Biomarkers for Risk of Tuberculosis and for Tuberculosis Treatment Failure and Relapse

Study Synopsis

Title: Biomarkers for Risk of Tuberculosis and for Tuberculosis Treatment Failure and Relapse

Phase: Observational Study

Population: Smear-positive pulmonary TB subjects attending Primary Health Centres (PHC) in Pondicherry and Tamil Nadu, India and their corresponding household contacts aged at least 6 years

Study Aims:

Aim 1. Identify biomarkers for risk of treatment failure in the TB case cohort of adults and children ≥ 6 years.

Aim 2. Identify biomarkers for risk of development of TB in the household contacts cohort.

Aim 3. In both cohorts, determine the impact of risk factors (diabetes mellitus, helminth infection, HIV, malnutrition, smoking, alcoholism, and anemia) on treatment outcome in pulmonary tuberculosis (PTB) and latent tuberculosis infection (LTBI) and define:

a. The odds ratio for the occurrence of TB, LTBI, and TB treatment failure comparing those with and without co-morbidities.

b. The impact of co-morbidities on transcriptomic risk profiles predictive of treatment failure and/or progression to active TB.

Aim 4. Perform network analysis of the transcriptome profiles to define stages in the continuum between LTBI and PTB and their immunologic concomitants.

Overview of Study Design

This protocol includes two cohorts: a cohort of patients with sputum smear positive pulmonary TB (PTB) and a second cohort of their household contacts (HHC). The clinical study will determine the impact of risk factors (diabetes mellitus, helminth infection, HIV, malnutrition, smoking, alcoholism, and anemia) on treatment outcomes in PTB and the association of these co-morbidities with the development of pulmonary TB (PTB) and latent

TB infection (LTBI). The fundamental research focused on the host transcriptome as a sensitive indicator of the replication of *Mycobacterium tuberculosis* (Mtb). The cohorts will provide descriptive data essential to local TB control including the prevalence of Mtb infection, HIV, drug resistance, and co-morbidities; and also will provide populations accessible for future trials of new TB diagnostics, treatments and preventive modalities including vaccines.

Study eligibility criteria for all index cases was based on health information that was collected by RNTCP as part of the routine TB care offered to all new TB cases. Household contacts were enrolled subsequently. Enrollment of study households was completed within 8 weeks of the initial index case evaluation; all study protocol activities will be completed within two years of the initial index case evaluation (up to 4 study visits to index cases, 2 visits to all household contacts including combined visit to index case and contacts at baseline, and an additional visit for TST+ controls at 24 months).

Study Definitions

Identification of Index TB Cases (IC): All consecutive pulmonary TB cases with sputum Ziehl-Neelsen stain positive for acid-fast bacilli (AFB) (≥1+) will be eligible to participate if they are Category I (new smear-positive PTB) patients and have no history of TB disease (i.e., no history of partial or complete treatment for a previous TB episode) (See inclusion/exclusion criteria below).

Treatment failure in index case will be defined as having a positive Ziehl-Neelsen sputum smear after five months of treatment and positive sputum culture for Mtb.

Treatment relapse index case will be defined as being diagnosed with a recurrent episode of TB confirmed by having a positive Ziehl-Neelsen sputum smear after five months of treatment and positive sputum culture for Mtb after the participant was declared cured or treatment completed/bacteriologic status indeterminate at the end of his/her most recent course of treatment.

IC Inclusion Criteria

1. All cases must meet these inclusion criteria:
2. At least 6 years old
3. Sputum Ziehl-Neelsen stain positive for AFB (≥1+)

4. Culture positive for Mtb; those who are smear+ but ultimately culture negative, will be included until their culture results return at which time they will retrospectively be removed from the study.
5. No history of TB treatment (i.e., no history of partial or complete treatment for a previous TB episode)
6. Intends to complete TB therapy for the recommended duration
7. Plans to enroll in the DOTS program for treatment
8. Intends to reside in the study area for the duration of their treatment

Index cases will be excluded if they refuse an HIV test and do not have documentation of a positive HIV test or a documented negative HIV test result within the past 3 months. They can be included regardless of their HIV status (known infected, known uninfected) or of the number of HHC fulfilling close contact criteria (see below).

IC Exclusion Criteria

1. An index case can be excluded if they:
2. Received > 1 week (five daily or 3 intermittent) doses of multi-drug TB therapy in the preceding 30 days
3. Known MDR or XDR-TB at diagnosis or known household contact of MDR or XDR-TB case
4. Chose not to initiate or complete the treatment course.
5. Received more than seven days of fluoroquinolone therapy or other drugs with anti-TB activity (e.g., rifampicin, ethambutol, clofazamine, aminoglycosides) for any reason in the preceding 30 days.
6. Too sick to enroll, defined as a Karnofsky score of 10 (moribund, fatal processes progressing rapidly) or less.

Household Contacts

Study Definitions

Identification of Household Contacts (HHC): A contact is defined as anyone age ≥6 years who on average had significant contact with the index case for at least 3 months before study enrollment as defined by:

- Sleeps under the same roof as the index case on average at least 5 days per week; or

- Shares at least one meal per day with the index case on average at least 5 days per week; or
- Watches television (or equivalent) with index case on average at least 5 days per week.

An HHC will be excluded if they:

- Have a previous history of TB (regardless of treatment history).

Up to 200 HHC found to have TB disease at the baseline evaluation will be enrolled as co-prevalent cases.

Incident PTB in the HHC will be defined as: culture or GeneXpert positive. Co-prevalent or incident cases in HHCs that are smear negative will be enrolled as cases for the case-control study.

HHC Inclusion Criteria

All HHC must meet these inclusion criteria:

- ≥6 years; and
- Intends to reside in the study area for the coming year; and
- Sleeps under the same roof as the index case on average at least 5 days per week; or
- Shares at least one meal per day with the index case on average at least 5 days per week; or
- Watches television (or equivalent) with index case on average at least 5 days per week.

HHC Exclusion Criteria

A household contact can be excluded if they:

- Have a previous history of TB (regardless of treatment history)

Schedule of Evaluations

Study Measurement	Screening (Days 0-14)	Baseline (at time of enrollment)	Follow-up (Month 2)	Follow-up (End of Rx)[3]	Follow-up (Month 12)	Follow-up (Month 24)
Index Cases						
Informed consent	X					
Medical history		X				
Anthropometrics		X				
Symptom/treatment questionnaire		X				
Sputum smear[1]	X	X	X	X		
Blood collection, RNA storage, helminth serology[2]		X	X	X		
CXR, sputum culture		X				
Pregnancy testing if applicable		X				
HIV testing if applicable[2]		X				
Co-morbidity testing (DM, etc.)		X				
Parasite testing: stool collection for ova and parasites		X		X		
Subset: treatment failure (n≈56) and 2 age and gender-matched controls responding to treatment (n≈112)						
Sputum culture				X		
Month 2 sample transcriptome analysis				X		
Parasite testing: stool collection for ova and parasites, helminths serology				X		
Co-morbidity questionnaire				X		
HIV testing if applicable				X		
Blood collection for transcriptome				X		
Household Contacts						
Informed consent		X				
Medical history and exposure questionnaire		X				
Anthropometrics		X				
TST		X				
Blood collection; helminth		X				

serology						
Status check if present for IC visit			X	X		
Parasite testing: stool collection for ova and parasites Sputum culture		X X	X		X X	X
Subset: incident cases (n≈70), 4 age and gender-matched controls per case (n≈280); co-prevalent cases (n≤200), matched controls (n≤800)						
Cases:						
CXR, sputum culture[2]					X	
Pregnancy testing					X	
HIV testing if applicable[2]					X	
Cases and Controls:						
Baseline sample transcriptome analysis					X	
Co-morbidity testing (DM, etc.)					X	
Parasite testing: stool collection for ova and parasites, helminths serology					X	
Co-morbidity questionnaire					X	
Controls:						
Status check TST[4]					X	X

[1] Collected in part by RNTCP, or study personnel as needed

[2] Only in consenting subjects

[3] Approximately month 5 for treatment failure IC, month 6 for IC with standard course of treatment, month 7 for IC with additional month of intensive phase treatment

[4]Only TST - controls