treatment so that the best drugs for your form of TB can be used.

It is entirely up to you to decide whether or not to take part in this study. If you do decide to take part, you will be asked to sign this consent form. We will then request you to provide us with an extra blood sample (40ml) that can be taken at the same time as your normal blood tests.

We will also ask that you have a TB skin test. This skin test is a standard test and has been performed on many millions of people. It is done by injecting a small amount of fluid under the skin. Over the next 2 days an area of redness and hardness at the site of the skin test may develop. This is called induration and we will measure it at 48 hours. If this is irritating we can give you a cream to stop it. We need to see you again in clinic in 2-3 days time to look at your skin test. We will pay 30 Rand travel expenses for this purpose. If you are HIV infected with no signs of active TB but the skin test or blood tests are positive you will be offered six months' treatment to reduce the risk you will develop TB in the future. Although blood testing very rarely causes problems, if anything goes wrong the University provides insurance to cover this possibility. This study will also be monitored by the Research Ethics Committee of the University of Cape Town. Their job is to ensure your safety and protect you during the study.

The decision to participate is entirely your own. IF YOU DECIDE NOT TO PARTICIPATE, YOUR TREATMENT WILL NOT BE DIASDVANTAGED IN ANY WAY. In addition, at any point during the trial you are free to withdraw without telling us why.

Since this is a research test, the results will not be made available to the participants. Throughout the trial your privacy will be maintained and nobody

other than the doctors and nurses looking after you will know that you are participating. Samples will be labelled with code numbers and hence the laboratory staff will not know your identity. When the results of the study become available, names of the participating patients will not be included.

Do you have any questions? During the study you may contact either the **Research Ethics Committee** (021 406 6492) or **Dr Marc Mendelson** (021 406 6793) if you have further questions. Please remember that Dr Mendelson will not be directly responsible for your medical care which will be conducted by your regular doctors and nurses.

Consent to participate in the study:

I have read the above / the above has been read to me and I have had the opportunity to discuss the

study with Dr _____ and ask any questions. I consent to take part in this study:
Signature _____

Name _____

Date _____

Name of person consenting _____

Signature _____

Date _____

Witness

Name _____

Signature _____

Date _____

Name of the Witness _____

Signature _____ Date _____

APPENDIX 6- PROTOCOL SUMMARY OF PRIMARY STUDY

Increasing prevention and treatment of TB through development of a rapid, sensitive, and affordable biological marker (genomic or proteomic) for diagnosis of TB in HIV positive and negative populations.

Summary

A simple, affordable diagnostic test for tuberculosis (TB) is urgently needed to improve detection of active TB particularly in HIV infected adults and children. Improved detection could reduce the global burden of transmission and address the key problem of delay in TB diagnosis leading to the increase in morbidity and mortality that characterizes the HIV/TB pandemic in the developing world. The major objectives of our programme are (1) to identify a biological marker(s) (either gene expression or protein) which distinguish patients with active TB from those other diseases prevalent in African populations with high HIV and TB prevalence. (2) To develop this marker(s) towards a rapid, simple and affordable diagnostic test for active TB, suitable for use in TB control in resource poor regions of the world most affected by the HIV pandemic.

As part of a multinational consortium of researchers we have recently been awarded a grant totaling € 4.5m to seek biomarkers of active tuberculosis. Work in Cape Town to achieve these objectives is envisaged as follows.

Aim 1: Establishment of patient cohorts

We will recruit adult cohorts of patients (50 per group) in Cape Town, an area of high HIV / TB co-infection. The cohorts will be defined as follows: (1) TB+ HIV- (2) TB+ HIV+ (3) LTBI (Latent TB) + HIV+ (4) LTBI+ HIV- (5) HIV+ with opportunistic infections other than TB (6) HIV- with acute and chronic infections other than TB.

Aim 2: Identification of Biomarkers

To identify (a) the pattern of gene expression using high density cDNA microarrays and (b) protein biomarker(s) using Surface Enhanced Laser Disorption Ionisisation Mass Spectrometry (SELDI) in blood collected from patient cohorts. Biomarker discovery will focus on the distinction of active TB from other disorders prevalent in both HIV infected and uninfected cohorts.

Aim 3: Biomarker characterization

To identify and characterize (a) the genes (identified in 2a above) and (b) proteins (identified in 2b above) that are specific to active TB using bioinformatic databases and protein sequencing.

Aim 4: Assay development

To develop a simple, robust RT-PCR based assay for gene biomarkers identified in 3a, and/or an ELISA based protein assay to quantify the candidate protein biomarkers identified in 3b.

Aim 5: Validation of Biomarkers

To validate the diagnostic performance of the defined biomarker assays in larger cohorts of (a) children in a separate study and (b) adults with TB, with and without concomitant HIV infection or other infections which mimic TB in South Africa

Aim 6: Production and Development of Diagnostic assays

Dissemination and reporting of knowledge and recruitment of industry partners for future development of affordable diagnostics based on our programme of biomarker discovery.

APPENDIX 7- ETHICS COMMITTEE APPROVAL FOR PRIMARY STUDY

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone (021) 406 6338 • Facsimile (021) 406 6411
e-mail: [illegible]

29 May 2009

REC REF: 012/2007

A/Prof RJ Wilkinson
IDMM
Medical School

Dear Prof Wilkinson

PROJECT TITLE: INCREASING PREVENTION AND TREATMENT OF TB THROUGH DEVELOPMENT OF A RAPID, SENSITIVE, AND AFFORDABLE BIOLOGICAL MARKER (GENOMIC OR PROTEOMIC) FOR DIAGNOSIS OF TB IN HIV POSITIVE AND NEGATIVE POPULATIONS.

Thank you for your letter to the Ethics Committee dated 26th May 2009.

It is a pleasure to inform you that the Ethics Committee has granted **annual approval** for the study to continue for another year.

Approval is granted till 01st June 2010.

We note progress report submitted.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

sm/ddw

APPENDIX 8- BUDGET AWARD LETTER FOR PRIMARY STUDY

EUROPEAN COMMISSION
EuropeAid Co-operation Office

Central management of thematic budget lines

Brussels, 31 MAI 2006
AIDCO 04/D (2006) 12341

Professor Michael Levin
Brighton and Sussex Medical School
Medical research building, Brighton and Sussex Medical School, University of Sussex
BN1 9PX, Biology road Brighton
The United Kingdom

Call for proposals **Call for proposal EuropeAid/121404/C/G/Multi "Aid for poverty-related diseases (HIV/AIDS, Tuberculosis and Malaria) in developing countries"**

Application ref.: **SANTE/2005/ 105-061 - 102**
ThemeII/ Increasing prevention and treatment of TB through development of a rapid, sensitive and affordable biological marker (genomic or proteomic) for diagnosis of TB in HIV positive or negative populations

Dear Sir,

With reference to the above application I am pleased to inform you that, on the recommendation of the Evaluation Committee, the European Commission has decided that your application may be awarded a European Community grant of a maximum **€ 4532158.00, i.e. 89.85 %** of the total eligible cost of the action.

A grant contract between the European Commission and your organisation will therefore be prepared. In this connection you will be contacted with a view to finalising it according to the recommendations of the Evaluation Committee.

Please note that this letter does not yet give you the right to the said grant. You will not acquire that right until both parties have signed the grant contract, and then your right will depend upon the terms of the contract.

Yours faithfully,

André Debongnie
Chairman of the Evaluation Committee

Commission européenne, B-1049 Bruxelles / Europese Commissie, B-1049 Brussel - Belgium. Telephone: (32-2) 299 11 11.
Office: L-41 03/153. Telephone: direct line (32-2) 2932 2 2951465.

APPENDIX 9- ETHICS COMMITTEE APPROVAL FOR THIS STUDY

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Amendment Form

Date	22 December 2011
HREC REF Number	012/2007
Protocol number (if applicable) & Protocol title	Increasing prevention and treatment of TB through development of a rapid, sensitive, and affordable biological marker (genomic or proteomic) for diagnosis of TB in HIV positive and negative populations
Principal Investigator	Robert J Wilkinson
Department / Office Internal Mail Address	Room 3.03 Wolfson Pavilion IIDMM Faculty of Health Sciences

List of Proposed Amendments with Revised Version Numbers and Dates

The aim of the original study was to identify biomarkers which distinguish patients with active TB from latent TB and other prevalent diseases. As part of this study, a cohort of HIV-infected adults with latent TB were recruited. As per national guidelines, these participants were provided with a 6-month course of isoniazid preventive therapy (IPT). Recruitment and IPT follow-up is now complete.

The proposed amendment seeks to describe the completion rates of IPT and to evaluate predictors of non-adherence using anonymous demographic data collected as part of the original study (using the same data collection tool approved by the ethics committee for the original study). This analysis forms the basis of a thesis for a Masters in Public Health degree for Tolu Oni, the study doctor.

Below is a synopsis of the thesis protocol. The complete protocol and the synopsis of the original study are submitted with this application.

RESEARCH ETHICS COMMITTEE
2012 -01- 11
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

HREC office use only (FWA00001637; IRB00001938)

☑ Approved	☐ Type of review: Expedited	☐ Full committee

This serves as notification that all changes and documentation described above are approved.

Signature Chairperson of the HREC		Date	11/1/2012

APPENDIX 10- INSTRUCTIONS TO AUTHOR FROM THE INTERNATIONAL JOURNAL OF TUBERCULOSIS AND LUNG DISEASE

1 Original articles on clinical or epidemiological research, intervention evaluation (health action, personnel training or health education programmes);

2 General reviews and technical updates in connection with the elaboration, implementation and assessment of national health programmes against tuberculosis and lung diseases.

The IJTLD can also be accessed electronically via the Union website (http://www.theunion.org). Access to all back issues is free. Access to the current 6 months is available to all paid-up members and subscribers using the number provided on their current membership card; non-members/non-subscribers can access and download individual articles using the 'Pay-per-view' option or contact The Union (membership@theunion.org) for information on how to become a member.

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All other correspondence, such as suggestions for review and perspectives articles, should be sent directly to: The Editorial Office, The Union, 68 boulevard Saint-Michel, 75006 Paris, FRANCE. e-mail: journal@theunion.org

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Each manuscript will be examined by a scientific editor and usually two referees. Notification of acceptance or rejection will be sent within 3 months from date of receipt. If a revised version is requested, it should be returned to the Editor no

later than 3 months after notification. A delayed revised article will be treated as a new manuscript.

The Editor reserves the right to make editorial and literary corrections.

Any opinions expressed or policies advocated do not necessarily reflect those of the Union.

AUTHORSHIP

All work must have been approved by all co-authors prior to submission.

Authorship credit should be based on the following criteria: 1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for intellectual content; and 3) final approval of the version to be published.

Multicentre groups: When a multicentre group has conducted the study, all individuals who accept direct responsibility for the manuscript should be identified. When submitting a group author manuscript, the corresponding author should clearly identify all individual authors, as well as the group name.

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The copyright form should be signed and scanned by the corresponding author and uploaded directly onto the website with the manuscript on submission. All authors must provide a signed form on acceptance.

PREPARATION OF MANUSCRIPTS

Manuscripts should conform to the *Uniform Requirements for Manuscripts submitted to Biomedical Journals* (http://www.icmje.org/index.html). Articles on clinical research should conform to the standards defined in the Helsinki Declaration.

Authors should ensure that they have followed the relevant recommendations for reporting their findings (CONSORT, STARD, MOOSE, STROBE, PRISMA, STREGA).

Details of ethics approval (or a statement that it was not required) should be provided in the Methods section of all research studies submitted to the Journal.

Authors may submit articles in English (US/UK), French or Spanish. Accepted articles can be translated into English for publication in the journal. Authors may publish in French or Spanish.

The article should have 1.5 or double spacing and continuous line numbering, and, on separate numbered pages:

Title page: This should contain: 1) a concise, informative title of not more than 110 characters and spaces, without abbreviations; 2) the names and all affiliations of all contributing authors, clearly indicating who is linked to each institution; 3) a running head of not more than 45 letters and spaces; 4) a word count of the text, excluding summary, references, tables and figures; 5) 3- 5 keywords that do not appear in the title.

Summary: An informative structured abstract of not more than 200 words should be provided that can be understood without reference to the text (see Ann Intern Med 1990; 113: 69-76). For optimal clarity, the author should use the headings Setting, Objective, Design, Results and Conclusion. Abstracts will be translated into the two other languages on acceptance for publication (authors are welcome to provide translations). Unstructured summaries may be submitted for review articles (250 words) and Short Communications (100 words).

Text: Headings should be appropriate to the nature of the article. Normally only two categories of heading are used. Major headings should be typed in capital letters. Minor headings can be typewritten in lower case letters (starting with a capital letter) at the left-hand margin. The subtitles should not be numbered either with figures or alphabetically.

The text should be written as objectively as possible. For word limits, please refer to the section ‘Length of text’.

Numerals should be spelt out in full from one to nine (except when referring to a measurement), and when beginning a sentence.

1. Research and experimental papers should follow the usual conventions, as follows:

Introduction Setting forth clearly the aim of the study or the main hypothesis, with reference to previous studies and indicating the method used.

Materials or *Study population and Methods:* NB: Indicate what measures were taken to assure the quality of the data.

Results Presented in logical sequence in the text, with tables and illustrations. All the results of the tables should not be repeated in the text; the most important results should be emphasised.

Discussion Related to the aims and results of the study. *Conclusions*

2. Other papers can be subdivided as the author desires; the use of headings enhances readability.

Acknowledgements: Acknowledge only persons who have made substantial contributions to the study, with their consent, all sources of support in the form of grants, and author contributions.

References (Vancouver format): The accuracy of references is the responsibility of the author. They must be numbered in the order in which they are cited in the text, and identified by Arabic numerals in superscript. References that are cited more than once should retain the same number for each citation. The list of references at the end of an article should be arranged in numerical order. NB: Numbering in tables/figures corresponds to where the tables/figures are cited in the text.

The only acceptable references are those of publications that can be consulted.

References to an article in a periodical should include the names of the authors, followed by their initials (list all authors when six or fewer; when there are more, list only the first three and add 'et al.'), the full title of the article in its original language, the name of the journal in its usual abbreviated form (Index Medicus), year of publication, tome or volume number, first and last page numbers in full:

e.g., Gordon J B, Bennett A M. Tuberculosis in reindeer. Scand Rev

Respir Dis 1978; 96 (Suppl): 217-219. *References to a piece of work* (book/monograph) should include

the names of the authors as above, the title of the piece of work in its original language, the number of the publication, the name of the editor, the place and year of publication, the number of the volume and the first and last page numbers.

References to a chapter in a book should include the names of the authors as above, the title of the chapter with the word "In" preceding the reference of the work as above.

e.g., Girling D J. The chemotherapy of tuberculosis. In: Ratledge C, Stanford J, Grange J M, eds. Biology of the mycobacteria. London, UK: Academic Press, 1989: pp 285-323.

Electronic references should be given only when an original citation is unavailable; as much information should be provided as possi- ble, including html address and date of access.

References to an article to be published should give the name of the journal with the mention '(in press)' and *only* appear after having been accepted. Articles under submission can be cited in the text.

Personal communications should be given in the text with the name of the individual cited *and with his/her consent.*

TABLES

Tables should be referred to consecutively in the text and placed after the references. They should be numbered in Arabic numerals which are used for reference in the text. A short descriptive title should appear above the table. Each column should have a short or abbreviated title. All abbreviations should be explained in a clear legend below the table. The number and size of the tables should be kept to a basic minimum to explain the most significant results.

FIGURES

Figures should be referred to consecutively in the text. They can be inserted into the Word document (after the tables) or uploaded separately as image files (.jpg, .ppt, .gif, .tif or .bmp).

Line drawings (curves, diagrams, histograms) should be in black and white, with solid black lines. For optimal clarity avoid shading.

The size of the symbols and lettering should be in scale with the figure. A sans serif font, such as Arial, should be used and be of uniform size. All figures should be the same point size. *Half-tone figures* should be clear and highly contrasted in black and white. Photo-micrographs should have internal scale markers where appropriate. X-ray films should bring out the detail to be illustrated with the area of importance clearly indicated.

Techniques (staining, magnification, etc) should be defined. *The cost of reproducing colour illustrations* (print or online) will be
covered by the authors.

Half tone and colour figures should be supplied at a resolution of a least 300 dpi (preferably 500 dpi).

Every Figure should have a brief explanatory legend that does not repeat information given in the text.

Patient confidentiality Where illustrations show recognisable individuals, consent must be obtained for publication. If not es- sential to the illustration, authors should indicate where it can be cropped, or mask the eyes.

After acceptance, figures should be supplied in editable format (e.g., .ppt, .xls) to allow editorial modifications.

Permission to reproduce illustrations or tables should be obtained from the original publishers and authors, and submitted with the article. They should be acknowledged as follows: *'Reproduced with the kind permission of (publishers) from (reference)'.*

ABBREVIATIONS AND UNITS

Avoid abbreviations in the title or summary. Abbreviations or unusual terms should be described at the first time of use.

Symbols and units of measure must conform to recognised scientific use, i.e., SI units. For more detailed recommendations, authors may consult the Royal

Society of Medicine publication *Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors.*

Designation of diseases must conform to the International Classification of Diseases. Designation of micro-organisms must con- form to the norms of biology. Proprietary names of drugs, instruments, etc., should be indicated by the use of initial capital letters. Names of instruments should be accompanied by the manufacturer's name, city, state and country.

LENGTH OF TEXT

Original articles: text up to 2500 words, a structured summary of 200 words, 7 moderate-sized tables/figures and 35 references.

Review articles: text up to 4500 words, a structured or unstructured summary of 250 words, 8 moderate-sized tables/figures and 90 references. *Pre-submission query required with one- page proposal.* Submitted to peer review.

Editorials: text up to 500 words and 5 references. Editorials are usually invited.

Perspective articles: text up to 2500 words and 35 references. *Pre-submission query required with one-page proposal.*

Technical notes and *Short communications:* text up to 1000 words, a summary of 100 words, 2 tables/figures and 10 references.

Notes from the Field: text up to 1000 words, a summary of 100 words, 2 tables and 10 references. Describe programme aspects that are of broad interest to readers: case finding, treatment, supervision, special populations or situations, new solutions, practical ideas, local experience. Format: Situation/setting, Aspect of interest, Discussion, Conclusion.

Case studies: text up to 1000 words, a summary of 100 words, 2 tables/figures and 10 references. Accepted *only* if they contain original and innovative material.

Correspondence: text up to 500 words without tables or figures and 5 references.

Papers that are too long must comply with editorial requirements. An excess page charge of 200€ per page is applied for all articles submitted as of 1 July 2011.

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