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Cox Proportional Hazards Analysis to Determine the Predictors of Time-to-Sputum Culture Conversion in Patients with Tuberculosis

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An abstract of
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in partial fulfillment of the requirements for the degree of Master of Public Health in Hubert
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Abstract

Cox Proportional Hazards Analysis to Determine the Predictors of Time-to-Sputum Culture Conversion in Patients with Tuberculosis

By

Tanushree Soni

Background: Tuberculosis continues to be one the deadliest infections in the world, infecting one fourth of the global population and claiming 5000 lives a day. Effective diagnosis and treatment regimens are imperative to reduce the burden of this disease. Sputum culture is used for accurate diagnosis, and conversion of serial sputum cultures from positive to negative is an important indicator of the efficacy of anti-tuberculosis treatment. In this study, we determine important predictors for sputum culture conversion in a cohort of patients admitted in a US hospital for the treatment of TB.

Methods and Findings: We abstracted medical records of patients admitted to a TB ward, 1985-2010. We compared patients who did and did not convert with respect to important social and medical characteristics using frequency tables, Kaplan Meier survival curves as well as Cox proportional hazards regression analysis to determine the time to conversion and the predictors of time to conversion. In our studies, we found that 61% of the cohort showed sputum culture conversion; the median time to conversion was 50 days and the maximum time was 56 days. Alcohol consumption, HIV infection IV drug use, race, baseline smear status, treatment with first-line drugs and the calendar period were significantly associated with time to conversion of sputum cultures. Patients with a HIV positive status and use of intravenous drugs had a shorter time to conversion. Treatment with certain drugs also significantly decreased the time to conversion. Cox proportional hazards regression analysis identified gender, baseline smear results, HIV, IV drug use, and race as independent predictors of conversion.

Conclusion: In conclusion, multiple variables can affect sputum culture conversion and should be taken into account for the management of TB patients.

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CHAPTER 1: Introduction

Background

The earliest written record of tuberculosis (TB) can be found on a clay tablet from the Assyrian Empire during the 6th Century BC [1]. Evidence of TB dates back to 2400-3400 B.C in mummies with spinal TB [2]. Even after so many centuries, TB continues to plague human health by infecting one-fourth of the world's population [3, 4]. Developing countries bear the highest burden of this disease with 95% of TB cases and TB related deaths [5]. Tuberculosis is the leading cause of death in people co-infected with HIV and such individuals are also 20-30 times more likely to develop active TB [5]. Tuberculosis is a bacterial infection that is preventable and treatable. The causative bacterium include species in the *Mycobacterium tuberculosis* complex. The most common disease manifestation is in the lungs, called pulmonary TB. *M. tuberculosis* is most commonly spread through the inhalation of aerosols containing the bacteria generated when a person with pulmonary TB disease coughs, sneezes, or spits. Infection with *M. tuberculosis* does not always manifest as active disease, and most commonly leads to a latent infection instead. Latently infected individuals have a 10% chance of developing active TB disease in their lifetime. Symptoms of active TB disease include persistent cough, night sweats and weight loss. As these symptoms can be mild and last for many months, individuals may not seek medical care immediately. Delays diagnosis and appropriate treatment could result in widespread transmission of the infection. Specific antibiotics are the choice of treatment for drug susceptible TB and standardized regimens are prescribed for six to nine months. Drug resistant TB, coinfection with HIV and other comorbidities such as diabetes and kidney failure complicate treatment with standard anti-tuberculosis drugs. Timely diagnosis and treatment are critical factors to prevent TB related morbidity and mortality. It is estimated that 49 million lives were saved between 2000-2015 because of proper diagnoses and treatment [6]. Decades of research and development have improved TB diagnosis, patient management and treatment; however, several gaps need to be addressed to decrease the TB incidence rate, especially in high disease burden settings.

Statement of the Problem

Ending the TB epidemic is one of the United Nations Sustainable Development Goals (SDG) [7]. The End TB strategy developed by the World Health Organization (WHO) describes three pillars essential to reach the program goals which are [8]:

- Pillar 1: integrated patient-centred care and prevention
- Pillar 2: bold policies and supportive systems
- Pillar 3: intensified research and innovation

Good quality patient care and prevention includes timely diagnosis and treatment. Pulmonary TB diagnosis includes microbiological analysis of respiratory samples and radiographic examination of the chest. In resource poor settings, microscopic analysis of sputum samples to detect acid-fast bacilli (AFB) is the diagnostic method most widely used. This method of diagnosis has some drawbacks, however due to the low costs, convenience and fast results, it remains as one the methods of diagnosis recommended by the WHO. Microbial culture is a more sensitive diagnostic test and involves the culturing of sputum and other samples to check for *M. tuberculosis* growth. This process can take up to several weeks but can be used to identify the strain as well as determine drug susceptibility. Microbial tests to detect *M. tuberculosis* are also used to determine treatment efficacy. Throughout the course of the treatment, sputum samples are collected at regular intervals and the results are used as indicators for drug effectiveness, drug resistance and decision to keep patient in isolation. Sputum culture conversion, defined by WHO as two samples at least 30 days apart that are negative for *M. tuberculosis* growth, is thus an important marker for TB case management. Tuberculosis treatment is very lengthy (6-9 months) and requires multiple drugs. Due to the long periods of treatment, patient non-compliance is a major hurdle face by TB elimination programs. When sputum conversion is delayed, treatment gets prolonged as well and non-compliance is exacerbated. Lack of adherence to anti-TB treatment then results in poor TB treatment outcomes such as relapse and increased drug resistance, resulting in a strain on healthcare systems and endangering public health. The WHO and CDC recommend patient isolation at home and in properly ventilated medical facilities when

diagnosed with active pulmonary TB as the risk of transmission among close contacts of this disease is high. A patient can be removed from isolation after sputum conversion; in the U.S., this generally means three properly collected and processed sputum samples are bacteriologically negative. Prolonged sputum positive samples increase the isolation period and suggest treatment may need to be changed. Other rapid diagnostic methods detect *M. tuberculosis* DNA through nucleic acid amplification tests (NAAT), and have been increasingly used to accelerate the diagnosis of TB [9].

There are several factors that affect the time taken to reach sputum conversion and should be accounted for during diagnosis as well as treatment. Extensive research has shown that bacillary load in the sputum at the initiation of treatment, presence of cavitary disease and drug resistant *M. tuberculosis*, coinfection with HIV, smoking, body mass index, and some comorbidities could delay the time to conversion.

Purpose of the Study

This study aims to determine the predictors of time-to-sputum culture conversion among patients hospitalized in University of Texas Health Science Centre at Tyler, from 1985 to 2010 for treatment of tuberculosis.

Significance Statement

Elucidation of the predictors of sputum culture conversion will contribute prognostic information that could help optimize the care of tuberculosis patients, leading providers toward longer or more intensive treatment in patients who are at risk for longer time to convert.

Acronyms and definitions

AFB: Acid Fast Bacilli.

Not decolorized by acid after staining, as bacteria that retain dye after an acid rinse.

AIDS: Acquired immunodeficiency syndrome

AII: Airborne Infection Isolation

BCG: Bacille Calmette-Guerin

CDC: Center for Disease Control and Prevention

CMI: Cell-Mediated Immunity.

Immune responses mediated by activated, antigen-specific T lymphocytes. These T cells may function as effector cells or may orchestrate propagation of the inflammatory response and cellular recruitment through their secretion of cytokines and chemokines.

CXR: Chest X-Ray (radiography)

DM: Diabetes Mellitus

DST: Drug Susceptibility Testing

DTH: Delayed-Type Hypersensitivity

EMB: Ethambutol

IGRAs: Interferon-Gamma Release Assays

INH: Isoniazid

FM: Fluorescent Microscopy

HIV: Human Immunodeficiency Virus

LJ: Lowenstein-Jensen

LTBI: Latent Tuberculosis Infection

The term used for people who test positive for tuberculosis (most commonly with a positive tuberculin skin test), but do not have any evidence of active infection. Currently one in three people worldwide are felt to harbor tuberculosis bacilli.

MAC: *Mycobacterium avium* complex

MDR-TB: Multi-Drug Resistant tuberculosis - Tuberculosis infection caused by *Mycobacterium tuberculosis* that is resistant to at least isoniazid and rifampin.

MTB: *Mycobacterium tuberculosis*

NAATs: Nucleic Acid Amplification Tests

NTM: Non-Tuberculous Mycobacteria

PCR: Polymerase Chain Reaction

PPD: Purified Protein Derivative

PTB: Pulmonary Tuberculosis

PZA: Pyrazinamide

RIF: Rifampicin

TB: Tuberculosis

TST: Tuberculin skin testing

WHO: World Health Organization

XDR-TB: Extensively Drug-Resistant Tuberculosis

Tuberculosis infection caused by *Mycobacterium tuberculosis* that is resistant to at least rifampicin and isoniazid as well as to any member of the quinolone family and at least one of the following second-line anti-TB injectable drugs: kanamycin, capreomycin, or amikacin.

ZN: Ziehl-Neelsen

CHAPTER 2: Literature Survey

Introduction

Tuberculosis (TB) is a contagious disease caused by *Mycobacterium tuberculosis* complex (MTB). The most common site of TB disease is the lungs; called pulmonary TB, however the bacteria can infect other parts of the body such as kidneys, spine, gastrointestinal tract and brain. TB infections in organs other than the lungs are called extrapulmonary TB. TB is an ancient disease known to occur throughout the history of mankind. The modern strain of *Mycobacterium tuberculosis* is theorized to have originated from a common ancestor about 20,000-15,000 years ago [10]. Historians have recorded TB under various names such as consumption, phthisis, scrofula, Pott's disease, and the White Plague. The disease earned the moniker “Captain Among these Men of Death” upon reaching epidemic proportions in the western world during the 18th and 19th centuries [10]. Tuberculosis epidemiology and TB drug discoveries helped reduced incident TB cases in the 20th century; however, the emergence of multi drug resistant strains of *M. tuberculosis* (MDR-TB) and human immunodeficiency virus (HIV) heralded the re-emergence of TB in the late 1980s and early 1990s in the U.S [11]. Currently TB ranks among the top 10 causes of death worldwide, with an estimated 10.4 million new TB cases and 1.8 million deaths in 2015 [12]. In 2014, the rate of TB in the US was 2.96 cases per 100,000 persons. Because of the global impact of TB, the Sustainable Development Goals (SDGs) for 2030 adopted the target to end the global TB epidemic [6, 7]. The WHO End TB strategy aims to reduce TB deaths by 90% and TB incidence rate by 80% as a part of the SDG goals [6].

TB transmission

Tuberculosis infection is a classic air-borne infection. *M. tuberculosis* is transmitted via respiratory secretions called droplet nuclei. These droplet nuclei are expelled into the air when people with pulmonary or laryngeal TB cough, sing, sneeze or talk. Person-to-person transmission depends on numerous factors. Patients with high bacillary loads in the sputum are most infectious and those with cavitary disease are more infectious than those without cavitary lung disease. Studies have found that effective transmission is linked to the density of droplet nuclei and duration of exposure [13]. The

particles are 1-5 μM in size and remain suspended in air, especially in areas with poor ventilation for prolonged periods. Therefore, extended exposure in closed spaces is a major risk for transmission [14].

Due to the nature of droplet nuclei, only those in close contact with an infectious TB source are at higher risk of contracting the disease. Even so, only 30-50% of close exposures actually get the tuberculosis infection [15]. Individuals with an immunocompromised condition (e.g. infection with Human Immunodeficiency Virus [HIV], transplantation, autoimmune disease, certain cancers), have a higher chance developing TB disease after infection, but there is no clear evidence of increased susceptibility to infection upon exposure to *M. tuberculosis* [13].

TB signs and symptoms

It is interesting to note that not all those infected with *M. tuberculosis* go on to develop the disease, in fact only 12% of the immune-sensitized individuals develop the disease [16]. Thus, two types of TB conditions exist: latent TB and TB disease (Fig.1.). Latent TB is characterized by the organism remaining inactive, localized to the lungs and not causing disease in healthy individuals. Such individuals could potentially develop active TB disease under conditions that compromise the immune system [17] (Fig.1.). When *M. tuberculosis* actively multiples in the human host it causes TB disease during which an individual can have any or all of the following symptoms:

- Persistent, productive cough for over 3 weeks, with or without blood in sputum
- Chest pains
- General weakness and exhaustion
- Gradual weight loss
- Lack of appetite
- Fever and chills
- Sleep hyperhidrosis, more commonly known as night sweats

Pulmonary TB

Tuberculosis most frequently presents as pulmonary TB (PTB), in which *M. tuberculosis* infects the lungs. PTB is also the most infectious form of TB and thus has great epidemiological relevance. Once inhaled, the organisms are lodged in the lung alveolus where they are ingested by alveolar macrophages. Phagocytosis by these macrophages is an important step in infection clearance [18]. Comorbidities and other conditions that affect the innate immune response exacerbate tuberculosis infections. When the bacteria can escape the macrophages, they can pread through the neighbouring lymph nodes to regional hilar and mediastinal lymph nodes and other extrapulmonary sites [19]. Cell mediated immunity (CMI) and delayed type hypersensitivity play a critical role in the control of *M. tuberculosis* multiplication 2-10 weeks post initial infection [18]. The side effects of the control of TB infection by CMI and delayed type hypersensitivity is the destruction of neighbouring tissues that leaves necrosis and cavitation. The delayed type hypersensitivity processes also result in the manifestation of pleural effusion 3-6 months post primary TB infections [19].

Latent TB infection (LTBI) is when intracellular *M. tuberculosis* remains dormant but viable [20]. Persons with LTBI have no signs or symptoms of disease and are not contagious to close contacts. In some cases, dissemination of *M. tuberculosis* via hematogenous spread allows the seeding of the bacteria in multiple organs. This condition, which involves the focal collection of a large number of TB bacilli is called miliary TB. Miliary TB is extremely difficult to diagnose due to atypical presentation of symptoms involving individual organs, however lung is the most commonly involved organ. Delayed diagnosis also leads to delay in treatment and poor disease outcomes [21].

Clinical presentation of the symptoms is often non-specific and depends on the age and individual immune status.

Signs and symptoms of TB disease include prolonged low grade fever, fatigue, weight loss, night sweats and persistent cough which could be non-productive in the initial stages but turns into

productive mucoid cough as the disease progresses. Cough is the most frequent symptom and could be accompanied by hemoptysis [22].

Diagnosis of tuberculosis

A crucial aspect of the global TB epidemic control measures is the accurate and timely diagnosis of TB. This aspect is often overlooked and lack of a sensitive TB test is a major obstacle for detection and clinical management of TB. A comprehensive evaluation for TB should include:

- Patient's medical history
- Possible exposure to someone with TB disease
- Physical examination
- HIV status
- Test for TB infection: Mantoux tuberculin testing (TST) and/or TB blood tests, also called interferon-gamma release assays (IGRAs)
- Chest radiography
- Diagnostic microbiology, including drug susceptibility tests, or nucleic acid amplification tests

TB symptoms are non-specific therefore a clinical diagnosis should be based on regional prevalence of the disease as well as multiple factors such as comorbidities, patient's exposure history, habitat and lifestyle choices.

Current immunodiagnostic test for TB

There are two types of immunodiagnostic tests for TB: a test of functional cellular immunity based tests and an in vitro T-cell based test. Neither directly measures the MTB antigen load, and they have variable sensitivities and specificities. The choice of test depends on the availability, costs and reason for testing [17].

The functional cellular immunity based test is the TST. This test is performed by intradermally injecting tuberculin purified protein derivative (PPD), to induce a localized type IV delayed-type hypersensitivity (DTH) reaction, manifested as a raised or swollen area at the site injection 48-72 hours after the injection. TST has many drawbacks, including poor specificity because it can be positive in Bacille Calmette-Guerin (BCG) vaccinated populations, and poor sensitivity in immunosuppressed individuals [23]. Meta-analyses have shown that TST is a sub-optimal test as it shows variable sensitivity and specificity for pulmonary and extrapulmonary TB and is also unable to distinguish between latent or active, remote or current infections and cross-reacts with infections due to nontuberculous mycobacteria (NTM) [24]. Despite its drawbacks, TST is widely used, especially in countries where BCG vaccination is uncommon.

The interferon-gamma (IFN-γ) release assays detect the anti-MTB cytokine IFN-γ, released by T-cells. IGRAs are the preferred tests for BCG vaccinated individuals as the MTB antigens are not found in the BCG vaccine. These tests are more specific than TSTs, however cannot differentiate between latent and active TB. The two FDA approved commercial IGRAs used are QuantiFERON®–TB Gold In-Tube test (QFT–GIT) and T–SPOT®.TB test (T–Spot).

Current laboratory based test for TB

Examination of sputum by microscopy and culture are the oldest and currently relevant diagnostic tests for detection of TB. The Centers for Disease Control and Prevention (CDC) and WHO recommendations include that all patients suspected of TB disease must have at least two sputum samples collected for acid fast bacilli (AFB) smear and culture analysis [17]. These samples should be consecutive sputum specimens collected in 8- to 24-hour intervals, in airborne infection isolation (AII) room or other isolated, well-ventilated area, preferably under the guidance of a trained healthcare professional. Smear microscopy involves the visualization of acid fast stained bacilli and is the most frequently and sometimes the only available test in low resource settings. There are two typesof staining methods Ziehl-Neelsen (ZN) stain for light microscopy and fluorescent dyes for fluorescent microscopy (FM). The ZN staining method is preferred because slides are examined under

the common light microscope. ZN has a very high specificity of 97% but the sensitivity varies from 30%-80% and is higher in immunocompetent individuals. FM is easier to examine, and requires a shorter period for a thorough and accurate microscopic examination. If resources can be secured, it is recommended for use over ZN. The major drawback of smear microscopy methods is that it cannot detect the *Mycobacterium* species, cannot distinguish viable and non-viable bacteria or drug resistant strains. Therefore, smear microscopy should ideally be supplemented with other diagnostic tests.

Bacteria belonging to the *Mycobacterium* genus are extremely slow growing organisms and can be cultured on special solid and liquid media. The egg based Lowenstein-Jensen (LJ) and Middlebrook 7H9 broth are the two most frequently used commercially available solid and liquid media respectively. Culturing is the most sensitive diagnostic method with liquid culture media having higher sensitivity compared to solid media. These tests are also invaluable for determining the drug sensitivity pattern of an isolate. The major disadvantage of culturing is that the results are available only after several (4-8) weeks of initial sample collection. Another feature is that it recovers all species of *Mycobacteria*; however rapid immunochromatographic assays can be used to provide definitive identification of species.

Nucleic Acid Amplification Tests (NAATs) are molecular based tests that amplify MTB specific nucleic acid sequences, including drug resistance genes, using real time polymerase chain reaction (PCR). These tests have good sensitivity and specificity for smear -positive samples and a lower sensitivity for smear-negative samples [24]. The Gene Xpert MTB/RIF® is the first automated NAAT assay system with a standardized protocol used in routine TB diagnosis in middle and high income countries as well as increasingly in low income countries. NAATs have many advantages over smear microscopy and culturing which include high throughput, increased speed of testing and lower biosafety requirements as well as high reproducibility [25].

Radiographic Screening

Chest radiography (CXR) is often done in conjunction with TST to screen for active pulmonary TB and used in algorithms to detect smear-negative PTB. Radiography results help in detection of current as well as prior infection or disease and the results in conjunction with TST results are used to categorize the infection as one of the three following categories: (a) TB infection, no disease; (b) TB infection, clinically active; and (c) TB infection, clinically inactive [13]. The degree of pulmonary involvement and radiologic manifestations depend on factors such as age, immune status duration of symptoms, and prior exposure to TB. There are two forms of pulmonary disease in immunocompetent individuals; primary and post-primary disease [19].

Primary disease occurs in patients who have no prior exposure to *M. tuberculsosis* and is more common in children. Common radiographic findings for primary TB include [26]

- Parenchymal consolidation: parenchymal opacities can be found mostly in lower and middle lobes, and is indistinguishable from bacterial pneumonia. Most frequently, the localized infection forms a tuberculoma or a caseating granuloma which undergoes calcification to form the Ghon lesion or focus. When the ipsilateral calcified hilar node is associated with the Ghon lesion it is called the Ranke complex. Both Ranke complex and Ghon lesion represent the ‘healed” primary PTB.
- Lymphadenopathy: lymph nodes enlargement is typically unilateral, involving the hilar and paratracheal stations. Lymphadenopathy is used in differential diagnosis of PTB in conjunction with parenchymal consolidation, however evidence of lymphadenopathy decreases with age. Lymphadenopathy is less common in post-primary cases.
- Pleural effusion: The collection of fluid around the lungs is more commonly seen in adults and may be the only chest radiographic manifestation.
- Airway involvement: When the enlarged nodules compress the adjacent airway, it results in distal atelectasis, erosion of the bronchi due to lymphadenopathy causes broncholithiasis, erosion into blood vessels leads to coughing blood.

- Miliary disease: characterized by the presence of homogenously distributed, innumerable, diffuse small 2–3-mm nodules that look like millet seeds. Prevalent in approximately 1%-7% of the patients, in the elderly, infants and immunocompromised individuals.

Post primary disease can develop from either reinfection with the *M. tuberculosis* or reactivation of tuberculosis and occurs primarily in adolescents and adults. The important features of post primary disease are absence of lymphadenopathy, multiple parenchymal manifestations mostly in the upper lobes and cavitary disease which is indicative of an active disease. Pleural effusions are detected in untreated cases. The presence of lung cavities is a characteristic feature of post-primary disease, seen in 20-50% of the cases. Cavities are thick, irregular walled patches in the center of nodules or mass or areas of consolidation and can be filled with air as well as fluid and develop because of caseous necrosis. The walls become thin and smooth upon successful treatment and resolution of the cavities often leads to scarring of the lung tissue [26]. Endobronchial spread of infection via the airways can be detected by X-rays in patients with active disease. Narrowing of the bronchial tube, a condition called bronchial stenosis occurs in about 10%-40% of active TB cases and can result is lung collapse, hyperinflation and obstructive pneumonia.

Chest radiography has a very low rate of false negative results in immunocompetent cases, however the sensitivity decreases in HIV-seropositive individuals [19]. PTB patients with HIV coinfection present with atypical pulmonary disease which is linked to low CD4 counts. Individuals with normal or near normal CD4 levels often present with typical cavitary pulmonary TB however, atypical infiltrates that include middle and lower lobe infiltrates, interstitial nodules, mediastinal lymphadenopathy and even normal chest radiographs are more common in patients with low CD4+ T -lymphocyte counts [27]. If available, CT scans are used in conjunction with CXR as needed, as the CT scans are more sensitive at detecting lymphadenopathies, bronchial stenosis, and very small cavities not evident in routine CXR.

Treatment

TB disease is characterized by the active multiplication of *M. tuberculosis* in the host. This is the time period during which the host shows clinical signs and symptoms. PTB is contagious to others, especially for people spending prolonged periods with such individuals. Prompt diagnosis and treatment is imperative for the prevention and control of the disease. *M. tuberculosis* is a slow growing intracellular bacterium that can remain dormant for long periods of time. Therefore, the current TB treatment regimen is prolonged and requires combination chemotherapy, to prevent relapse and the selection of strains which acquire resistance to anti-TB drugs through spontaneous mutations. Anti-TB drugs are classified into four groups as shown in figure 1. The first group or first line drugs includes isoniazid (INH), the broad spectrum antibiotic rifampicin (RIF), ethambutol (EMB) and pyrazinamide (PZA). These drugs, used in combination, should be the first choice of drugs for treatment of persons with TB disease caused by susceptible strains. The second line of drugs includes fluoroquinolones, aminoglycosides, polypeptides, and other drugs that are used for treatment of multi-drug resistant TB (MDR-TB). *M. tuberculosis* strains that are resistance to at least isoniazid and rifampicin are categorized as MDR strains. MDR strains that are also resistant to both a fluoroquinolone and a second line injectable anti-TB drug are sub-classified as extensively drug resistant (XDR). Third-line drugs include drugs that could be used for treatment of MDR-TB but are less well studied, less effective, or cause more adverse reactions. Drugs used to treat TB are shown to interact with other drugs that maybe be used to treat concomitant illnesses [28-30]. One of the most important interactions is observed with rifampicin, as this drug induces the production of drug metabolizing enzymes such as hepatic cytochrome P450 (CYP) 3A4 and intestinal and hepatic P-glycoprotein. This induction can result in increased metabolism of certain other drugs co-administered with rifampicin, thereby reducing serum concentrations and drug effectiveness [28, 30].

Treatment of drug susceptible TB

TB treatment is a long regimen totalling six- months of chemotherapy for new patients. Retreatment must be individualized based on DST result. TB treatment has two phases; the first intensive phase

requires a daily dose of INH, RIF, PZA, and EMB for 8 weeks, followed by the continuation phase that requires a daily dose of INH and RIF for an additional 18 weeks [31].

Treatment of drug resistant TB

- MDR-TB treatment regimens are extremely complicated, in part because the optimal number of drugs, their combination, and treatment duration have not been standardized by randomized controlled trials. The treatment regimen should be tailored to the drug susceptibility test results. However, treatment can be initiated before the DST results become available based on the probability of susceptibility or resistance to each available anti-TB drug. This probability is determined by the patient's prior history of anti-TB treatment if any, the prevalence of drug resistance in the population, and if there are any known contacts then their drug resistance profiles[32]. This treatment has been called an expanded empiric MDR-TB regimen and includes the four first-line drugs and two or more additional second line drugs. The expanded empiric treatment is warranted if the infection is life threatening, there is high suspicion for MDR-TB, if the patient was born or residing in countries with high burden of MDR-TB and patients with a history of contact with MDR-TB cases. It is important to revise the treatment plan once the DST results are available [32].

Adherence to TB treatment

According to WHO adherence can be defined as “the extent to which the patient's history of therapeutic drug-taking coincides with the prescribed treatment” [33]. Patients on TB treatments have been documented to have problems with adhering to the drug regimen because of the number and duration of drugs administered [34]. Non-compliance to TB treatments is the biggest hurdle in the global control of TB because it leads to prolongation of the infectious period, disease progression, emergence of resistance, relapse, and death [33, 34].

Epidemiology

TB is a major public health concern worldwide. In 2015, of the estimated 10.6 million incident cases, 56% were men, 34% women, 10% children and 11% were HIV positive [6]. Survey data shows that 3.9% (95% CI: 2.7-5.1%) of the incident cases and 21% (95% CI: 15-28%) of the previously treated cases were identified as MDR or rifampicin resistant (RR) TB. In terms of mortality, there were approximately 1.4 million TB deaths in 2015, with 0.4 million deaths resulting from TB disease among HIV positive population and 250,000 of these deaths were from MDR/RR TB [6]

One-fourth of the global population is infected by MTB and six countries India, Indonesia, China, Nigeria, Pakistan and South Africa contribute about 60% of the cases [6] (Fig.2.). The frequency of TB disease is inversely related to the socio-economic conditions and therefore TB burden is higher in low income countries [35]. Low to middle income countries like India and South Africa account for almost one-third of TB related deaths [35]

The case fatality ratio (CFR) of TB ranges from below 5% in some countries to over 20% in most countries in the WHO African region. The inequality in CFR is interpreted to correlate with the inequalities in access to TB treatment and diagnosis among persons living in these countries, as well as to the prevalence of HIV infection [6]

A preliminary report by the U.S. National Tuberculosis Surveillance System indicates a levelling of TB incidence in the United States at 3.0 cases per 100,000 persons during 2013–2015. This rate is approximately 13 times higher in the foreign born US population compared to US-born persons [13].

Significance of bacteriological tests in PTB

Microscopic analysis of sputum smears and culture analysis of sputum (and other biological specimens) are the most reliable diagnostic methods. These two methods are also invaluable the in monitoring and management of response to tuberculosis treatment [23, 28, 29]. Microscopic smear analysis for AFB (by ZN or FM) is the preferred test in low resource setting, and culture is a standard

practice in places with adequate resources. Sputum samples are tested for AFB positivity monthly after initiation of anti-tuberculosis therapy [23]. The median time to smear and culture conversion to negative, i.e., the absence of AFB in smears and no *M. tuberculosis* growth in media, is generally 2-4 weeks following initiation of effective therapy in drug-susceptible TB and 3 months in cases with MDR/XDR-TB [36].

Sputum Smear Analysis

As per WHO recommendations, new TB cases or relapse cases with smear positive PTB, sputum smear microscopy should be performed at the end of the intensive phase of treatment with first line TB drugs. It is important to note that the intensive phase can last for two or three months [28]. If the new TB cases test positive at the end of two-months treatment, then they need to be retested at three months and if the sample tests positive at the third month, then drug susceptibility testing should be performed. Treatment failure for new PTB patients with a positive smear at the start of treatment occurs when the treatment fails to achieve sputum conversion at month five or six or have MDR-TB strains at any time [28]. These patients should then be started on the MDR-TB treatment regimen. Failure of sputum conversion at the end of the intensive phase could be the result of poor adherence to treatment, incorrect dosing, comorbidity such as diabetes, presence of resistant strains, or extensive cavitation at start of treatment [37-40]. These factors can also play an important role in increasing the time to conversion and thus lengthen the required treatment duration [41-45]. Treatment for PTB patients could be considered successful when three consecutive samples taken 30 days apart are consistently negative after the completion of treatment.

Smear positive cases are more infectious than smear negative cases with the potential to transmit the disease; therefore hospital infection control measures include respiratory isolation of such patients and institution of other air-borne infection isolation control measures as well as contact investigations [46]. Microscopic analysis of sputum is important to inform the timely removal of patients from isolation [47, 48]. A patient should be removed from isolation only after they have met the following criteria [13]:

1. Successfully initiated anti-TB therapy
2. Shows clinical improvement
3. Has three consecutive AFB-negative smears that are taken 8-24 hours apart. At least one the three specimens should be an early morning specimen.

Culture analysis

Microscopic analysis of sputum suffers from some inherent flaws in that it cannot differentiate between viable and non-viable bacteria, *M. tuberculosis* from other NTM, and drug-resistant from drug susceptible strains. Therefore, sputum cultures are better indicators of treatment progress and should be performed at least monthly [49]. According to WHO, positive treatment outcome based on bacteriological examination is considered when patients with a positive culture at the initiation of treatment have two negative cultures taken at least 30 days apart [28, 49]. In case of MDR-TB, a patient is said to be cured if the treatment was completed and showed at least five consecutive negative cultures in the final year of treatment [49]. If only one is weakly positive during this period and is followed by three consecutive negative tests done 30 days apart, with no clinical deterioration, then the patient can still be classified as cured [49].Factors influencing sputum and culture conversion

Numerous factors influence the time to sputum and culture conversion, and include age, immune status, presence of cavitary disease, comorbidities (e.g., diabetes, HIV, kidney disease), nutritional status, smoking status, alcohol abuse, and bacillary load at the initiation of treatment. Some of the important baseline predictors of sputum culture conversion in PTB are discussed below

HIV

The incidence of tuberculosis in USA increased to an excess of 20% cases in 1985 and 1990, a period that coincided with the AIDS epidemic [11, 50]. With this increase in TB cases, it was established that TB is the most common opportunistic infection in people who are co-infected with HIV and is the most common cause of death in people with AIDS [12, 51] As of 2013, about 12% of the patients in the US who developed TB were coinfected with HIV and globally this number was 14.8% [52].

Susceptibility to tuberculosis and progression of latent TB to active disease is largely dependent on the immune status of an individual. Immunocompromised hosts, like those with HIV- induced immunodeficiency, have increased susceptibility to primary TB infection, reinfection and risk rapid progression from the latent phase to the active phase [53, 54]. TB patients with HIV coinfection often present with concurrent extrapulmonary TB [51, 55]. Along with increased morbidity rates, HIV coinfection also increases the TB related mortality rates [55].

Diagnosis of tuberculosis for patients with HIV coinfection

Diagnosis of TB with HIV coinfection is particularly challenging due to atypical chest radiographs and frequent negative sputum smears [56]. The rate of smear positivity in such cases is directly correlated to the extent of cavitary disease in patients with active TB. Patients with extensive cavitary disease generally have positive sputum smears [52]. The use of culture diagnostic methods is essential to confirm diagnosis in HIV seropositive patients with suspected TB [57]. Confirmation of TB diagnosis with HIV coinfection, is also complicated due to the high rates of extrapulmonary and disseminated TB in HIV positive individuals. Therefore, multiple specimen sampling and more sensitive methods of diagnosis such as Gene Xpert MTB/Rif® may be required to confirm diagnosis may be required to confirm the diagnosis [52]. In the past, the use of culture and NAAT diagnostic methods has often been unavailable in resource poor countries. However, resources made available by the Global Fund for AIDS, Tuberculosis, and Malaria have been mobilized in recent years to provide increased access to these improved diagnostic modalities. An algorithm provided by WHO aids the identification of TB for people with HIV associated TB that are smear negative or present with extrapulmonary TB, in low resource settings. The guidelines use expanded case definition along with the use of broad spectrum antibiotics and co-trimoxazole prophylaxis to prevent other common opportunistic infections [56]. HIV also has implications in the screening for latent TB in patients with HIV. TST and IGRA are primary methods to screen for latent TB and a positive PPD signifies the presence of TB infection. The sensitivity of TST is reduced in cases of HIV associated latent TB infections as a higher proportion of these patients are anergic, and thus PPD negative [58].

Treatment of tuberculosis for patients with HIV coinfection

Standard anti-tuberculosis regimens are generally used to treat TB with HIV coinfection with good treatment outcomes. However, the duration of treatment could be prolonged in cases of patients presenting with extensive cavitary disease at baseline, non-sputum converters after two months of treatment or if pyrazinamide is not used in the intensive phase [29]. Generally, Rifabutin, instead of rifampin is the choice of anti-TB drug when antiviral drugs are also being administered because rifabutin does not reduce antiretroviral serum levels to the extent as rifampicin does.

The drug regimen of drug resistant TB and HIV coinfections must be determined based on the sensitivity patterns of the bacterial isolate. Antiretroviral treatment (ART) is recommended alongside anti-tuberculosis irrespective of CD4 counts as ART is associated with a decreased in TB related mortality, and the progression of HIV disease [59]. There is substantial increase in adverse reactions seen during anti-tuberculosis therapy from drug-drug interaction, and additive drug toxicities during the co-administration of ART and TB drugs, especially rifamycin derivatives [60]. The most common adverse reactions are gastrointestinal intolerance, skin rashes, peripheral neuropathy, and hepatotoxicity, a condition commonly seen during anti-tuberculosis therapy but exacerbated in patients with HIV infections [61].

A meta-analysis study by Akolo C *et al* concluded that preventive anti-TB therapy with any first line TB drug reduces the incidence of progression to active TB (RR 0.68, 95% CI 0.54 to 0.85) in HIV positive patients. The decrease is more pronounced and statistically significant in patients with a positive TST compared to those with a negative TST (RR 0.38, 95% CI 0.25 to 0.57 v/s RR 0.89, 95% CI 0.64 to 1.24) [58].

Diabetes

Diabetes is an important comorbidity to consider for people with tuberculosis. The association between TB and diabetes has been established for centuries and diabetes has often been shown to be an attributable risk for poor TB outcomes such as increased mortality, incidence of relapse, and

treatment failure [62-65]. The current diabetes epidemic is unabated with 422 million people afflicted with type 2 diabetes and globally [66]. A recent study found the population attributable fraction of diabetes for tuberculosis to range from 3.4% to 16% in 22 of the high burden countries [67]. Case-control and cohort studies have shown that the relative odds of developing TB in diabetics is 2.44-8.33 compared to people without diabetes [68]. This risk is especially high in the Hispanic population as this population has increased prevalence of latent TB [69]. There are two types of diabetes; type1 (insulin dependent) and type 2 (non-insulin dependent). Studies show that the risk of developing TB in people with type 1 diabetes is higher than those with type 2 [68]. The probability of developing TB was also shown to be higher in patients with poor glycemic control as indicated by hemoglobin A 1 c blood levels [70]. Complications of poor management of diabetes include vascular complications leading to strokes and cardiovascular diseases, complications of the eyes, kidneys, nerves and increased susceptibility to infections. The immune response is impaired in diabetic patients and the impairment is irreversible even with administration of insulin [71]. Phagocytes, such as alveolar macrophages and lymphocytes that are part of the cell based immune response, play a critical role in clearing and control of *M. tuberculosis* and their functions have been shown to be adversely affected in diabetics [71]. Diabetes also affects other cell based responses such as T-cell functions that are hypothesized to increase susceptibility to TB [68].

Treatment of both diabetes and TB in patients is complicated because of drug interactions. Rifampicin interacts with hypoglycemic drugs and affects glycemic control resulting hyperglycemia [30]. It has been suggested that diabetes also affects the pharmacokinetics of TB drugs, especially slower absorption due to decreased gastrointestinal motility, however there is not enough evidence and more research is needed to make any conclusions [72].

Studies to determine the effect of diabetes on sputum -culture conversion have not been conclusive with relative risks that ranged from 0.79 to 3.25 [63] . However, in two reports, diabetic patients undergoing TB treatment have longer sputum-culture conversion times compared to control groups

(42 vs 37 days p-value=0.03) [62, 73]. Some studies suggest that bacterial clearance takes longer in diabetics due to higher baseline bacillary loads [68].

Body Mass Index (BMI)

Malnutrition and TB share a bidirectional relationship in which malnutrition is an important risk factor for the occurrence of TB disease as well as the clinical manifestation of TB and TB contributes to nutritional depletion [74]. The widespread prevalence of both malnutrition and TB poses an immense public health concern in low income countries, especially with regards to the control of TB. Studies to determine the effects of nutritional status on tuberculosis is complicated, however malnutrition adversely affects cell mediated immunity, a critical host defence against tuberculosis, thus rendering these individuals susceptible to primary TB infections and increased frequency of the progression of latent infections to active TB [75]. TB is often referred to as consumption or wasting because active disease is associated with altered metabolism, malabsorption of macro and micro-nutrients and loss of appetite [76]. These conditions are also the adverse reactions of the TB treatment drugs in many patients [76].

Results from an old study involving US Navy recruits showed that a higher proportion (4-fold increase) of positive TSTs were seen in individuals who were at least 10% underweight at baseline compared to those who were 10% overweight [77]. Body mass index (BMI) is defined as "body weight in kilograms divided by the square of the body height, and is universally expressed in units of kg/m^2, resulting from mass in kilograms and height in meters" and is an accurate representation of the nutritional status. A prospective cohort study also showed that the relative risk of acquiring TB was five-fold higher in the low BMI group as compared to the high BMI cohort, even after controlling for various factors [78]. More recent studies concluded that the risk of TB relapse and mortality is higher in individuals who had a body mass index of less than 18.5 kg/m^2 [79-81]. This research also showed that if a patient has a low baseline BMI, then weight gain of 5% or less during the intensive TB treatment phase, is significantly associated increased relapse risk [79]. A baseline BMI of less than 18.5 kg/m^2 was also determined to be an independent predictor of poor MDR-TB outcome in a

retrospective cohort study [41]. HIV positive individuals with negative smear and extrapulmonary TB were shown to have increased mortality (adjusted hazard ratio 4.05, 95% confidence interval 2.77–5.91, $p<0.001$) if they remained underweight or showed a decline in BMI after the initiation of TB treatment [82]. Low BMI, low serum albumin levels and anemia which is a manifestation of malnutrition are also associated with delayed sputum conversion in PTB infections [83-86].

Tobacco/Smoking

The causal association between smoking and TB has been investigated for many years. There is enough evidence that shows a dose-response relationship between smoking and active TB [87-89]. Active and passive smoking increase the risk of infection if exposed to tuberculosis. This risk increase is seen both in adults as well as children and is related to the number of cigarettes smoked and duration of smoking [90]. The TB-associated mortality risk is almost doubled in active smokers [91]. Treatment failure along with relapse is also common among smokers [90, 91]. Culture conversion was affected by cigarette smoking with smokers having increased odds (OR 2.28, 95% CI 1.02–5.33, $p = 0.04$) of remaining culture positive at the end of a two-month anti-TB regimen [92]. Other studies also corroborate these findings and some have demonstrated that the adjusted odds of culture non-conversion to be six times higher in current smokers compared to ex-smokers and this effect depended on the number of cigarettes smoked per day [92, 93]. Smoking also prolongs the time to sputum and culture conversion, thus resulting in the increased duration of treatment [94, 95].

Alcohol

Alcohol dependency has been shown to increase the risk of developing pulmonary diseases, such as tuberculosis and pneumonia, due to the adverse effect of alcohol on host immune response [96]. Two separate meta-analysis evaluated the association between TB disease and alcoholism, and yielded a pooled relative risk of 2.94 (95% CI: 1.89-4.59) for TB in heavy alcohol users, compared with non-heavy alcohol users, demonstrating a strong and significant association between heavy alcohol use (>

40g of alcohol/day) and /or alcohol use disorders (AUD) [97, 98]. An international study of 14 countries with high TB endemicity concluded higher risks of active TB in diabetic men who drank alcohol [99]. A time-series analysis found an association between tuberculosis and certain alcohol consumption indicators in Russia suggesting the role of alcohol in the incidence of TB in Russia [100]. Other research demonstrates that alcohol consumption resulted in poor TB treatment outcomes and reactivation of latent TB [98, 101]. Alcohol interferes with the absorption and metabolism of isoniazid, thus altering its pharmacokinetics [98]. Isoniazid serum concentrations were lowered and the drug had a shorter half-life due to interaction with alcohol [98]. There are no studies indicating the role of alcohol in smear/culture conversion or its effect on diagnosis of TB.

CHAPTER 3: Manuscript

Introduction

Tuberculosis is one of the oldest diseases known to mankind and is currently one of the top 10 causes of mortality worldwide. There were an estimated 10.4 million incident TB cases globally in 2015 with 1.4 million TB-related deaths [6]. The most common form of tuberculosis is pulmonary TB in which the causative bacteria, *Mycobacterium tuberculosis* complex, affects the lungs. Pulmonary TB is also the infectious form of TB because of aerosolization of droplet nuclei containing the tubercle bacilli are expelled during coughing, spitting, or sneezing. An estimated 85% of new, drug-susceptible TB cases are successfully treated and as a result 49 million TB-related deaths have been averted globally between 2000 and 2015 [6].

Sputum microscopy, which was developed more than a century ago, and culture methods to detect *M. tuberculosis* are critical components of diagnosis as well as monitoring of treatment for pulmonary TB. The absence of *M. tuberculosis* in a sputum smear sample when screened microscopically is indicative of a non-infectious condition. One performance indicator for TB treatment success is three consecutive, negative sputum cultures collected on different days [36]. Culture conversion, where no growth is observed in three consecutive sputum samples is the ideal indicator for response to therapy and lack of infectiousness [36]. The results from these bacteriological examinations of sputum are also used in hospital guidelines for patient isolation. Due to the high transmissibility of pulmonary or laryngeal TB infection, patients are often kept in respiratory isolation until they show conversion to negative sputum cultures [36].

There are several factors that influence the time to sputum culture conversion from positive to negative. Some of these factors include age, nutritional status, comorbidities such as diabetes and kidney disease, immunocompetency of the patient, HIV infection, presence of cavitary disease, history of tuberculosis, smoking habits and alcohol intake [39, 42, 43, 45, 92, 99]. In this study, we determined the predictors of time to culture conversion in patients admitted at a Texas Hospital between 1985-2010. Using statistical analysis, it was determined that HIV coinfection, use of IV

drugs, presence of multidrug-resistant TB and use of second line drugs for treatment were significantly associated with time to culture conversion in our studies. We also found that patients admitted before 1996 had longer average time to conversion compared to those who were admitted after 1996.

Methods

Study design

This was a retrospective cohort study based on data collected from medical and laboratory records at the University of Texas Health Science Center at Tyler (UTHSCT), from 1985 to 2010 for 961 patients. Patients were followed until the end of treatment outcome.

Laboratory results prior to admission at the University of Texas Health Science Center and patients with negative cultures at baseline were excluded from our studies. Based on these exclusion criteria, the analysis was limited to a final dataset of 706 patient.

Laboratory methods

Bacteriological methods included culturing of sputum samples using at least two culture media. In the initial years of the study, Löwenstein-Jensen (LJ) and Middlebrook 7H10 (M7H10) were used for culturing *M. tuberculosis.* In 1988, the semi-automated radiometric liquid culture method, BACTEC 460TB (Becton Dickinson, Sparks, MD) was added, replacing LJ media and itself being replaced 10 years later by BACTEC MGIT 960 fully automated non-radiometric liquid culture method. M7H10 was also used for drug susceptibility testing. AFB microscopy of sputum smears, using the Ziehl-Neelsen (ZN) staining method were routinely performed.

Definitions

A positive culture was defined as ≥ 1 colony of *Mycobacterium tuberculosis* and a positive smear was defined as ≥ 1 acid fast bacilli (AFB) per 100 high-power fields (HPF) [102].

Culture and smear conversion were defined as ≥3 consecutive negative cultures or smears from different sputum samples. Culture conversion definition also included sequential negative results even if there were missing culture(s) between them [39].

Multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB, first line drugs (FLD), second line drugs (SLD) and group 5 drugs were defined according to WHO guidelines [103]. For our analysis, the XDR data consisted of very few patients and these were therefore grouped with the MDR TB patients.

Time to conversion was defined in days from the date of admission to the sputum collection date for the first of three consecutive negative cultures.

Predictive variables

Predictive variables were selected based on previous studies that examined factors that determine conversion and time to conversion. Patient medical and clinical records were used to extract data for each variable. Variables included in our studies are demographic and clinical variables such as age, gender, race and body mass index, comorbidity conditions such as diabetes, cardiovascular disease and HIV status, TB-related variables include sputum smear results, previous history of TB, cavitary disease, MDR-TB and anti-TB drugs. Other possible influential factors included use of alcohol, tobacco and intravenous drugs. Information from discharge codes and on-going medicines were used to address missing values for diabetes and HIV. Hospital discharge diagnoses (ICD-9-CM codes) obtained from medical record summaries were used to determine the presence of cavitary disease. A calendar period variable was created where period 1 included 1985-1996 and period 2 included 1997-2010.

Outcome variables

Sputum culture conversion and time to conversion were the two outcome variables of interest and are detailed in the definitions section of methods above. Patients who did not have sputum culture conversion were censored on the date of their last sputum specimen.

Statistical analyses

All the patient information from medical records were double entered followed by data cleaning. Data was transferred to SAS version 9.4 (SAS Institute Inc., Cary, NC).

Statistical analyses of the database were performed using SAS software. A P value ≤ 0.05 was considered statistically significant. Chi-square analysis was performed to determine the association between categorical variables and sputum culture conversion. Wilcoxon rank-sum test was used to compare the medians for the continuous variables.

The primary outcome of interest, time to conversion of sputum cultures, was analyzed using the Kaplan-Meier (K-M) method stratified by each independent variable. Statistical significance of the difference between K-M plots was determined using the log-rank test. Cox proportional hazard regression modeling was used to identify independent predictors of time to sputum culture conversion.

Proportional hazards assumptions were assessed by graphically comparing estimated ln(-ln) survivor curves, and Schoenfeld's goodness-of-fit test. Extended Cox models were used to address issues of non-proportionality. Hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated from the regression model for the effect of each variable on time to initial culture conversion. Backward selection was used to arrive at the final model. Adjusted survival curves for each significant variable from the model was plotted.

Ethical considerations

The study design was reviewed and approved by the Institutional Review Board of Emory University, CDC and UTHSCT.

Results

Bivariate analysis of predictive variables with sputum conversion

Data analysis was performed for 706 patient records. Average age of the cohort was 46.2 years (±16.6 SD), with 148 females. Sputum culture conversion was achieved by 60.84% of the patients in this study.

Descriptive analysis for the cohort, comparing those with and without sputum culture conversion is shown in Table 1. The average age of the converters was 46.25 years (±14SD) with 85.8% males. Per the bivariate analysis, sputum conversion was significantly associated with alcohol consumption, gender, race, history of TB, use of intravenous drugs, presence of cavitary disease, MDRTB and treatment with first line drugs (FLDs), injectable drugs, fluoroquinolones, second line drugs (SLDs) and group 5 drugs.

Of the 706 patients records, 35 patients had missing data and the 671 patients with data to document the presence of diabetes, 97 (14.46%) were diabetic; however, no association was identified between sputum conversion and diabetes.

Among the race-ethnicity category, there was a larger percentage of sputum culture conversion among African Americans and Asians, 70% and 64%, respectively, than among non-Hispanic whites (53%), while 60% of Hispanics had culture conversion (p-value=0.001).

A greater proportion of patients with cavitary disease had sputum culture conversion by the end of the study as compared to those without cavitary disease (67% v/s 58%, p-value=0.034).

Of those with a previous history of TB, 70% of individuals successfully converted to sputum culture negative compared to 57% of new TB cases (p-value=0.001). A greater percentage of non-IV drug users converted compared to IV drug users (74% vs 60%, p-value=0.032).

Of the 82 MDRTB patients, 77% were found to have sputum culture conversion in our analyses. Among the non-MDRTB cohort, 59% converted and this difference between the two groups is significant (p-value=0.002).

In bivariate analysis, treatment with FLDs, injectable drugs, fluoroquinolones, WHO Group 5 drugs, and second line drugs in general was also found to be significantly associated with sputum conversion (Table 1). A greater percentage of sputum conversion was seen in patients treated with injectable drugs (69%) and second line drugs (68%) as compared to those who were not treated with these drugs (53% and 52%, respectively). A higher number of patients not treated with fluoroquinolones and Group5 drugs had sputum conversion compared to those treated with these drugs (Table 1).

Variables associated with time to conversion (TOC)

Kaplan-Meier analysis was done to determine the association between the variables and time to conversion (Table 2).

The median time to conversion was calculated to be 50 days (IQR 46-56). Alcohol consumption, HIV infection IV drug use, race, baseline smear status, treatment with FLDs and the period of analysis were significantly associated with time to conversion based on the log-rank statistics.

Among the race-ethnicity categories, Whites had the longest time of conversion (TOC) with a median of 63 days (IQR 50-70) and African Americans had the shortest median TOC (42 days, IQR: 36-50) compared to the other race categories. AFB smear positives took longer to convert as opposed to those who were AFB smear negative at the time of admission (57 days; IQR:51-62 v/s 26 days; IQR: 20-34). Both HIV positive patients and IV drug users had a shorter time to conversion (27 and 42 days respectively) as compared to HIV negative and non-IV drugs users (51 and 50 days), respectively.

These differences were found to be significant (Table 2). As expected, presence of drug resistant *M. tuberculosis* delayed the time to conversion (54 days for MDRTB v/s 50 days for non-MDRTB), however this difference was not statistically significant.

IN bivariate analysis, patients treated with first line drugs took longer to convert compared to those not treated with FLDs (Table 2). Those treated with two FLDs had the longest median time to conversion (62 days; IQR: 29-90) and those treated with four FLDs had the shortest median conversion time (50 days; IQR:43-55).

Patients admitted before 1996 had a slightly longer, but significant, time to conversion compared to those admitted after 1996 (51 vs 50 days, p-value= 0.021). This was true even for patients with or without MDRTB, although the results were not statistically significant for patients with MDRTB (Table 2).

Multivariable Analysis

Extended Cox regression model was performed as Schoenfeld residual analysis showed that treatment with injectable drugs violated the proportional hazards assumption. Based on the extended models, HIV infection (aHR= 1.69, 95% CI: 1.14-2.52) and IV drug use (aHR=1.54, 95% CI: 1.10-2.16) are independently associated with higher rates of culture conversion (Table 3). Among the race-ethnicity subgroup, African Americans (aHR=1.62, 95% CI: 1.26-2.07) had a higher rate of culture conversion compared to Whites and this was the only group that was significantly different in the analysis. A positive smear result at the time of admission (aHR=0.41, 95% CI: 0.32-0.53) is independently negatively associated with conversion. Lastly, the females in our cohort had lower conversion rates compared to males (aHR=0.70, 95% CI:0.52-0.95). In terms of treatment, only injectable drugs remained significantly associated with conversion.

Adjusted survival curves

Adjusted conversion curves for each variable that was independently and significantly associated with conversion were plotted. Participants without HIV, non-IV drug users and smear positive patients had faster conversion probability compared to those who were HIV positive (Fig.3B), IV drug users (Fig.4B) and smear negative (Fig. 5B). Compared to males, females had faster conversion probabilities (Fig. 6B).

Discussion

In this retrospective cohort study of 706 TB patients, 60.84% had sputum culture conversion in a median of 50 days. Using extended Cox regression modelling, predictive factors independently and significantly associated with conversion were gender, HIV, IV drug use, race, and smear results at the time of admission. It is important to note that in the present study, a hazard ratio of less than one indicates delayed conversion, while a hazard ratio greater than one indicates accelerated conversion.

HIV status was found to be significantly associated with time to conversion in KM curve analysis, where HIV negative patients showed longer time-to-sputum conversion. HIV was also found to be independently and significantly associated with conversion in multivariate analysis. Patients coinfected with HIV had a 69% higher hazard rate of culture conversion compared to those without HIV. Association of HIV infection with conversion and delayed time to conversion for HIV negative patients has also been reported by other studies [42, 43, 104]. The explanation for decreased time to conversion in patients with HIV coinfection offered by these studies and findings from our studies is that TB patients with HIV generally do not present with cavitary disease, have lower bacterial burdens, and therefore appear to have faster response to TB treatment. In our cohort, a significantly lower percentage of HIV positive patients had cavitary disease, 12% compared to 33% of HIV negative individuals. However, it is important to note that the frequency of negative sputum samples is also very high in cases with HIV coinfections [105]. Therefore, shorter culture conversion times should be interpreted with caution, as they may not always signify clearing of infection.

Like HIV status, there was a significant association between IV drug status, conversion and time to culture conversion in bivariate and multivariate studies. Interestingly, IV drug users took half as long to convert their cultures compared to non-IV drug users; these patients had a 54% greater hazard rate of culture conversion. A study by A. Domínguez-Castellano *et al*, is the only other study that found drug addiction to be independently associated with culture conversion [42]. This study also reported that drug addiction is associated with shorter time to culture conversion, however no explanation for these findings were given.

A positive or negative sputum smear at the time of admission or treatment is an important indicator of the time it takes for culture conversion. Patients with greater bacillary load take longer to convert their cultures, as determined by several studies [39, 42-45, 106, 107]. In our analyses, we show that participants with positive smears took almost 2.2 times longer to convert their culture to negative compared to those with negative smears. In multivariate analysis, smear results at the time of admission was significantly associated with culture conversion; patients with positive baseline smears had a slower rate of conversion, presumably because of a high bacterial burden. This has important clinical implications and stresses the importance of early diagnosis and treatment. It would be interesting to determine whether the baseline smear grade would be associated with delayed culture conversion and have adverse treatment outcomes, as shown in other studies [38, 108, 109].

We were interested in determining if the time to conversion differed during the study period. For this, we divided the data into two groups; period 1 contained data for patients admitted between 1985 and 1996 and period 2 had patient data from 1997 to 2010. Interestingly, we found that the time to conversion was slightly but significantly better for patients admitted after 1996 (by 1 day), irrespective of the MDRTB status. To our knowledge, this is the first study to report these findings. Possible explanations for these findings include improvements in use of standardized treatment regimens, reflected by updated treatment guidelines, following the resurgence of TB in the U.S., coupled with the increasing prevalence of HIV over the course of this analysis and the advent of highly active anti-retroviral combination chemotherapy for. As mentioned earlier, a resurgence of TB

was documented in the U.S.in the late 1980s which coincided with the HIV epidemic and emergence of MDRTB [11, 110]. The modification of treatment regimens, based on the presence of HIV and MDRTB, could have contributed to shortened conversion times seen in our studies.

There are several limitations to our analyses. This is a retrospective cohort study of available data, and not a prospective randomized controlled study. The data were collected from clinical records which could have missing or incorrectly entered data. As this study uses a hospital cohort, the results are not generalizable. However, because the patients were hospitalized until sputum culture conversion, they were mostly well controlled for treatment adherence and had detailed follow-up. The stratified sampling is accompanied by unequal probabilities of selection, however for statistical analysis we assumed that this is a simple random sampling of a larger population. Statistical methods for complex sample designs were beyond the scope of this study.

Conclusion

In this study, predictive factors of sputum culture conversion were evaluated through the duration of tuberculosis treatment. Most patients achieved culture conversion within 8 weeks from the initiation of treatment. The patients' HIV status, IV drug use, gender, race and baseline smear results are important determinants of culture conversion. These factors should be evaluated to determine which patients would be anticipated to have a slow culture conversion to negative, and would aid in more intensive clinical management of TB. Further studies could be designed to understand the effect of these predictors on the treatment outcomes in this cohort.

CHAPTER 4: Conclusion and Public Health Implications

The present study evaluated the factors affecting sputum culture conversion during the course of tuberculosis treatment. Survival analysis demonstrate that alcohol consumption, HIV infection IV drug use, race, baseline smear status, treatment with FLDs and the period of analysis were associated with time to culture conversion. Of these, only gender, HIV coinfection, use of IV drugs, race and baseline smear status were independently associated with conversion.

There are several public health implications of our findings. Culture conversion is not only used for monitoring treatment, but also considered an important outcome indicator of treatment efficacy in non-MDRTB and MDRTB infections in clinical studies [39, 111, 112]. Persistent positive cultures after two months of treatment is generally indicative of treatment failure and/or presence of MDR *M. tuberculosis* strains [39, 41]. Currently, treatment of MDRTB and XDRTB is one of the biggest hurdles in ending the TB epidemic [3, 103, 113]. The existing treatment of MDRTB includes the use of second line drugs that are expensive, less effective, and have more adverse reactions compared to first line drugs. Moreover, MDRTB treatment is extremely long and commonly lasts two years [113]. There are several ongoing clinical trials to develop new tuberculosis therapies and there is an increasing interest in using culture conversion as a surrogate, prognostic marker for endpoints in new anti-TB and anti-MDRTB clinical trials [114, 115]. Apart from use in clinical trials, sputum culture conversion is also used as a proxy biomarker of long-term cure [94, 116]. These findings, along with other studies could contribute towards using culture conversion as prognostic indicators.

Tuberculosis is a transmissible disease especially among close contacts and healthcare workers and is a recognizable risk in healthcare settings. Both CDC and WHO have guidelines for infection control that require a minimum patient isolation period which depends on the infectiousness of the patient [46, 48, 117]. Sputum culture conversion results are used to determine the period of isolation. A patient can be removed from isolation after three properly collected and processed sputum samples are bacteriologically negative [46, 48]. Predictors of culture conversion can be used to approximate

isolation periods and enhance patient management. This is especially useful in resource poor settings where there are inadequate infection control practices in place.

It is a common misconception that concomitant infection with HIV increases tuberculosis infectiousness. The present study indicates that HIV is associated with faster sputum conversion and higher rates of conversion to negative cultures, which are indicative of decreased infectivity. These results, along with other observational studies refute the hypothesis that TB patients with HIV coinfection are highly contagious. When started on effective therapy, HIV coinfected patients can be rendered non-contagious in a relatively short time [43]. These findings can be used to improve HIV-TB awareness programs and prevent further stigmatization associated with HIV.

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Figures and Tables

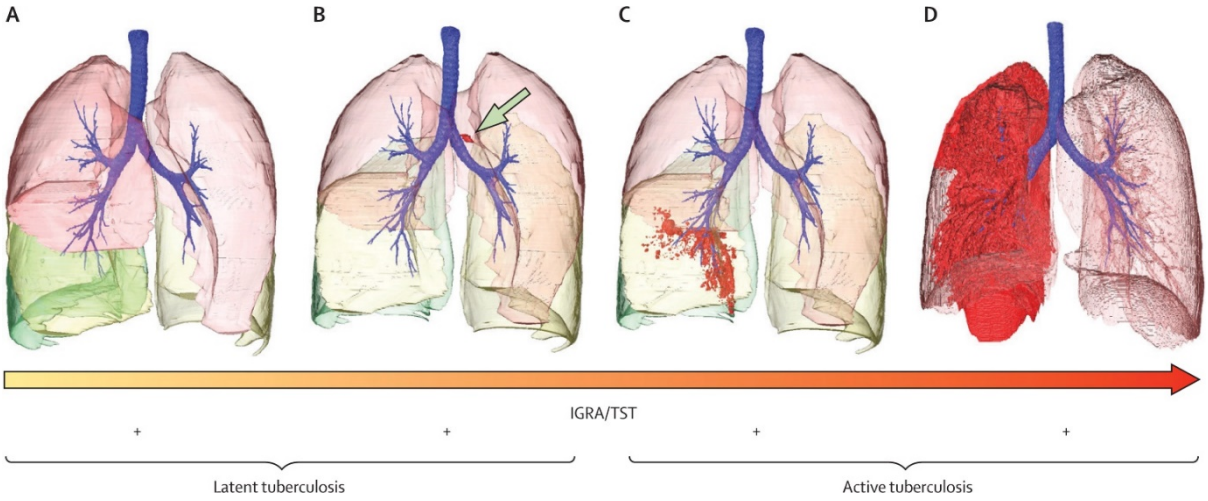


Figure 1. Outcomes of infection with *Mycobacterium tuberculosis* [20]

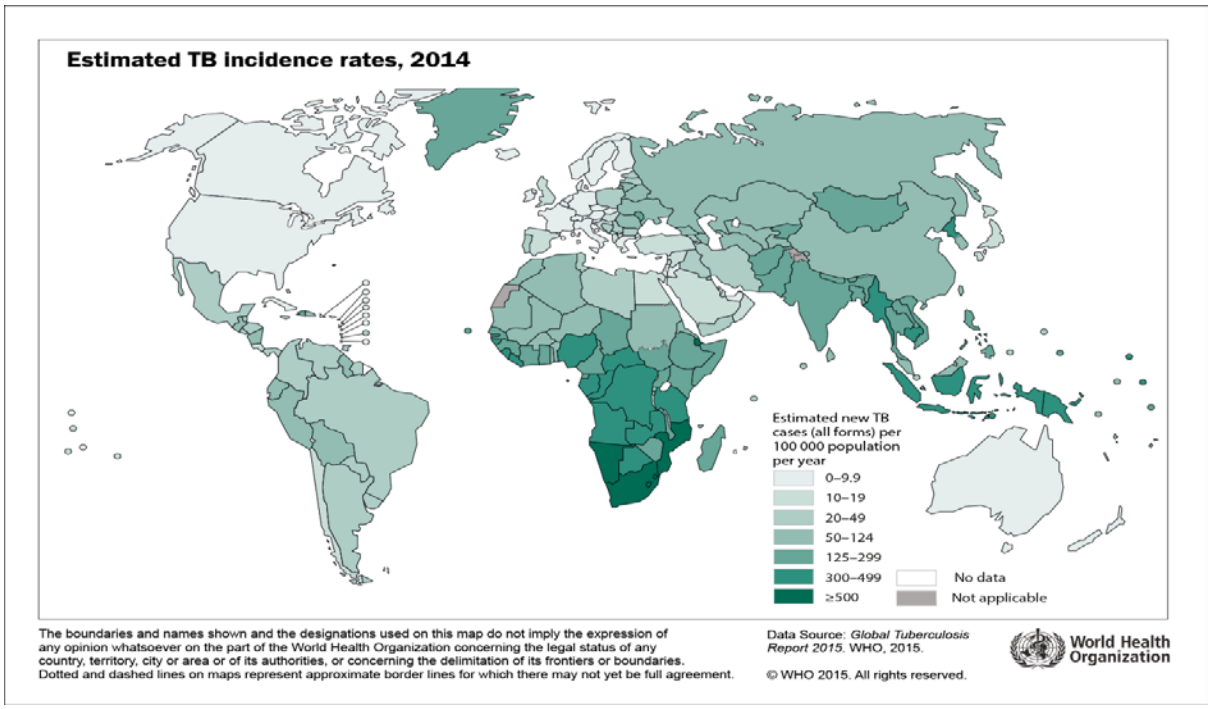
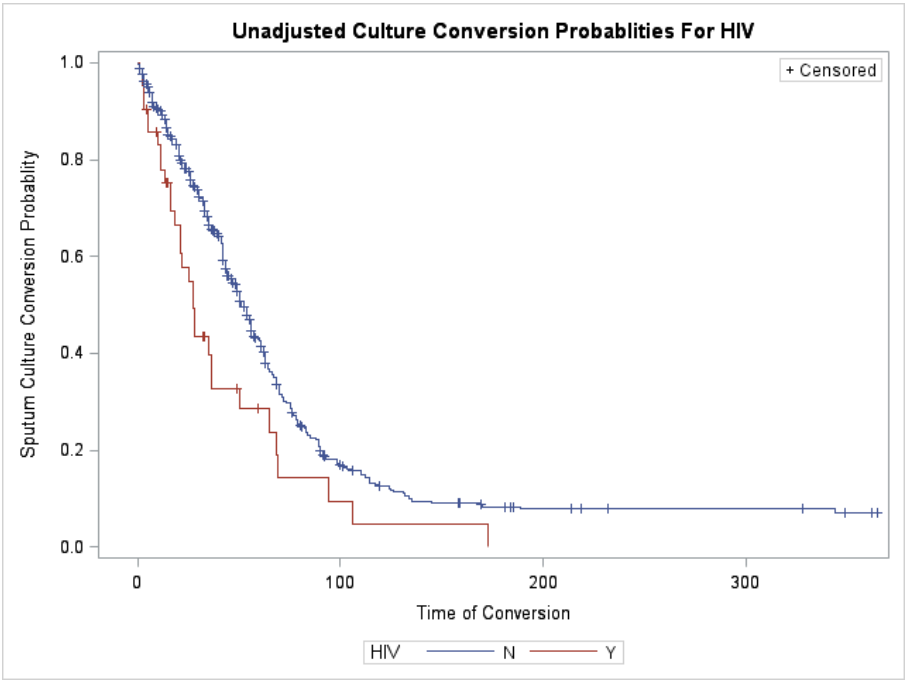


Fig.2. Estimated global TB incidence rates [118].

A



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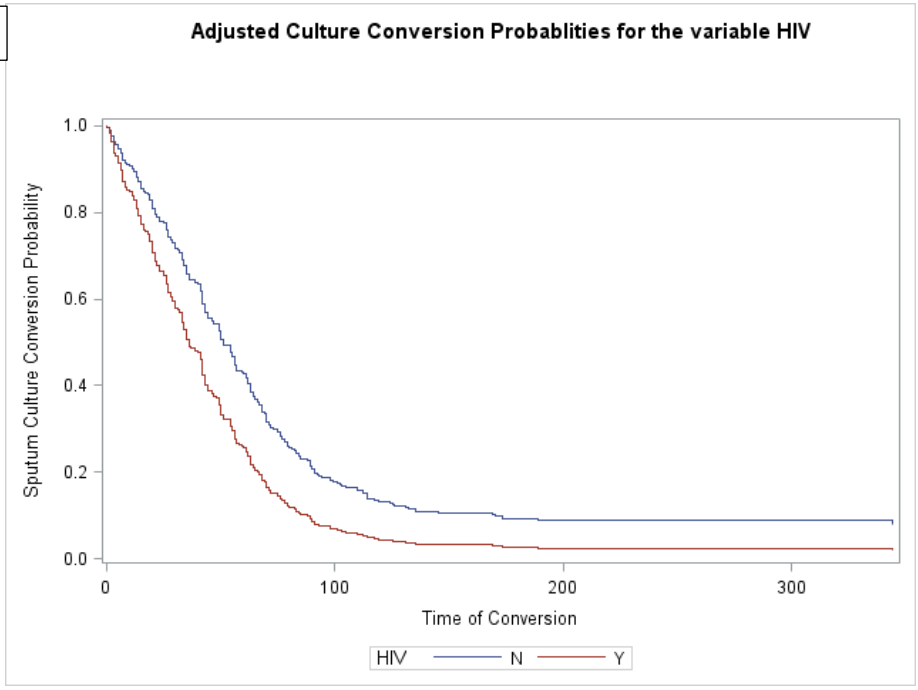
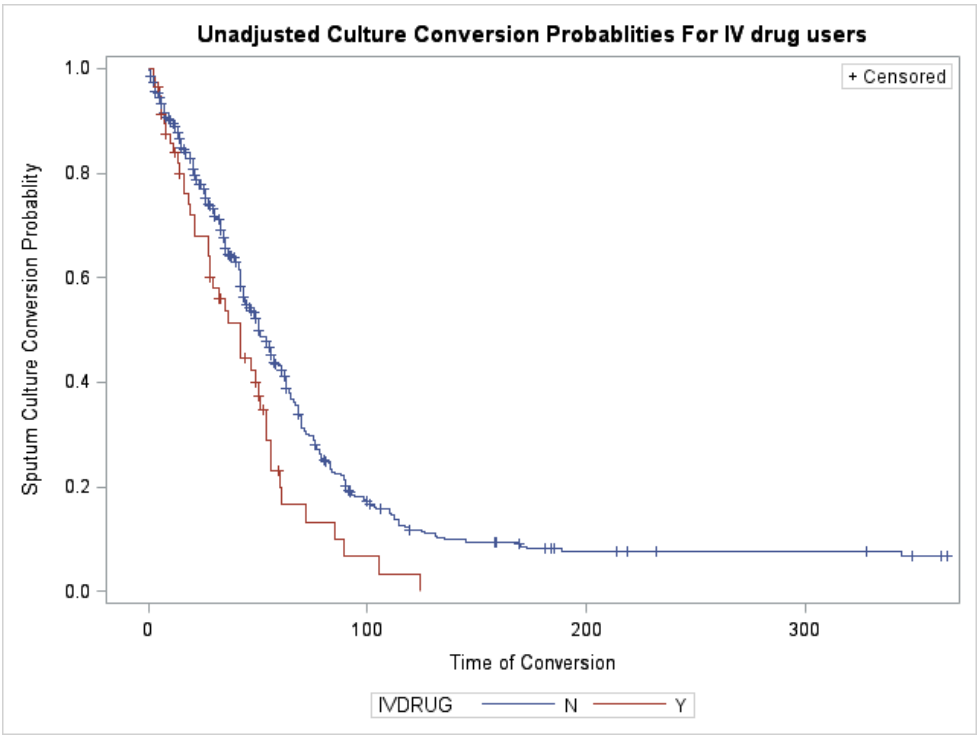


Figure 3A and B: Unadjusted and Adjusted survival curves for HIV coinfection; N=HIV negative, Y=HIV positive.

A



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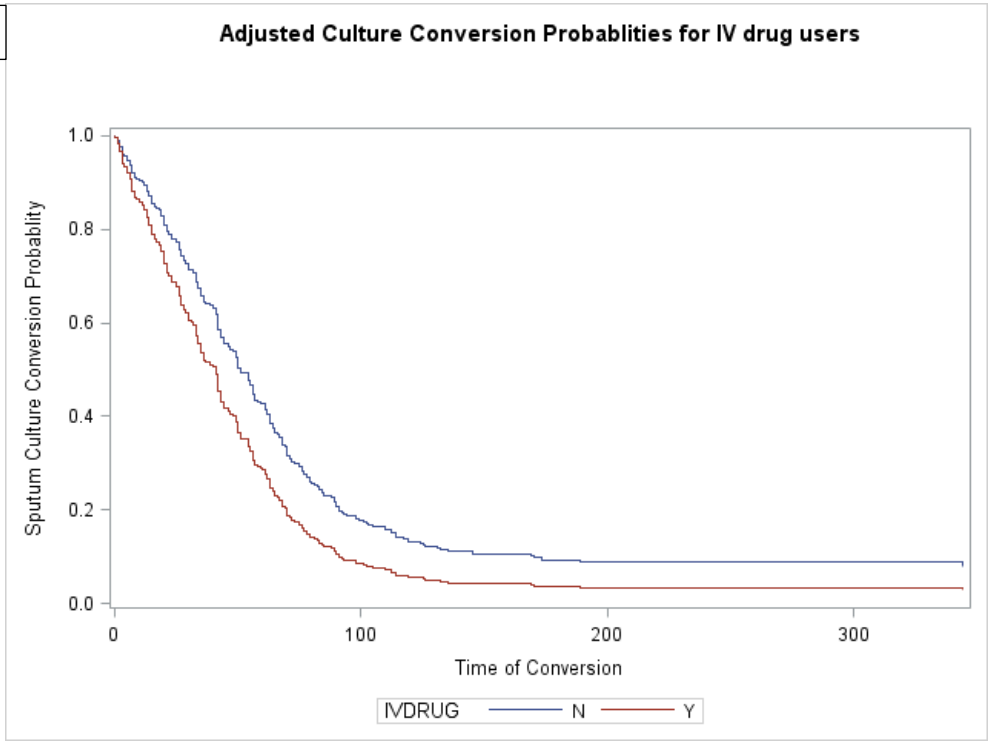
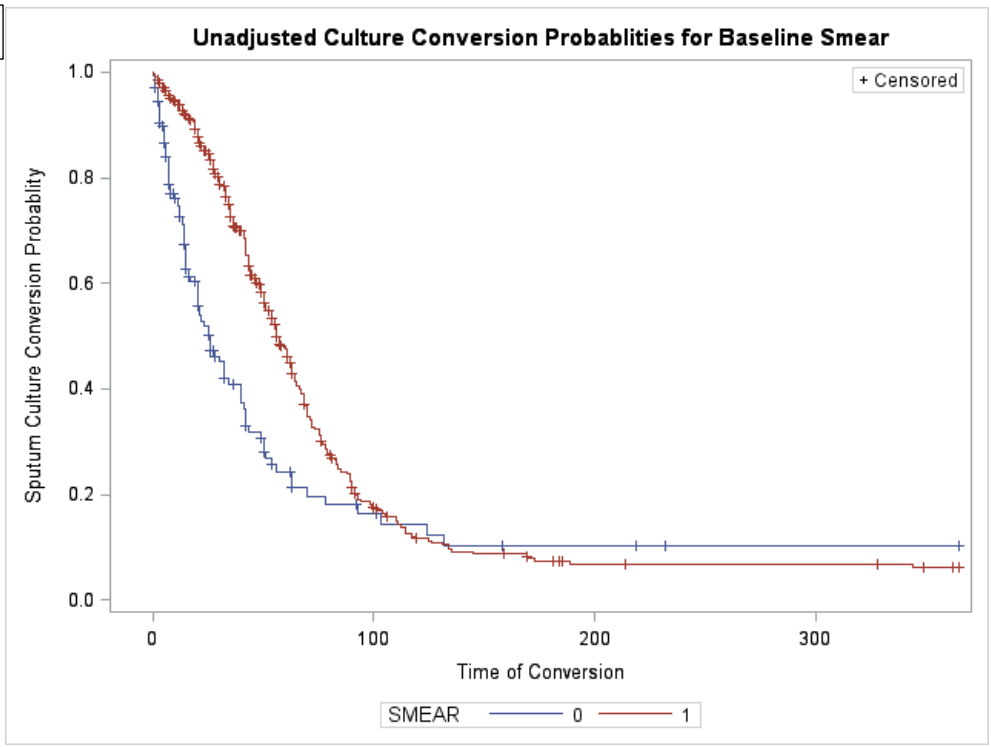


Figure 4A and B: Unadjusted and Adjusted survival curves for IV drug use; N=Non-IV drug user, Yes=IV drug user.

A



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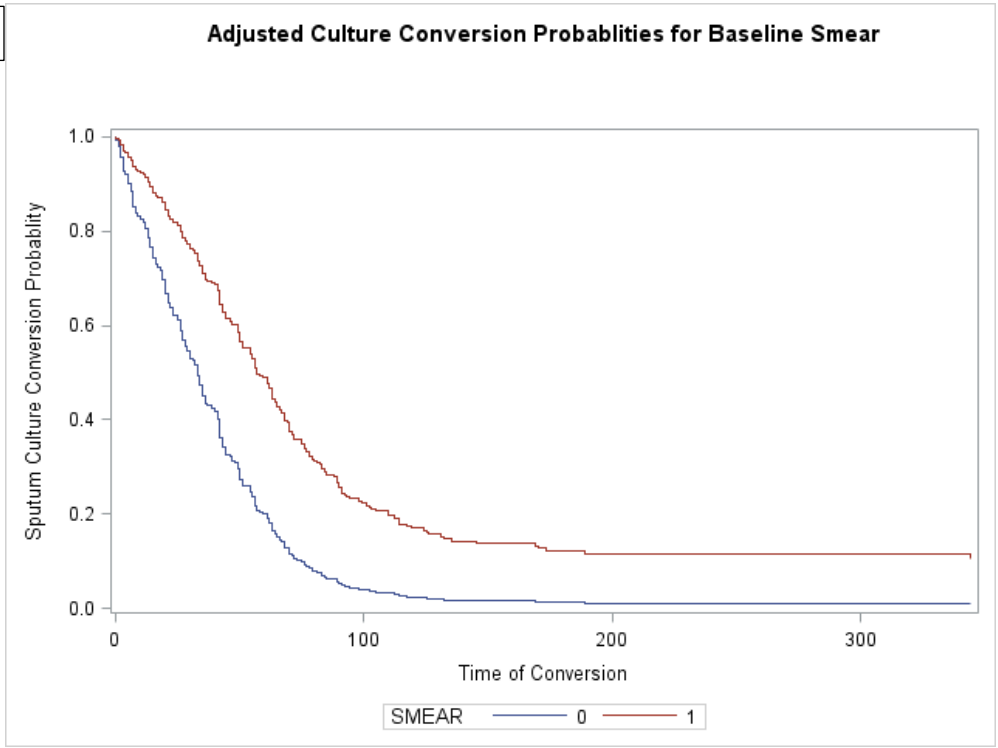
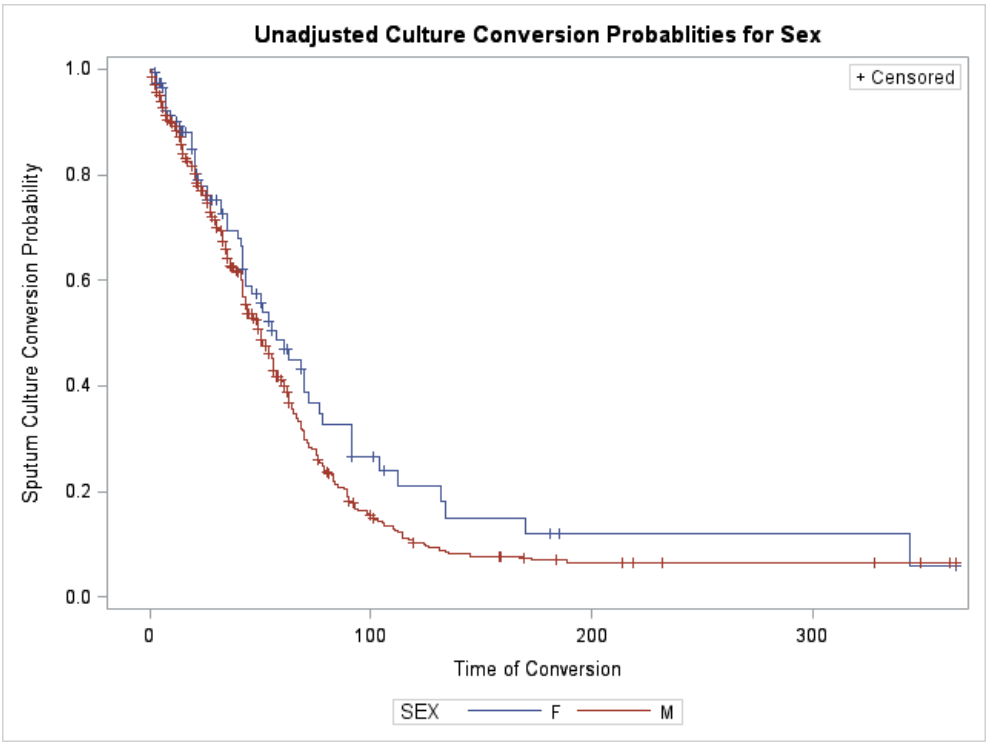


Figure 5A and B: Adjusted survival curves for baseline smear; 0=smear negative, 1=smear positive.

A



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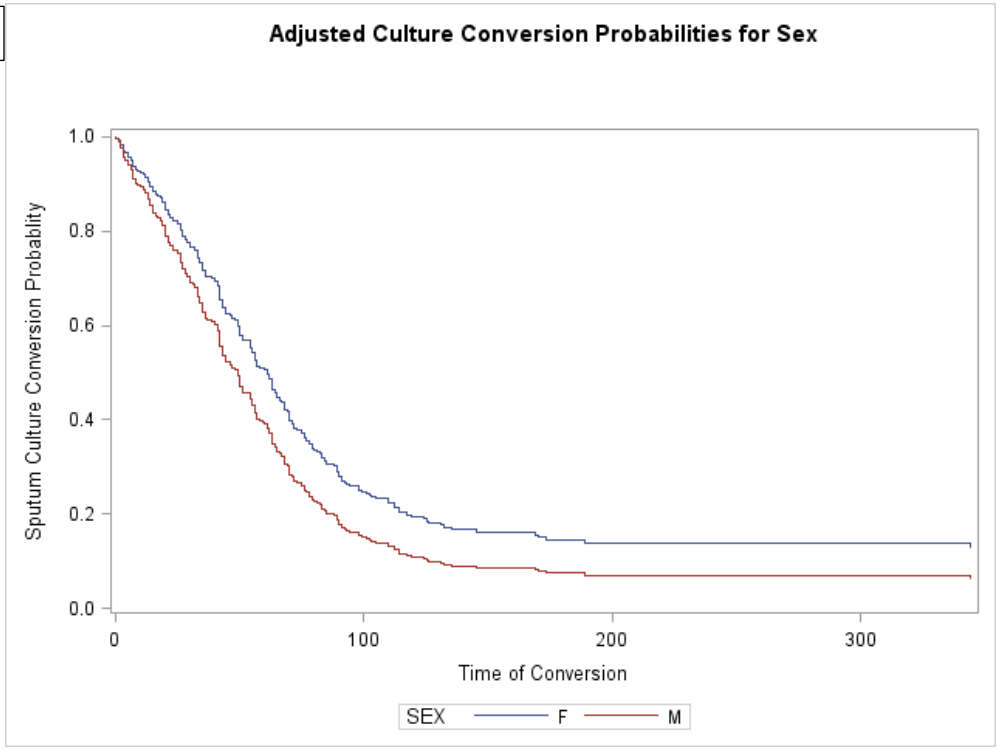

Figure 6A and B: Unadjusted and Adjusted survival curves for Sex; F=Female, M=Male.

Table 1: Demographic data of patients with tuberculosis who show sputum culture conversion at the end of the study.

Variable	Value	Effective sample size[1]	Total Number n (column%)	Number of converters n (row %)	Number of non-converters n (row %)	p-Value
Sex	Female	686	148 (21.57)	59 (39,.86)	89 (60.14)	<.0001
	Male		538 (78.43)	358 (66.50)	180 (33.50)	
Alcohol	Yes	679	294 (43.42)	206 (70.07)	88 (29.93)	<.0001
	No		385 (56.70)	211 (54.81)	174 (45.19)	
BMI category	0 (<18.5 kg/m^2)	622	218 (35.04)	142 (65.14)	76 (34.86)	0.133
	1 (18.5-24.5 kg/m^2)		330 (53.05)	201 (60.91)	129 (39.09)	
	2 (25-30 kg/m^2)		48 (7.71)	25 (52.08)	23 (47.92)	
	3 (>30 kg/m^2)		26 (4.18)	12 (46.15)	14 (53.85)	
Cardiovascular disease	Yes	633	272 (42.96)	171 (62.90)	101 (37.10)	0.672
	No		361 (57.03)	221 (61.22)	140 (38.78)	
Cavitary Disease	Yes	687	216 (31.44)	144 (66.67)	72 (33.33)	0.034
	No		471 (68.55)	274 (58.17)	197 (41.83)	
Diabetes	Yes	671	97 (14.45)	55 (56.70)	42 (43.30)	0.353
	No		574 (85.54)	354 (61.67)	220 (38.33)	
HIV	Yes	671	42 (6.25)	31 (73.81)	11 (26.19)	0.081
	No		629 (93.75)	379 (60.30)	250 (39.70)	
History of TB	New	686	497 (72.44)	284 (57.14)	213 (42.86)	0.001
	Previous		189 (27.55)	133 (70.37)	56 (29.63)	
IV Drug Use	Yes	664	58 (8.73)	43 (74.14)	15 (25.86)	0.032
	No		606 (91.26)	362 (59.74)	244 (40.26)	
MDRTB	Yes	687	82 (11.93)	63 (76.83)	19 (23.17)	0.002
	No		605 (88.06)	355 (58.68)	250 (41.32)	
Race	Asian	676	28 (4.14)	18 (64.29)	10 (35.71)	0.001
	Black		235 (34.76)	165 (70.21)	70 (29.79)	
	Hispanic		167 (24.70)	100 (59.88)	67 (40.12)	
	White		246 (36.39)	130 (52.85)	116 (47.15)	
Smear result at the time of admission	Positive	683	505 (73.93)	314 (62.18)	191 (37.82)	0.252
	Negative		178 (26.07)	102 (57.30)	76 (42.70)	
Tobacco Use	Yes	676	367 (54.28)	234 (63.76)	133 (36.24)	0.086
	No		309 (45.72)	177 (57.28)	132 (42.72)	
Treatment						
First Line Drugs (FLDs)	0	687	33 (4.80)	15 (45.45)	18 (54.55)	0.017
	1		37 (5.30)	26 (70.27)	11 (29.73)	

[1] Effective sample size is less than 706 due to a variable number of missing values.

Variable	Value	Effective sample size[1]	Total Number n (column%)	Number of converters n (row %)	Number of non-converters n (row %)	p-Value
	2		77 (11.20)	41 (53.25)	36 (46.75)	
	3		200 (29.11)	112 (56.00)	88 (44.00)	
	4		340 (49.49)	224 (65.88)	116 (34.12)	
Injectable Drugs	Yes	687	344 (50.07)	237 (68.90)	107 (31.10)	<.0001
	No		343 (49.93)	181 (52.77)	162 (47.23)	
Fluoroquinolones	Yes	687	180 (26.20)	133 (73.89)	47 (26.11)	<.0001
	No		507 (73.80)	285 (56.21)	222 (43.79)	
Group 5 Drugs	Yes	687	20 (2.90)	17 (85.00)	3 (15.00)	0.025
	No		667 (97.10)	401 (60.12)	266 (39.88)	
Second Line Drugs (SLD) overall	Yes	687	385 (56.04)	262 (68.05)	123 (31.95)	<.0001
	No		302 (43.96)	156 (51.66)	146 (48.34)	
Period	1 (1985-1996)	687	372 (54.14)	226 (60.75)	146 (39.25)	0.957
	2 (1997-2010)		315 (45.85)	192 (60.95)	123 (39.05)	

Table 2: Potential predictors for sputum culture time of conversion (TOC) for patients with TB

Variable	Value	TOC Median days (IQR)	Log-Rank p-value
Sex	Female	57 (43-72)	0.093
	Male	50 (44-56)	
Alcohol	Yes	49 (42-55)	0.014
	No	54 (42-55)	
BMI category	0 (<18.5 kg/m^2)	50 (42-56)	0.356
	1 (18.5-24.5 kg/m^2)	50 (44-57)	
	2 (25-30 kg/m^2)	66 (24-81)	
	3 (>30 kg/m^2)	33 (18-46)	
Cardiovascular disease	Yes	50 (44-58)	0.085
	No	50 (42-56)	
Cavitary Disease	Yes	50 (43-57)	0.18
	No	51 (44-61)	
Diabetes	Yes	51 (33-65)	0.71
	No	50 (44-56)	
HIV	Yes	27 (18-36)	0.002
	No	51 (47-56)	
History of TB	New	51 (44-57)	0.655
	Previous	50 (42-56)	
IV Drug Use	Yes	42 (27-51)	0.003
	No	50 (44-56)	
MDRTB	Yes	54 (34-73)	0.102
	No	50 (44-56)	
Race	Asian	50 (20-83)	0.002
	Black	42 (36-50)	
	Hispanic	57 (43-63)	
	White	63 (50-70)	
Smear result at the time of admission	Positive	57 (51-62)	<.0001
	Negative	26 (20-34)	
Tobacco Use	Yes	54 (42-63)	0.96
	No	50 (46-56)	
Treatment			
First Line Drugs (FLDs)	0	41 (27-91)	0.032
	1	54 (29-64)	
	2	62 (29-90)	
	3	55 (42-63)	
	4	50 (43-55)	
Injectable Drugs	Yes	50 (44-57)	0.882
	No	54 (42-58)	
Fluoroquinolones	Yes	50 (42-55)	0.476
	No	51 (44-59)	

Variable	Value	TOC Median days (IQR)	Log-Rank p-value
Group 5 Drugs	Yes	63 (22-94)	0.899
	No	50 (44-56)	
Second Line Drugs (SLD)	Yes	51 (46-57)	0.861
	No	50 (42-58)	
Period	1 (1985-1996)	51 (44-61)	0.02
	2 (1997-2010)	50 (42-56)	
Period 1	MDRTB: Yes	54 (35-91)	0.063
	MDRTB: No	51 (43-61)	
Period 2	MDRTB: Yes	46 (15-73)	0.063
	MDRTB: No	50 (42-56)	

Table 3: Multivariable analysis of predictors of sputum culture conversion in patients with TB

Variable		Unadjusted HR	95% CI	Adjusted HR	95% CI
Sex	M	1	NA	1	NA
	F	0.792	0.60-1.04	0.703	0.52-0.95
Smear result at time of admission	Negative	1	NA	1	NA
	Positive	0.529	0.42-0.66	0.411	0.32-0.53
HIV	No	1	NA	1	NA
	Yes	1.759	1.22-2.54	1.694	1.14-2.52
IVDRUG	No	1	NA	1	NA
	Yes	1.592	1.16-2.19	1.542	1.10-2.16
Race	White	1	NA	1	NA
	Asian	1.099	0.67-1.80	1.045	0.63-1.74
	Black	1.553	1.23-1.96	1.616	1.26-2.07
	Hispanic	1.224	0.94-1.59	1.314	0.99-1.75
Treatment with FLDs	No	1	NA	1	NA
	1	1.379	0.73-2.61	1.368	0.71-2.65
	2	0.838	0.46-1.52	0.844	0.45-1.57
	3	0.964	0.56-1.65	0.821	0.46-1.45
	4	1.265	0.75-2.14	1.304	0.75-2.26
Treatment with Injectable drugs	No	1	NA	1	NA
	Yes	0.986	0.81-1.20	1.332	1.07-1.66

APPENDIX A

SAS Code

```
libname tblab 'H:\Thesis';
options nocenter ls=255 ps=13000;

proc freq data=tblab.tbresults;
tables spectype;
run;
*COMBING LJ3 AND LJ6;
data tblab.PTBlab;
set tblab.tbresults;
IF LJ3 ='POS' AND LJ6='POS' THEN LJ='POS';
IF LJ3 ='POS' AND LJ6='NEG' THEN LJ='POS';
IF LJ3 ='POS' AND LJ6='N/D' THEN LJ='POS';
IF LJ3 ='POS' AND LJ6='CON' THEN LJ='POS';
IF LJ3 ='POS' AND LJ6='NTM' THEN LJ='POS';
IF LJ3 ='NEG' AND LJ6='POS' THEN LJ='POS';
IF LJ3 ='NEG' AND LJ6='NEG' THEN LJ='NEG';
IF LJ3 ='NEG' AND LJ6='N/D' THEN LJ='NEG';
IF LJ3 ='NEG' AND LJ6='CON' THEN LJ='NEG';
IF LJ3 ='NEG' AND LJ6='NTM' THEN LJ='NEG';
IF LJ3 ='N/D' AND LJ6='POS' THEN LJ='POS';
IF LJ3 ='N/D' AND LJ6='NEG' THEN LJ='NEG';
IF LJ3 ='N/D' AND LJ6='N/D' THEN LJ='';
IF LJ3 ='N/D' AND LJ6='CON' THEN LJ='';
IF LJ3 ='N/D' AND LJ6='NTM' THEN LJ='';
IF LJ3 ='CON' AND LJ6='POS' THEN LJ='POS';
IF LJ3 ='CON' AND LJ6='NEG' THEN LJ='NEG';
IF LJ3 ='CON' AND LJ6='N/D' THEN LJ='';
IF LJ3 ='CON' AND LJ6='CON' THEN LJ='';
IF LJ3 ='CON' AND LJ6='NTM' THEN LJ='NEG';
IF LJ3 ='NTM' AND LJ6='POS' THEN LJ='POS';
IF LJ3 ='NTM' AND LJ6='NEG' THEN LJ='NEG';
IF LJ3 ='NTM' AND LJ6='N/D' THEN LJ='NEG';
IF LJ3 ='NTM' AND LJ6='CON' THEN LJ='NEG';
IF LJ3 ='NTM' AND LJ6='NTM' THEN LJ='NEG';
RUN;
PROC FREQ DATA=tblab.PTBlab;
TABLES LJ*LJ3*LJ6/NOROW NOCOL NOPERCENT;
RUN;
PROC FREQ DATA=tblab.PTBlab;
TABLES LJ*LJ3*LJ6/LIST MISSING NOCUM;
RUN;


**COMBINING LJ+M7H10;

Data tblab.PTBlab1;
set tblab.PTBlab;
IF LJ ='POS' AND M7H10='POS' THEN SOLID='POS';
IF LJ ='POS' AND M7H10='NEG' THEN SOLID='POS';
IF LJ ='POS' AND M7H10='N/D' THEN SOLID='POS';
IF LJ ='POS' AND M7H10='CON' THEN SOLID='POS';
IF LJ ='POS' AND M7H10='NTM' THEN SOLID='POS';
IF LJ ='NEG' AND M7H10='POS' THEN SOLID='POS';
IF LJ ='NEG' AND M7H10='NEG' THEN SOLID='NEG';
```

```
IF LJ ='NEG' AND M7H10='N/D' THEN SOLID='NEG';
IF LJ ='NEG' AND M7H10='CON' THEN SOLID='NEG';
IF LJ ='NEG' AND M7H10='NTM' THEN SOLID='NEG';
IF LJ ='' AND M7H10='POS' THEN SOLID='POS';
IF LJ ='' AND M7H10='NEG' THEN SOLID='NEG';
IF LJ ='' AND M7H10='N/D' THEN SOLID='';
IF LJ ='' AND M7H10='CON' THEN SOLID='';
IF LJ ='' AND M7H10='NTM' THEN SOLID='NEG';
RUN;
PROC FREQ DATA=tblab.PTBlab1;
TABLES SOLID*LJ*M7H10/NOROW NOCOL NOPERCENT;
RUN;
PROC FREQ DATA=tblab.PTBlab1;
TABLES SOLID*LJ*M7H10/LIST NOCUM;
RUN;
PROC FREQ DATA=tblab.PTBlab1;
TABLES BACTEC SOLID;
RUN;


**COMBING SOLID+BACTEC;

Data tblab.PTBlab2;
set tblab.PTBlab1;
IF SOLID='POS' OR BACTEC='POS' THEN CUL='POS';
IF SOLID='NEG' AND BACTEC NE 'POS' THEN CUL='NEG';
IF BACTEC='NEG' AND SOLID NE 'POS' THEN CUL='NEG';
IF BACTEC NOT IN ('NEG','POS') AND SOLID NOT IN ('NEG','POS') THEN CUL=' ';
RUN;
PROC FREQ DATA=tblab.PTBlab2;
TABLES CUL*SOLID*BACTEC/LIST NOCUM MISSING;
RUN;

*SUBSETTING SPECTYPE WITH ONLY PTB SAMPLES;
data tblab.PTBlab3;
set tblab.PTBlab2;
where spectype in ('BAL',  'BRONCH',  'LUNG',  'NASALCAV', 'RESP_NOS'
'SPU',  'TRACHEA',  'TRACHEAL');
run;

proc freq data=tblab.PTBlab3;
tables spectype;
run;


*sorting;
proc sort data=tblab.PTBlab3;
by PTINDEX DATETBLAB Descending CUL;
run;
data tblab.PTBlab4;
set tblab.PTBlab3;
by PTINDEX;
IF FIRST.PTINDEX THEN NUMSPU=0;
NUMSPU+1;
DT1=LAG1(datetblab);
DT2=LAG2(datetblab);
IF NUMSPU=1 THEN DO; DT1=.; DT2=.; END;
IF NUMSPU=2 THEN DT2=.;
IF LAST.PTINDEX THEN DATEOFLASTSPU=DATETBLAB;
run;
```

```
proc print data= tblab.PTBlab4;
run;
PROC SORT DATA=tblab.PTBlab4;
BY PTINDEX NUMSPU;
RUN;

*CONVERSION;
data tblab.PTBlab5;
set tblab.PTBlab4;
BY PTINDEX;
IF FIRST.PTINDEX THEN NUMNEG=0;
IF CUL='NEG' THEN NUMNEG+1;
IF CUL='POS' THEN NUMNEG=0;
IF NUMNEG=3 THEN CONVERT='YES';
Else convert='NO';
IF NUMNEG=3 THEN DATECON=DT2;
RUN;

proc print data= tblab.PTBlab5 (OBS=100);
VAR PTINDEX DATETBLAB DT1 DT2 CUL CONVERT DATECON NUMSPU NUMNEG;
run;


DATA tblab.PTBlab6 ;
SET tblab.PTBlab5 ;
IF CONVERT='YES';
RUN;
PROC PRINT DATA=tblab.PTBlab6;
VAR PTINDEX NUMSPU CONVERT;
RUN;

PROC SORT DATA=tblab.PTBlab6;
BY PTINDEX NUMSPU;
RUN;

DATA tblab.PTBlab7;
SET tblab.PTBlab6;
BY PTINDEX NUMSPU;
IF FIRST.PTINDEX;
KEEP PTINDEX PTID CONVERT DATECON DATEOFLASTSPU;
RUN;

* Merging TB MYCO dataset with ABCD all dataset;
proc contents data=tblab.PTBlab7;
run;
proc contents data=tblab.tstball;
run;
proc sort data=tblab.PTBlab7;
by PTIndex;
run;
proc sort data=tblab.tstball;
by ptindex;
run;
data tblab.TBlabmerge;
merge tblab.tstball tblab.PTBlab7;
by PTIndex;
run;
proc contents data=tblab.TBlabmerge;
run;
proc print data=tblab.TBlabmerge;
```

```
run;

* Merging TB treatment dataset with TB Lab merge datset;
proc sort data=tblab.TBlabmerge;
by PTIndex;
run;
proc sort data=tblab.tx4tanu;
by ptindex;
run;
data tblab.TBlabTXmerge;
merge tblab.TBlabmerge tblab.tx4tanu;
by PTIndex;
run;
proc contents data=tblab.TBlabTXmerge;
run;
proc print data=tblab.TBlabTXmerge;
run;

*Assigning no to variable convert with blanks and rounding off PTINDEX TO
AN INTEGER.;
data tblab.TBlabmerge1;
set tblab.TBlabTXmerge;
IF Convert ='' THEN Convert='NO';
IF PTID=. THEN PTID=ROUND(PTINDEX,1);
run;

proc freq data= tblab.TBlabmerge1;
tables ptid convert;
run;

*Conversion of height and weight to calculate BMI;
Data tblab.TBlabmerge2;
set tblab.TBlabmerge1;

if 4<=htin<=7 then htin=12*floor(htin)+(10*(htin-floor(htin)));



if ptindex=1048.01 then awtkgs=.;

if ptindex=1053.01 then do; awtlbs=.; dwtlbs=.; htin=.; end;

if ptindex=1106.01 then do; age=.; awtkgs=.; htin=.; end;

if ptindex=1126.01 then htcm=.;

if ptindex=2034.01 then htin=.;

if ptindex=5009.01 then awtkgs=dwtlbs*0.4536-1;


/* calculate weight in kg and height in m */

weightkg=awtkgs;

if awtkgs=. then weightkg=awtlbs*0.4536;

heightm=htcm/100;

if htcm=. then heightm=htin*0.0254;
```

```
/* calculate BMI */

BMI=weightkg/heightm**2;

run;
proc print data=tblab.TBlabmerge2 noobs;
var heightm weightkg BMI;
run;

* Creating BMI categories 0=underweight, 1=normal, 2=overweight, 3=obese;
data tblab.TBlabmerge3;
set tblab.TBlabmerge2;
if BMI < 18.5 then BMIcat=0;
if  18.5<BMI<=24.9 then BMIcat=1;
if  25<BMI<=29.9 then BMIcat=2;
if  BMI>= 30 then BMIcat=3;
If BMI= . then BMIcat=.;
run;

Proc freq data=tblab.TBlabmerge3;
tables BMIcat;
run;

PROC CONTENTS DATA=tblab.TBlabmerge3;
RUN;

PROC UNIVARIATE DATA=tblab.TBlabmerge3;
      VAR age;
      HISTOGRAM age/ NORMAL;
      PROBPLOT age / NORMAL (MU=EST SIGMA=EST);
RUN;
*CORRECTIONS FOR RACE= ' ' AND RACE=X data;
PROC PRINT DATA=tblab.TBlabmerge3;
where Race= 'X';
run;
PROC PRINT DATA=tblab.TBlabmerge3;
where Race= '';
run;

DATA tblab.TBlabmerge4;
SET tblab.TBlabmerge3;
If PTINDEX= 447.02 then RACE= 'A';
If PTINDEX= 447.03 then RACE= 'A';
If PTINDEX= 3515.04 then RACE= 'U';
If PTINDEX= 3515.05 then RACE= 'U';
If PTINDEX= 4086.01 then RACE= 'A';
If PTINDEX= 4086.02 then RACE= 'A';
If PTINDEX= 4105.01 then RACE= 'A';
If PTINDEX= 6547.01 then RACE= 'A';
If PTINDEX= 9025.02 then RACE= 'B';
RUN;

PROC FREQ DATA=tblab.TBlabmerge4;
TABLE RACE;
RUN;

* CORRECTIONS FOR DIABETES AND HIV  DATA;
DATA tblab.TBlabmerge5;
SET tblab.TBlabmerge4;
If PTINDEX= 439.03 THEN DIABETES= 'Y';
```

```
If PTINDEX=454.02  THEN DIABETES= 'Y';
If PTINDEX= 454.02 THEN DIABETES= 'Y';
If PTINDEX=454.02  THEN DIABETES= 'Y';
If PTINDEX=454.02  THEN DIABETES= 'Y';
If PTINDEX=1007.01 THEN DIABETES= 'Y';
If PTINDEX=1007.01 THEN DIABETES= 'Y';
If PTINDEX=1022.03 THEN DIABETES= 'Y';
If PTINDEX=1022.04 THEN DIABETES= 'Y';
If PTINDEX=1039.01 THEN DIABETES= 'Y';
If PTINDEX=1039.01 THEN DIABETES= 'Y';
If PTINDEX=1060.01 THEN DIABETES= 'Y';
If PTINDEX=2028.02 THEN DIABETES= 'Y';
If PTINDEX=4024.03 THEN DIABETES= 'Y';
If PTINDEX=4024.03 THEN DIABETES= 'Y';
If PTINDEX=4059.01 THEN DIABETES= 'Y';
If PTINDEX=4059.01 THEN DIABETES= 'Y';
If PTINDEX=4059.01 THEN DIABETES= 'Y';
If PTINDEX=4059.01 THEN DIABETES= 'Y';
If PTINDEX=4059.01 THEN DIABETES= 'Y';
If PTINDEX=4059.01 THEN DIABETES= 'Y';
If PTINDEX=4129.01 THEN DIABETES= 'Y';
If PTINDEX=5522.01 THEN DIABETES= 'Y';
If PTINDEX=5522.01 THEN DIABETES= 'Y';
If PTINDEX=8004.01 THEN DIABETES= 'Y';
If PTINDEX=5513.01 THEN DIABETES= 'Y';
If PTINDEX=5513.01 THEN DIABETES= 'Y';
If PTINDEX=5504.01 THEN DIABETES= 'Y';
If PTINDEX=5504.01 THEN DIABETES= 'Y';
If PTINDEX=5504.01 THEN DIABETES= 'Y';
If PTINDEX=5504.01 THEN DIABETES= 'Y';
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If PTINDEX=5504.01 THEN DIABETES= 'Y';
If PTINDEX=5504.01 THEN DIABETES= 'Y';
If PTINDEX=5547.01 THEN DIABETES= 'Y';
If PTINDEX=5547.01 THEN DIABETES= 'Y';
If PTINDEX=5547.01 THEN DIABETES= 'Y';
If PTINDEX=5547.01 THEN DIABETES= 'Y';
If PTINDEX=5547.01 THEN DIABETES= 'Y';
If PTINDEX=5547.01 THEN DIABETES= 'Y';
If PTINDEX=5547.01 THEN DIABETES= 'Y';
If PTINDEX=5547.01 THEN DIABETES= 'Y';
If PTINDEX=5547.01 THEN DIABETES= 'Y';
If PTINDEX=6528.01 THEN DIABETES= 'Y';
*CORRECTIONS FOR HIV;
If PTINDEX=11.01 THEN HIV='N';
If PTINDEX=  18.01 THEN HIV='N';
If PTINDEX=2065.01 THEN HIV='N';
If PTINDEX=4086.02 THEN HIV='N';
If PTINDEX=4090.03 THEN HIV='N';
If PTINDEX=4095.02 THEN HIV='N';
If PTINDEX=4100.04 THEN HIV='N';
If PTINDEX=4103.05 THEN HIV='N';
If PTINDEX=4107.02 THEN HIV='N';
If PTINDEX=4108.02 THEN HIV='N';
If PTINDEX=102.01  THEN HIV='N';
If PTINDEX=1103.01 THEN HIV='N';
If PTINDEX=1132.01 THEN HIV='N';
If PTINDEX=5079.01 THEN HIV='N';
If PTINDEX=5085.01 THEN HIV='N';
If PTINDEX=8100.01 THEN HIV='N';
If PTINDEX=8101.01 THEN HIV='N';
If PTINDEX=8109.01 THEN HIV='N';
```

```
If PTINDEX=8118.01 THEN HIV='N';
RUN;

PROC FREQ DATA=tblab.TBlabmerge5;
TABLES RACE DIABETES HIV;
RUN;


PROC FREQ DATA=tblab.TBlabmerge5;
TABLES CONVERT ALCOHOL HXTB ILLDRUG IVDRUG TOBAC DISP;
RUN;

*EXTRAPULMONARY TB;

PROC FREQ DATA=tblab.TBlabmerge5;
TABLES KID LYM MEM PLR SKE;
RUN;

PROC PRINT DATA=tblab.TBlabmerge5;
WHERE DISP='';
RUN;

*Merging ABCD_ALL WITH SUMDST_RESULTS;

PROC SORT DATA=tblab.TBlabmerge5;
BY PTINDEX;
RUN;
PROC SORT DATA=tblab.SUMDST_RESULTS;
BY PTINDEX;
RUN;

DATA TBLAB.TBLABDST;
MERGE tblab.TBlabmerge5 tblab.SUMDST_RESULTS;
BY PTINDEX;
RUN;

PROC CONTENTS DATA=TBLAB.TBLABDST;
RUN;

PROC PRINT DATA=TBLAB.TBLABDST;
WHERE ALCOHOL= 'U';
VAR PTINDEX;
RUN;

PROC FREQ DATA=TBLAB.TBLABDST;
TABLES ALCOHOL;
RUN;
PROC PRINT DATA=TBLAB.TBLABDST;
 WHERE PTINDEX IN
 (6.01
 1021.01
 1045.01
 1069.02
 1072.01
 2036.01
 2098.01
 4025.01
 7044.01
 7047.01);
VAR DCDX1 DCDX2 DCDX3 DCDX4 DCDX5 DCDX6 DCDX7 DCDX8 DCDX9 PTINDEX;
```

```
RUN;


*REASSIGNMENT OF MISSING AND UNKNOWN VALUES FOR ALCOHOL IVDRUGS COPD CARDIO
BASED ON DCDX2 DATA;
DATA TBLAB.TBLABDST1;
SET TBLAB.TBLABDST;
If PTINDEX=6.01 THEN ALCOHOL= 'Y';
If PTINDEX=448.04 THEN ALCOHOL= 'Y';
If PTINDEX=4107.02 THEN ALCOHOL= 'Y';
If PTINDEX=455.02 THEN IVDRUG= 'Y';
If PTINDEX=5074.02 THEN IVDRUG= 'Y';
If PTINDEX=430.02 THEN IVDRUG='Y';
If PTINDEX=26.01 THEN COPD= 'Y';
If PTINDEX=1013.02 THEN COPD= 'Y';
If PTINDEX=1024.05 THEN COPD= 'Y';
If PTINDEX=1052.02 THEN COPD= 'Y';
If PTINDEX=2029.05 THEN COPD= 'Y';
IF PTINDEX=448.03 THEN CARDIO='Y';
IF PTINDEX=448.04 THEN CARDIO='Y';
IF PTINDEX=484.02 THEN CARDIO='Y';
IF PTINDEX=1112.01 THEN CARDIO='Y';
IF PTINDEX=3133.01 THEN CARDIO='Y';
IF PTINDEX=5064.01 THEN CARDIO='Y';
IF PTINDEX=5075.01 THEN CARDIO='Y';
IF PTINDEX=8129.01 THEN CARDIO='Y';
IF PTINDEX=8131.01 THEN CARDIO='Y';
IF PTINDEX=9101.01 THEN CARDIO='Y';
*REAASIGNMENT OF ALCOHOL AND TOBAC CATEGORIES INTO YES/NO OR MISSING
REASSIGNMENT OF RACE OTHER AND UNKNOWN CATEGORIES AS MISSING
HXTB AS NEW AND PREV;
IF ALCOHOL = 'C' THEN ALCOHOL='Y';
IF ALCOHOL = 'F' THEN ALCOHOL='N';
IF ALCOHOL = 'U' THEN ALCOHOL='';
IF TOBAC= 'C' THEN TOBAC= 'Y';
IF TOBAC = 'F' THEN  TOBAC='N';
IF TOBAC = 'U' THEN TOBAC='';
IF RACE= 'O' THEN RACE='';
IF RACE='U' THEN RACE='';
IF HXTB='OCC' THEN HXTB='PREV';
IF HXTB='OTH' THEN HXTB='PREV';
IF HXTB='REL' THEN HXTB='PREV';
IF HXTB='REC' THEN HXTB='PREV';
RUN;

PROC FREQ DATA=TBLAB.TBLABDST1;
TABLE  ALCOHOL TOBAC RACE HXTB IVDRUG COPD CARDIO;
RUN;

PROC FREQ DATA=TBLAB.TBLABDST1;
TABLE  SUMDST_INH SUMDST_RIF;
RUN;
*CLASSIFICATION OF MDRTB AND XDRTB;

DATA TBLAB.TBLABDST2;
SET TBLAB.TBLABDST1;
IF SUMDST_INH= 'R' AND SUMDST_RIF='R' THEN MDRTB='Y';
ELSE MDRTB='N';
RUN;

PROC FREQ DATA=TBLAB.TBLABDST2;
```

```
TABLE   SUMDST_INH SUMDST_RIF MDRTB;
RUN;

      DATA TBLAB.TBLABDST3;
      SET TBLAB.TBLABDST2;
      IF (SUMDST_CIP= 'R' OR SUMDST_LEV='R' OR SUMDST_OFL='R' OR
SUMDST_MOX='R')
      AND (SUMDST_CAP='R' OR SUMDST_KAN='R' OR SUMDST_AMK='R') THEN
XDRTB='Y';
      ELSE XDRTB='N';
      RUN;

PROC FREQ DATA=TBLAB.TBLABDST3;
TABLE  XDRTB;
RUN;

PROC PRINT DATA=TBLAB.TBLABDST3;
WHERE XDRTB= 'Y';
VAR PTINDEX;
      RUN;
* REASSIGNMENT OF  MDRTB=Y BASED ON DCDX DATA Inclusion of XDRTB as MDRTB;
 DATA TBLAB.TBLABDST4;
SET TBLAB.TBLABDST3;
IF PTINDEX= 1102.02 THEN MDRTB= 'Y';
IF PTINDEX= 4023.01 THEN MDRTB= 'Y';
IF PTINDEX= 4035.02 THEN MDRTB= 'Y';
IF PTINDEX= 5009.01 THEN MDRTB= 'Y';
IF PTINDEX= 1102.02 THEN MDRTB= 'Y';
IF PTINDEX= 490.14 THEN MDRTB='Y';
IF PTINDEX=3513.01 THEN MDRTB='Y';
IF PTINDEX=4086.01 THEN MDRTB='Y';
IF PTINDEX=4087.01 THEN MDRTB='Y';
RUN;

PROC FREQ DATA=TBLAB.TBLABDST4;
TABLES MDRTB XDRTB;
RUN;

*CREATING THE TIME TO CONVERSION(TOC)VARIABLE;

DATA TBLAB.TBLABDST5;
SET TBLAB.TBLABDST4;
IF CONVERT= 'YES' THEN TOC=DATECON-DATEADMIT;
RUN;
PROC FREQ DATA=TBLAB.TBLABDST5;
TABLE TOC;
RUN;

PROC Print DATA=TBLAB.TBLABDST5;
WHERE TOC < 0;
VAR PTINDEX TOC;
RUN;

*Derivation of tblab.first_toc dataset;

data one;
set tblab.tblabdst5; run;
proc sort data=one;
by ptid ptindex;
run;
```

```
proc freq data=one noprint;
tables ptindex/out=numindex;
run;

proc freq data=numindex;
tables count;
run;

proc freq data=one;
tables convert/missing;
run;

proc sort data=one;
by ptindex
descending convert;
run;

data one1;
set one;
lagindex=lag(ptindex);
run;

data one2;
set one1;
if ptindex=lagindex then delete;
if convert=' ' then convert='NO';
run;

proc freq data=one2;
tables convert;
run;
proc sort data=one2;
by ptindex;
run;

data two;
set tblab.lastcx;
rename datetblab=datelastlab;
run;
proc sort data=two;
by ptindex;
run;

data onetwo;
merge two one2;
by ptindex;
if toc=. then toc=datelastlab-dateadmit;
run;

proc univariate data=onetwo;
class convert;
var toc;
run;

proc sort data=onetwo;
by ptid ptindex;
run;


data first;
set onetwo;
```

```
by ptid;
if first.ptid;
run;

proc means data=first;
class convert;
var toc;
run;

proc univariate data=first nextrval=20;
class convert;
var toc;
run;


proc freq data=first;
table toc;
run;

data tblab.first;
set first;
run;

data tblab.first_toc;
set first; if toc < 0 then delete;
run;
*Merging first_toc with AFB dataset;

Proc contents Data=tblab.first_toc;
run;

Proc contents Data=tblab.afb4tanu;
run;


Proc sort Data= tblab.first_toc;
by PTINDEX;
run;
Proc sort Data=tblab.afb4tanu;
by PTINDEX;
run;
Data tblab.first_tocafb;
Merge tblab.first_toc (IN=A) tblab.afb4tanu;
by PTINDEX;
If A;
run;
Proc print data=tblab.first_tocafb;
run;
*Merging first_toc with treatement dataset;
Proc sort Data= tblab.first_tocafb;
by PTINDEX;
run;
Proc sort Data=tblab.tx4tanu;
by PTINDEX;
run;
Data tblab.first_tocafbtx;
Merge tblab.first_tocafb (IN=A) tblab.tx4tanu;
by PTINDEX;
If A;
run;
Proc print data=tblab.first_tocafbtx;
```

```
run;
Proc contents data=tblab.first_tocafbtx;
run;

*Creating CAVITARY variable; *REAASIGNMENT OF ALCOHOL AND TOBAC CATEGORIES
INTO YES/NO OR MISSING
REASSIGNMENT OF RACE OTHER AND UNKNOWN CATEGORIES AS MISSING
HXTB AS NEW AND PREV;
data tblab.first_tocafbtx1;
set tblab.first_tocafbtx;
ICD9DCDX1=COMPRESS(TRANWRD(ICD9DCDX1,'.',''));
ICD9DCDX2=COMPRESS(TRANWRD(ICD9DCDX2,'.',''));
ICD9DCDX3=COMPRESS(TRANWRD(ICD9DCDX3,'.',''));
ICD9DCDX4=COMPRESS(TRANWRD(ICD9DCDX4,'.',''));
ICD9DCDX5=COMPRESS(TRANWRD(ICD9DCDX5,'.',''));
ICD9DCDX6=COMPRESS(TRANWRD(ICD9DCDX6,'.',''));
ICD9DCDX7=COMPRESS(TRANWRD(ICD9DCDX7,'.',''));
ICD9DCDX8=COMPRESS(TRANWRD(ICD9DCDX8,'.',''));
ICD9DCDX9=COMPRESS(TRANWRD(ICD9DCDX9,'.',''));
ICD9DCDX10=COMPRESS(TRANWRD(ICD9DCDX10,'.',''));

TEMP1=SUBSTR(ICD9DCDX1,1,4);
TEMP2=SUBSTR(ICD9DCDX2,1,4);
TEMP3=SUBSTR(ICD9DCDX3,1,4);
TEMP4=SUBSTR(ICD9DCDX4,1,4);
TEMP5=SUBSTR(ICD9DCDX5,1,4);
TEMP6=SUBSTR(ICD9DCDX6,1,4);
TEMP7=SUBSTR(ICD9DCDX7,1,4);
TEMP8=SUBSTR(ICD9DCDX8,1,4);
TEMP9=SUBSTR(ICD9DCDX9,1,4);
TEMP10=SUBSTR(ICD9DCDX10,1,4);
CAVITY=0;
IF TEMP1='0112' THEN CAVITY=1;
IF TEMP2='0112' THEN CAVITY=1;
IF TEMP3='0112' THEN CAVITY=1;
IF TEMP4='0112' THEN CAVITY=1;
IF TEMP5='0112' THEN CAVITY=1;
IF TEMP6='0112' THEN CAVITY=1;
IF TEMP7='0112' THEN CAVITY=1;
IF TEMP8='0112' THEN CAVITY=1;
IF TEMP9='0112' THEN CAVITY=1;
IF TEMP10='0112' THEN CAVITY=1;
IF CONVERT='YES' THEN CON=1;
ELSE CON=0;
IF ALCOHOL = 'C' THEN ALCOHOL='Y';
IF ALCOHOL = 'F' THEN ALCOHOL='N';
IF ALCOHOL = 'U' THEN ALCOHOL='';
IF TOBAC= 'C' THEN TOBAC= 'Y';
IF TOBAC = 'F' THEN  TOBAC='N';
IF TOBAC = 'U' THEN TOBAC='';
IF RACE= 'O' THEN RACE='';
IF RACE='U' THEN RACE='';
IF HIV='U' THEN HIV='';
IF HXTB='OCC' THEN HXTB='PREV';
IF HXTB='OTH' THEN HXTB='PREV';
IF HXTB='REL' THEN HXTB='PREV';
IF HXTB='REC' THEN HXTB='PREV';
IF IVDRUG='U' THEN IVDRUG='';
IF afbsmear='N/D' THEN afbsmear='';
If AFBSMEAR="POS" then SMEAR=1;
IF AFBSMEAR="NEG" then SMEAR=0;
```

```
IF TOC=0 then TOC=0.001;

*grouping SLD,Creating the variable period and truncating TOC;

if tx_sld>2 then tx_sld=3;
if tx_sld>0 then sld=1; else sld=2;

yearadmit=year(dateadmit);
if yearadmit<1996 then period=1; else period=2;

if toc >365 then toc=365;
RUN;

*Merging first_tocsfbtx1 with basecult_posneg to create BLPOS dataset for
final analysis;

PROC SORT DATA=tblab.first_tocafbtx1;

BY PTINDEX;

RUN;



PROC SORT DATA=tblab.basecult_posneg;

BY PTINDEX;

RUN;



DATA ADDBLCX;

MERGE tblab.first_tocafbtx1 tblab.basecult_posneg;

BY PTINDEX;

RUN;



DATA BLPOS;

SET ADDBLCX;

IF BLCX= 'POS';
run;

Proc contents data=BLPOS;
run;
Proc Freq data=BLPOS;
Tables con;
Run;
*ASSOCIATION WITH CATEGORICAL VARIABLES and CONVERT;
PROC FREQ Data=BLPOS;
TABLE  convert * (sex race alcohol smear bmicat cardio cavity diabetes HIV
HXTB IVDRUG MDRTB TOBAC TX_FLD TX_INJ TX_FQ TX_GRP5 SLD) / CHISQ EXPECTED
NOPERCENT NOCOL;
RUN;
PROC FREQ Data=BLPOS;
```

```
TABLE  HIV*cavity/ CHISQ EXPECTED NOPERCENT NOCOL;
RUN;
*ASSOCIATION WITH AGE and CONVERT;
PROC TTEST DATA=BLPOS;
VAR AGE;
CLASS CONVERT;
RUN;
*SURVIVAL ANALYSIS;
ODS html sge=on;
PROC LIFETEST DATA= BLPOS PLOTS=(survival LLS) METHOD=KM;
TIME TOC*CON(0);
STRATA race; *ANALYSIS BY VARIABLE OF INTEREST;
RUN;
ODS html sge=off;

*Hazard Ratio Regression analysis;

OPTIONS PS=10000;
*Backward Selection;
PROC PHREG DATA=BLPOS;
CLASS SEX(ref='M' param=ref) ALCOHOL (ref='N' param=ref)  BMICAT CAVITY
(ref='0' param=ref) cardio DIABETES (ref='N' param=ref) HIV(ref='N'
param=ref)
HXTB IVDRUG(ref='N' param=ref)MDRTB (ref='N' param=ref) RACE(ref='W'
param=ref) SMEAR (REF='0' param=ref)TOBAC (ref='N' param=ref) TX_FLD TX_INJ
TX_FQ TX_GRP5 SLD (REF='2' param=ref);
MODEL TOC*CON(0)=AGE SEX ALCOHOL BMICAT CARDIO CAVITY DIABETES HIV HXTB
IVDRUG MDRTB RACE SMEAR TOBAC TX_FLD TX_INJ TX_FQ TX_GRP5 SLD /SELECTION=B
RISKLIMITS;
RUN;
*Forward Selection;
PROC PHREG DATA=BLPOS;
CLASS SEX(ref='M' param=ref) ALCOHOL (ref='N' param=ref)  BMICAT CAVITY
(ref='0' param=ref) cardio DIABETES (ref='N' param=ref) HIV(ref='N'
param=ref)
HXTB IVDRUG(ref='N' param=ref)MDRTB (ref='N' param=ref) RACE(ref='W'
param=ref) SMEAR (REF='0' param=ref)TOBAC (ref='N' param=ref) TX_FLD TX_INJ
TX_FQ TX_GRP5 SLD (REF='2' param=ref);
MODEL TOC*CON(0)=AGE SEX ALCOHOL BMICAT CARDIO CAVITY DIABETES HIV HXTB
IVDRUG MDRTB RACE SMEAR TOBAC TX_FLD TX_INJ TX_FQ TX_GRP5 SLD  /SELECTION=F
RISKLIMITS;
RUN;
*Stepwise Selection;
PROC PHREG DATA=BLPOS;
CLASS SEX(ref='M' param=ref) ALCOHOL (ref='N' param=ref)  BMICAT CAVITY
(ref='0' param=ref) cardio DIABETES (ref='N' param=ref) HIV(ref='N'
param=ref)
HXTB IVDRUG(ref='N' param=ref)MDRTB (ref='N' param=ref) RACE(ref='W'
param=ref) SMEAR (REF='0' param=ref)TOBAC (ref='N' param=ref) TX_FLD TX_INJ
TX_FQ TX_GRP5 SLD (REF='2' param=ref);
MODEL TOC*CON(0)=AGE SEX ALCOHOL BMICAT CARDIO CAVITY DIABETES HIV HXTB
IVDRUG MDRTB RACE SMEAR TOBAC TX_FLD TX_INJ TX_FQ TX_GRP5 SLD  /SELECTION=S
RISKLIMITS;
RUN;

*MODEL BASED ON BACKWARD SELECTION is selected because there was no
difference in the -2lnL and AIC between the models, however backward
selection had more variables of importance;
*Model Diagnostics using Schoenfel residual analysis with variables
selected by Backward selection;
```

```
PROC PHREG DATA=BLPOS;
CLASS SEX(ref='M' param=ref) HIV(ref='N' param=ref) IVDRUG(ref='N'
param=ref) RACE(ref='W' param=ref) SMEAR (REF='0' param=ref) TX_FLD TX_INJ
;
MODEL TOC*CON(0)=SEX  HIV IVDRUG RACE SMEAR TX_FLD TX_INJ / RISKLIMITS;  *
Gives 95% CI's;
OUTPUT OUT=tblab.resid1 RESSCH= r_SEX  r_HIV r_IVDRUG r_RACE r_SMEAR
r_TX_FLD r_TX_INJ;  *one term for each predictor;
RUN;

** Create a dataset containing only observations where an 'event' was
experienced **;
DATA tblab.events;
      SET tblab.resid1;
      IF con=1;
RUN;

** Rank these observations according to survival time **;
PROC RANK DATA=tblab.events OUT=tblab.ranked TIES=mean;
      VAR TOC;
      RANKS timerank; *creates a new variable for the ranked survival time
values;
RUN;

** Test for significant correlation between residuals & survival time ranks
**;
PROC CORR DATA=tblab.ranked;
      VAR r_SEX  r_HIV r_IVDRUG r_RACE r_SMEAR r_TX_FLD r_TX_INJ;
      WITH timerank;
RUN;

* Cox model estimation with interaction between log(TOC) and TX_INJ;
PROC PHREG DATA=BLPOS;
CLASS SEX(ref='M' param=ref) HIV(ref='N' param=ref) IVDRUG(ref='N'
param=ref) RACE(ref='W' param=ref) SMEAR (REF='0' param=ref) TX_FLD(ref='0'
param=ref)  TX_INJ (ref='0' param=ref) ;
MODEL TOC*CON(0)=SEX  HIV IVDRUG RACE SMEAR TX_FLD TX_INJ INJXTOC /
RISKLIMITS;  * Gives 95% CI's;
INJXTOC=TX_INJ*TOC;
RUN;

*Adjusted survival curves;
ODS GRAPHICS ON;
ODS html sge=on;
PROC PHREG DATA=BLPOS PLOTS(OVERLAY)=survival;
CLASS SEX(ref='M' param=ref) HIV(ref='N' param=ref) IVDRUG(ref='N'
param=ref) RACE(ref='W' param=ref) SMEAR (REF='0' param=ref) TX_FLD
(ref='0' param=ref) TX_INJ (ref='0' param=ref) ;
MODEL TOC*CON(0)=SEX  HIV IVDRUG RACE SMEAR TX_FLD TX_INJ / RISKLIMITS;  *
Gives 95% CI's;
baseline covariates=BLPOS outdiff=Diff1 out=pred survival=_all_/diradj
group=TX_INJ;
run;
ODS html sge=on;

proc print data=pred; run;

*CALCULATION OF UNADJUSTED HR;
PROC PHREG DATA=BLPOS;
Class SMEAR (REF='0' param=ref);
MODEL TOC*CON(0)=Smear/ RISKLIMITS;  * Gives 95% CI's;
```

```
RUN;

*Adjusted survival curves option2;
PROC PHREG DATA=BLPOS;
CLASS SEX(ref='M' param=ref) HIV(ref='N' param=ref) IVDRUG(ref='N'
param=ref) RACE(ref='W' param=ref) SMEAR (REF='0' param=ref) TX_FLD(ref='0'
param=ref)  TX_INJ (ref='0' param=ref) ;
MODEL TOC*CON(0)=SEX  HIV IVDRUG RACE SMEAR TX_FLD TX_INJ / RISKLIMITS;  *
Gives 95% CI's;
Baseline out=out2 survival=S2 LOGLOGS=LS2;
run;
Proc GPLOT data=out2;
PLOT S2*TOC=HIV;
TITLE Adjusted Survival of HIV;
run;

Proc GPLOT data=out2;
PLOT LS2*TOC=HIV;
TITLE LOG-LOG curves adjusted for sex, HIV, IVDrugs, race, smear, Treatment
with FLDs and injectables;
run;
```