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DIAGNOSIS AND PREVENTION OF ACTIVE TB DISEASE

By

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Doctor of Philosophy

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Abstract

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By Amyn Abdul Malik

Tuberculosis (TB) is an infectious disease that results in over 10 million cases each year. One million of these cases occur in children with 25% (250,000) dying from TB. More than 80% of the deaths occur in children younger than 5 years old and nearly all of them (96%) were not receiving treatment. One of the main reasons for children not being on treatment is under-diagnosis due to poor performance of available diagnostic tools in children. Another group at high risk of developing active TB disease is household contacts of TB patients. Unfortunately for contacts exposed to drug-resistant TB at home, there is no consensus on the use of preventive treatment.

In **Aim 1**, we attempted to validate the use of monocyte-lymphocyte ratio (MLR) as a tool for TB diagnosis in children and created a risk score to improve diagnosis. We pooled data from Vietnam, Cameroon, South Africa and Kenya for our analysis. Using multivariable logistic regression with ROC curve analysis, we created an internally validated risk score for diagnosis of active TB disease in children.

In **Aim 2**, we estimated the effectiveness of fluroquinolone-based preventive treatment for drug-resistant TB exposure in household contacts. We used follow-up data from a cohort of household contacts in Pakistan that received preventive treatment. We estimated the counterfactual risk in these contacts based on reports from the literature to assess the effectiveness. We found a substantial reduction in risk of active TB disease in contacts who received preventive treatment.

In **Aim 3**, we identified risk factors for adverse events in household contacts receiving fluoroquinolone-based preventive treatment. We used survival analysis methods for recurrent events for our analysis

using the data from Pakistan. We found preventive treatment to be safe with low incidence of adverse events with better tolerability in younger children.

In conclusion, we derived a risk score for diagnosis of active TB disease in children and found preventive treatment for drug-resistant TB exposure to be safe and effective. Household contacts of drug-resistant TB patients including children, who are free of active TB disease after evaluation, should be offered preventive treatment.

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CHAPTER 1

Background and Dissertation Aims

A. BACKGROUND AND RATIONALE

1) Tuberculosis and TB infection

Tuberculosis (TB), caused by *Mycobacterium tuberculosis (M. tuberculosis)* complex, is the leading infectious cause of death globally, overtaking HIV/AIDS in 2015.[1] TB is an ancient disease with modern strains of *M. tuberculosis* probably originating some 20,000 years ago[2] and the present diversity originating 250-10,000 years ago.[3,4,5]

The infectious nature of TB was demonstrated by Jean-Antoine Villemin in 1865 when he inoculated a rabbit with some purulent material extracted from the lungs of a patient who died of TB.[6] It was in 1882 that Robert Koch discovered the causative agent to be *M. tuberculosis* complex.[7]

M. tuberculosis is a bacillus with a cell wall containing mycolic acid, which makes it resistant to desiccation and is also impervious to gram stain. Hence, it can appear either as gram positive or gram negative. It is classified as acid-fast bacillus (AFB) as it forms acid-stable complexes with certain arylmethane dyes. The most common staining method used for *M. tuberculosis* is Ziehl-Neelsen stain. *M. tuberculosis* is highly aerobic and has a cell division time of 15-20 hours.[8]

M. tuberculosis primarily infects the lung but is capable of dissemination to any part of the human body. TB infection can present clinically as a spectrum from asymptomatic infection to active disease, which can be life-threatening. Generally, patients with TB are classified as either having latent TB Infection (LTBI) or active TB disease with lungs being the most common site depending on the clinical presentation. Patients

with LTBI are asymptomatic and do not transmit the disease while patients with active TB disease experience symptoms such as cough, fever, fatigue, loss of appetite and weight loss. Some patients with active TB disease may also be asymptomatic.[9]

Epidemiology and transmission.

According to Global TB Report 2018, 10.4 million individuals developed TB disease in 2017 and 1.5 million died of the disease. TB is a major cause of morbidity and mortality in low and middle income countries, with 80% of the incidence occurring in 22 high burden countries and India, Indonesia, China, Nigeria, Pakistan, and South Africa accounting for over half the notified cases.[1] The primary mode of spread of TB is from person-to-person through respiratory contact. It is estimated that each diseased individual can infect up to 10 individuals with TB each year.[10]

Our understanding of TB transmission is limited because of the complex interplay between host, pathogen and environmental factors. There is heterogeneity in infectiousness among TB patients; transmission is influenced by both host and pathogen factors including HIV coinfection and lineage of *M. tuberculosis*; and even extremely low aerial concentrations of *M. tuberculosis* can lead to infection.[11-16] The current paradigm in TB transmission is that close contact in households accounts for the majority of TB transmission. Studies have documented an infection rate of 30-50% amongst household contacts.[17,18]

Of adults infected with TB, 10-20% will develop active disease over their life time with increased lifetime risk for HIV infected patients and children <2 years old.[19,20] Immunocompetent children less than 2 years old have 10-30% risk of developing active pulmonary TB disease following primary infection. The risk decreases to 2-5% in children between 2-10 years of age and increases to 10-20% in children older than 10 years.[21] Patients with end-stage renal disease or diabetes mellitus have moderate risk of progression.[19,20] Approximately 5% of those infected progress to active TB disease following infection within 1 to 2 years

while another 10-15% progress from re-activation of LTBI over their life time.[22-26] As such, household contact tracing and preventing household transmission has long been a key element of TB control.[27,28]

Progression from LTBI to active disease and the biological basis for latency are poorly understood. An important contributor seems to be activation of antigen-specific CD4+ T-cells.[29] The role of tumor necrosis factor (TNF) has also been shown with emerging data suggesting that there is an ideal level of TNF required for control; excess of TNF or lack of it may worsen the existing immunopathology or lead to lack of immune containment.[9] Progression can be subtle, with over 25% of the patients showing no clinical symptoms, but are still capable of spreading the disease.[30,31] It is important, therefore, to distinguish between TB infection and active TB disease.

Diagnosis of TB infection

There are two tests currently available to diagnose infection with *M. tuberculosis*: Tuberculin Skin Test (TST)/Mantoux Test (MT) and Interferon Gamma Release Assay (IGRA). TST/MT is a skin test and requires injecting 0.1 ml of purified protein derivative (PPD) in the skin intradermally and the induration is measured 48-72 hours later. People with cell-mediated immunity against these antigens show a hypersensitivity reaction resulting in an induration.[32] The size of the induration is measured and interpreted based on the context.[9] TST can be false positive in individuals with a BCG vaccine or exposure to non-tuberculous mycobacteria.[33]

IGRAs are blood tests that measure the cell-mediated immunity in response to *M. tuberculosis* antigens and are more specific for *M. tuberculosis* and not affected by BCG vaccination.[34-36]

Both TST and IGRA are imperfect and are unable to distinguish between LTBI and active TB disease. These tests do not actually measure the infection but cell-mediated immunity and are unable to differentiate

between recent infection and remote infection.[37-39] Both of the tests have limited predictive value for development of active disease, with only a subset of positive individuals progressing to active TB disease.[37,40] These tests stay positive even after someone has been treated for LTBI.[41-43] In addition, the tests have imperfect sensitivity and may be negative in the setting of active TB disease; negative TST/IGRA among patients with TB are associated with increased mortality risk.[44,45] Furthermore, performance characteristics vary by age and nutritional status, with decreased sensitivity among children under 5 years old, and even lower sensitivity for children under 2 years old.[46-48]

Diagnosis of TB disease

There is no perfect diagnostic test for TB. The four main modalities used to help diagnose active TB disease include 1) radiology, 2) microscopy (AFB sputum smear), 3) AFB culture and drug resistance testing (DST) and 4) molecular testing.[9]

Chest radiography is often used for screening but as it lacks specificity and must be accompanied with microbiologic testing (culture and/or PCR).[9] Sputum smear microscopy (described below) is not confirmatory as it does not distinguish different mycobacterial species from each other.

Sputum smear microscopy is the most widely used test for diagnosis in low and middle-income countries due to availability,[49] although it is being replaced by molecular testing (Xpert MTB/RIF assay) gradually with WHO making a conditional recommendation for using Xpert MTB/RIF as the first-line diagnostic test in adults and children suspected of having active TB disease.[50,51] Sputum smear microscopy requires direct visualization of *M. tuberculosis*. Sensitivity of sputum smear microscopy ranges from 32-94% and specificity ranges from 50-99%. LED fluorescence microscopy improves sensitivity by 10%. Microscopy requires presence of $\geq 10^4$ CFU/ml for detection.[52] Specificity is imperfect as positive AFB does not

distinguish M. tuberculosis from other mycobacterial species which can cause disease in particular among HIV-infected individuals or young children.

AFB culture and DST is much more sensitive (~90%) and specific (>99%) than microscopy requiring 10^1 to 10^2 CFU/ml but requires specialized facilities and a long incubation period (10-21 days). It is also not widely available in many TB-endemic countries.[53]

Molecular testing including Xpert MTB/RIF® (Cepheid, Sunnyvale, CA, United States) assay rely on DNA amplification and are much faster than AFB culture and DST. Sensitivity of Xpert MTB/RIF assay, the most widely available molecular test, ranges from 67-99% while its specificity is 99%. Xpert MTB/RIF provides the added benefit of drug-susceptibility testing (DST) as compared to sputum smear microscopy. Although WHO has made a conditional recommendation to use Xpert MTB/RIF as a first-line diagnostic test, its cost is much higher than microscopy and it is not easily accessible in many low and middle-income countries.[54]

Lipoarabinomannan (LAM) is a component of the outer cell wall of *M. tuberculosis* and is detectable in the urine of individuals with active TB, with modest diagnostic performance in HIV infected individuals. It is recommended by WHO in a highly selected group of HIV-infected individuals with low CD4 count or serious illness.[55]

Sputum smear microscopy, AFB culture and DST and molecular testing (Xpert MTB/RIF) all rely mostly on sputum specimen for diagnosis of pulmonary TB. Proper specimen collection including sufficient volume, optimal transportation time (less than 1 day) and prevention from environmental contamination are essential for optimal performance characteristics of *M. tuberculosis* culture.[56] Sputum samples are comparatively easily collected for adults but can be difficult to collect in children as they are often unable to expectorate sputum. Sputum induction and gastric aspirate can be used as alternative approaches in

children to obtain a sample for testing.[57] Gastric aspirate is useful as children often swallow sputum instead of expectoration.

Table 1-1: Available technologies recommended by WHO for diagnosis of active TB disease (adapted from Pai et al.)[9]

Test	Principle	Sensitivity (%)	Specificity (%)
Chest X-ray	Visualization of the lungs	87 Children: 75	89 Children: 85
Conventional sputum smear microscopy	Visualization of *M. tuberculosis* using light microscopy	32-94 Children: 26	50-99 Children: 100
LED fluorescence microscopy	Visualization of *M. tuberculosis* using fluorescence microscopy	52-97	94-100
Culture with DST	*M. tuberculosis* culture on liquid media	89 Children: 30-50	>99 Children: 100
Xpert MTB/RIF assay	PCR (DNA amplification)	98 Children: 62	99 Children: 99
LAM lateral flow assay	Antigen detection	44 (54 in HIV+) Children: 48 (47 in HIV+)	92 (90 in HIV+) Children: 61 (82 in HIV+)

2) Pediatric/Childhood TB and gap in diagnosis

In 2017 an estimated 1 million children developed active TB and 250,000 died of the disease.[1] It is estimated that 10% of all new cases globally occurred in children.[58,59] Most of the deaths (>80% of all deaths in children under 15 years with TB) occur in children less than 5 years of age with 96% occurring in children not receiving treatment.[60]

The spectrum of active TB disease in children is different from adults with a higher percentage of extra-pulmonary TB (up to 30-40% of the cases).[61] Children and adolescents are at higher risk of progression to disease after *M. tuberculosis* infection with 50-80% of the infected children under 2 years developing radiological abnormalities.[62,63] The natural history of TB in children from pre-chemotherapy literature shows that the risk of progression to active disease is highest in infants with *M. tuberculosis* infection (20-40%), the risk then decreases for children 2-10 years of age reaching its lowest level in children 5-10 years of age. The risk then rises again in adolescence.[64] In high TB transmission settings, incidence of TB disease increases rapidly in adolescence.[65]

Diagnosis of active TB disease in children is complex. Children have nonspecific symptoms and are usually unable to produce sputum samples, which is the gold standard biospecimen for diagnosis in adults. Many childhood TB clinics do not have the ability to collect sputum samples by induction or gastric aspiration due to lack of resources, often leading to clinical diagnosis and missed cases, as well as over-diagnosis. Childhood TB is also associated with low bacterial count (pauci-bacillary disease) resulting in lower sensitivity of diagnosis as less bacilli are expectorated in the sputum or in gastric aspirate.[57] Of children who have samples collected for culture, only 15-50% have *M. tuberculosis* confirmed by culture.[66]

In contexts where immediate microbiologic confirmation is not possible, current TB diagnosis in children requires an algorithmic approach with reliance on clinical presentation, contact history with a patient with

active TB disease, evidence of *M. tuberculosis* infection (TST/MT of IGRA positive), chest radiography, and microbiological testing (AFB sputum smear, AFB culture and molecular testing). This approach has a number of limitations as children often have non-specific symptoms and all investigations may not be available in low resource settings, resulting in over or under-diagnosis. In areas with prevalent diseases with overlapping features, missed or mis-diagnosis is a challenge.[67] A number of different structured clinical approaches exist for diagnosis of childhood TB, providing a logical and reproducible progression based on clinical judgement, but most of these have not been validated against a gold standard and were developed in a hospital setting and may not be valuable in community settings.[67] Study by Hatherill *et al* comparing 9 such structured approaches showed poor concordance with significant variation (6.9-89.2%) for the frequency of diagnosis of childhood TB depending on the system used.[68]

There has been widespread attention to the challenges faced in diagnosis of Childhood TB recently but under-diagnosis remains a problem.[69] A major advantage of obtaining a microbiological diagnosis that is lost with clinical diagnosis is the ability to perform drug susceptibility testing (DST) and start treatment based on appropriate drugs, which is crucial in the era of drug-resistant TB.

To standardize diagnosis for research, based on the above, children are classified using Clinical Case Definitions (table 2) developed by an expert panel convened by National Institute of Health (NIH) as confirmed TB (microbiological confirmation), unconfirmed TB (2 or more of: TB symptoms, abnormal chest x-ray, positive TST or TB exposure, and response to TB treatment) and unlikely TB (not meeting the criteria for confirmed and unconfirmed TB).[66]

Table 1-2: Clinical Case Definitions for TB classification for research studies (adapted from Graham et al.)[66]

Case definition	Criteria
Confirmed tuberculosis	Bacteriological confirmation obtained Requires Mycobacterium tuberculosis to be confirmed (culture or Xpert MTB/RIF assay) from at least 1 respiratory specimen
Unconfirmed tuberculosis	Bacteriological confirmation NOT obtained AND at least 2 of the following: • Symptoms/signs suggestive of tuberculosis (as defined) • Chest radiograph consistent with tuberculosis • Close tuberculosis exposure or immunologic evidence of M. tuberculosis infection • Positive response to tuberculosis treatment (requires documented positive clinical response on tuberculosis treatment—no time duration specified) - With M. tuberculosis infection • Immunological evidence of M. tuberculosis infection (TST and/or IGRA positive) - Without M. tuberculosis infection

	• No immunological evidence of M. tuberculosis infection
Unlikely tuberculosis	Bacteriological confirmation NOT obtained AND Criteria for "unconfirmed tuberculosis" NOT met - With M. tuberculosis infection • Immunological evidence of M. tuberculosis infection (TST and/or IGRA positive) - Without M. tuberculosis infection • No immunological evidence of M. tuberculosis infection

Newer/alternative approaches to TB diagnosis in children

Induced sputum, nasopharyngeal aspirate and gastric aspirate/lavage can increase the diagnostic yield, the likelihood that the test will establish a diagnosis, in children but require trained individuals and resources, for which most primary care settings in low and middle income countries are rarely equipped.[70-74] The diagnostic yield in culture varies by population, but from gastric lavage is generally 10-30% while that from induced sputum can be as high as 28%. Yield from nasopharyngeal aspirate can be about 22%.[57,75-77]

LAM (described above) has been recommended by WHO only in a highly selected group of HIV-infected children with low CD4 count or serious illness.[55] It is not recommended for HIV negative children. Pooled sensitivity of LAM in HIV-infected patients with CD4 count ≤100 is 56% while pooled specificity is 90%. This is mostly based on adult data.[55]

Xpert MTB/RIF assay on stool samples in children with HIV or severe pulmonary disease has also shown promise (sensitivity: 31.9-68%; specificity: 98-99.7%) and can be used, but is labor-intensive requiring stool processing.[74,78-80] A proof of concept for stool processing without centrifugation has been published, but more data are still needed.[81]

Use of Xpert MTB/RIF Ultra ® for diagnosis of pulmonary TB in children can increase sensitivity of diagnosis by decreasing the limit of detection to 16 CFU/ml from 114 for Xpert MTB/RIF assay, but still requires a sputum sample.[16,82] Xpert MTB/RIF Ultra can also be used on stool samples.

Approaches using host biomarkers and gene transcription profiling have shown promise in children.[83] Using flow cytometry to measure *M. tuberculosis* specific T-cell activation markers for both CD4 and CD8 t-cells has shown sensitivity of 81% and specificity of 87% with an AUC of 0.89 (95% CI: 0.83-0.93) in adults.[84] A new immunodiagnostic T-cell activation marker–tuberculosis (TAM-TB) assay – in a proof-of-concept study in children has shown sensitivity of 83% (95% CI: 59-96) and specificity of 97% (95% CI: 89-100).[85] Two studies using multi-transcript signatures from blood have reported sensitivity of 80% (95% CI: 78-83%) and specificity of 90% (95% CI: 84-96%) for diagnosis of active TB disease in children.[86,87] These biomarker and gene transcriptional based approaches require specialized equipment and training and are costly.

One approach that has generated interest is the use of monocyte-lymphocyte ratio (MLR). The absolute number of monocytes or lymphocytes in peripheral blood or their ratio has been shown to predict the risk of TB disease in HIV-infected and HIV-exposed uninfected infants within 2 years (aHR: 1.17 per unit increase in MLR; 95% CI: 1.01 to 1.34) and in children born to HIV-infected mothers. It is hypothesized that elevated MLR in TB disease results from monocyte phagocytosis of TB bacilli and relative monocyte proliferation compared to lymphocytes. MLR may also have utility as a diagnostic biomarker, since MLR was higher in patients with active TB disease compared to those with LTBI or no TB.[88-93] A prospective

longitudinal study of close HIV negative household contacts from Madagascar showed increased MLR was significantly associated with appearance of TB symptoms in contacts with aHR of 4.97 (95% CI: 1.3 to 18.99) and monocytes ≥ 7.5% had aHR of 6.25 (95% CI: 1.63 to 23.95) for active TB disease.[94] A study by La Manna et al has shown an MLR of 0.285 to have a sensitivity of 91% and specificity of 93% to differentiate active TB patients from healthy controls using ROC curve analysis in adults without HIV. In a cohort of HIV-infected Kenyan children, MLR of greater than 0.38 was associated with confirmed TB with a sensitivity of 79% and specificity of 77% in HIV infected children.[95] Attractive features of MLR that can render it a feasible tool in low resource setting include use of blood instead of sputum, availability and affordability of differential blood count in most laboratories, and easy calculation of the ratio.

3) Drug-resistant TB (DR-TB)

In 2017, almost 600,000 cases of TB globally were due to DR-TB.[1] Drug resistance in TB was first reported in 1948 as soon as streptomycin, the first anti-TB drug, was used for treatment.[96,97] Drug resistance in the TB bacillus develops due to genetic mutations either through target modification for the drug or through introducing a defect in an enzyme that is necessary for drug effect.[9,98] Drug resistant strains are described based on the resistance profile with the two main ones being multi-drug resistant TB (MDR-TB) strain, which is resistant to isoniazid and rifampicin, the two first-line drugs used for TB treatment, and extensively drug resistant TB (XDR-TB) strain, which in addition to being MDR, is resistant to fluoroquinolone and to at least one of the three injectable agents: kanamycin, amikacin or capreomycin.[99] Although it was initially hypothesized that DR-TB strains would be less transmissible due to fitness cost incurred by mutation, studies from South Africa and other countries have shown DR-TB strains to be just as transmissible and that drug resistance in TB is mostly spread by primary transmission of the resistant strain as opposed to secondary development during treatment.[100-102]

4) TB treatment: active TB disease and LTBI

The current standard of treatment for active drug-sensitive (DS) TB is a 6-month treatment with an intensive phase of 4 drugs, isoniazid, rifampicin, ethambutol and pyrazinamide for 2 months followed by a continuation phase with isoniazid and rifampicin for 4 months.[103] This regimen has an over 85% success rate under routine programmatic conditions.[1] Due to the long duration of treatment, certain patients develop adverse events, with the most common ones being liver toxicity, neuropathy, musculoskeletal issues and skin rash. This can lead to issues with adherence and can result in treatment failure and/or development of DR-TB because of selection of resistant mutants during intermittent cycles of killing.[9]

In 2017, 30% of estimated incident MDR-TB were diagnosed and 25% started on treatment globally.[1] Treatment regimens for active DR-TB are complex and depend on the resistance profile of the patient. They usually last 18-20 months (WHO has endorsed a shorter regimen of 9-12 months under certain specific conditions) and use second-line medications that are less effective and more toxic resulting in severe adverse events. WHO recommends using at least 4 drugs that are likely to be effective against the bacterium.[104-106] The treatment success rate for MDR-TB under programmatic conditions is approximately 50%.[1] Almost 20% of the patients discontinue treatment before being cured because of the long duration and adverse events.[107] DR-TB treatment can cost over USD 26,000 for XDR-TB, not accounting for patient-incurred cost.[108,109] This represents a significant burden on already overstretched health systems especially in countries where DR-TB is prevalent. In countries with DR-TB and HIV coinfection, overlapping toxicities and possibly synergistic adverse events can lead to poorer outcomes.[110]

Treatment for LTBI and household contact investigation: DS-TB

In 2018, WHO published its updated and consolidated guidelines for treatment of LTBI for DS-TB recommending that HIV positive individuals, contacts of patients with active pulmonary TB and those with

immunocompromising conditions should be offered LTBI treatment with either 6-9 months of daily isoniazid, 3-4 months of daily isoniazid and rifampicin, 3 months of daily rifampicin or 3 months of weekly rifapentine and isoniazid.[20]

Studies by Dye et al at WHO have shown that to meet the WHO 2050 elimination target of less than one case per million population, treatment for latent TB infection (LTBI) will have to be incorporated in programs, given the reservoir of LTBI that exists (estimated to be 1/4 of the world population[111]).[112] Dye's model, where he considered a hypothetical country in 2015 with a poorly controlled epidemic and an incidence of 1100 per million per year with a 65% case detection and 70% cure rate, showed that improving case detection and treatment would bring the incidence to 130 per million per year by 2050, but reactivation and relapse of old cases would continue to give rise to over 100 cases per million population. In this context, one of the most efficient uses of resources is household contact investigations, a critical activity for TB programs for two reasons: early identification of TB patients and treatment of latent TB infection (LTBI).

Treatment for LTBI and household contact investigation: DR-TB

There is no currently approved treatment for latent DR-TB infection and no randomized control trial data are available to guide the approach. Observational studies from the Federated States of Micronesia, US, UK, South Africa and other countries have shown efficacy of fluoroquinolone-based preventive treatment in adults and some children as summarized below and in table 3.[25,113-126]

Between 1995 and 2003, 51 children with MDR LTBI were started on preventive treatment with second line drugs in New York City with 38 of them completing treatment. None of the 51 children developed TB during the study period or 2 years after. The treatment was generally well tolerated.[115]

In July 2008, outbreaks of MDR-TB with ongoing household transmission were identified on Chuuk Island in Federal States of Micronesia by a CDC team. The investigation team identified 232 individuals with significant contact with MDR TB patients. Of these 232 contacts, 20 were found to have disease (5 DS TB and 15 MDR TB) and 119 were found to be infected using TST. Of these 119 contacts, 104 were started on preventive treatment with a fluoroquinolone-based regimen for 12 months under DOT. None of the contacts initiated on preventive treatment developed TB disease over 3 years of follow up and the treatment was found to be well tolerated with minimal adverse effects. Of the 15 contacts that refused treatment, 20% (3 individuals) developed MDR-TB. In this observational cohort, 43 of the 104 contacts that started treatment were less than 18 years old and the treatment regimens were found to be safe in this sub-population as well. This study demonstrated the operational feasibility and tolerability of preventive treatment for contacts infected with MDR-TB.[114]

In an observational cohort of 186 children infected with MDR-TB strain sensitive to ofloxacin (both HIV positive and negative) in Western Cape in South Africa between 2007 and 2009 that were started on 3 drug preventive therapy, a study reported that 6 children developed incident TB in 219 patient-years of follow up. Nine of the children started on therapy were HIV positive of which 2 developed TB.[116]

A 2002 study from Western Cape Province of South Africa documented giving prophylaxis to 41 children under 5 years exposed to MDR-TB, of which 5% (2 children) developed the disease as compared to 64 children not receiving prophylaxis of which 20% (13 children) developed the disease. Those put on prophylaxis were at higher risks, but the rate of development of disease was lower suggesting effective treatment.[25]

A 2014 study from California, USA showed that putting children on preventive treatment with pyrazinamide and levofloxacin resulted in increased adverse events and out of the 26 children put on treatment by the study, only 8 completed therapy. None developed active TB disease.[113] Another study from

California put 22 contacts on pyrazinamide and levofloxacin regimen for 12 months. In the study, 13 contacts stopped treatment early due to adverse events with 3 developing serious adverse effects. None of the contacts developed TB disease.[120]

Similarly, 17 contacts in Canada were treated with the same regimen and 14 developed adverse effects with none completing treatment.[124] This suggests that a combination of pyrazinamide and levofloxacin is not well tolerated, resulting in high incidence of adverse events and non-completion of preventive treatment.

Attamna et al treated 89 contacts with different regimens between 1998 and 2006 in Israel and followed up 387 other contacts not provided preventive treatment. None developed TB disease.[119]

Between 1995 and 2010, Denholm et al treated 11 of the 49 eligible contacts in Australia with different regimens for TB infection. None developed the disease at the end of the study.[126]

Gracia-Prats et al in 2013 treated 24 children in Cape Town, South Africa with 6-month regimen of ethambutol, ofloxacin and high-dose isoniazid. Of these, 2 children developed adverse effects and 1 stopped treatment early, but none developed TB disease.[123]

In Japan between 1998 and 2002, 41 contacts were treated with different regimens for LTBI. Of these, 13 developed TB disease, showing that choosing the right regimen is essential while providing prophylaxis for preventing disease.[121]

William et al treated 8 children in UK with different two drug combinations of first and second line drugs for LTBI with none developing TB disease.[122]

The evidence base described above is made up solely of observational data and shows fluoroquinolone to be effective. There are 2 clinical trials underway that are using levofloxacin to treat contacts exposed to MDR-TB, but their results will not be available until 2020 at the earliest.[127]

Although there have been concerns about the safety of extended fluoroquinolone use for children, a significant body of evidence has shown it to be safe, with American Academy of Pediatrics and WHO supporting their use in infants and children with TB.[116,128-130]

Based on the observational studies described above, in 2018 WHO recommended treating DR-TB infection for household contacts at high risk for developing disease, but there are no current definitive recommendations for what treatment regimen to use, and few national programs recommend it.[20,131]

In the observational studies described above, the most common adverse effects experienced by contacts on treatment included gastrointestinal, hepatic and musculoskeletal signs or symptoms. Although between 10-50% of the contacts in these studies experienced some adverse events, 4-5% of the contacts discontinued treatment due to these.[25,113-118,120,123-126]

Table 1-3: Summary of the findings of some of the observational studies of DR-TB preventive treatment (adapted from HMS Policy Brief on Post-Exposure Management of Multidrug-Resistant Tuberculosis Contacts: Evidence-Based Recommendations)[117]

First Author	Year	Country	Regimen used	Number of individuals in the study and those who developed disease	Adverse Events
Adler-Shohet	2014	USA	Lfx and PZA	26 children treated for TB infection. None developed TB disease.	Only 8 completed treatment due to AEs.
Attamna	1998-2006	Israel	Cfx and PZA	12 contacts treated for TB infection with tailored regimen: 71 given H, 6 other treatments and 387 given nothing. None developed TB disease.	No information provided.
Denholm	1995-2010	Australia	Various drugs including FQ	Of 49 eligible contacts, 11 treated for TB infection.	4 of 11 contacts developed AE; 2 stopped early.

				None developed TB disease.	
Feja	1995-2003	USA	Regimen tailored to index patient's DST	51 contacts treated for TB infection. None developed TB disease	8 out of 22 contacts developed AE; 2 stopped early
Garcia-Prats	2013	South Africa	Ofx, EMB and high dose INH	24 children treated for TB infection. None developed TB disease.	2 developed AE; 1 stopped early.
Lou	1999	USA	Lfx and PZA	57 organ transplant patients treated for TB infection. None developed TB disease.	32 stopped early due to AE.
Morris	2007-2010	Micronesia	Lfx/Mfx +/- EMB/ETO	104 contacts treated for TB infection. None developed disease.	4 stopped early due to AE.
Papastavros	2000	Canada	Lfx and PZA	17 contacts treated for TB infection. None developed TB disease.	14 contacts developed AE; all 14 stopped treatment.

Ridzon	1997	USA	Ofx and PZA	22 contacts treated for TB infection. None developed TB disease.	13 contacts stopped early due to AE.
Sasaki	1998-2002	Japan	Various drug combinations	41 contacts treated for TB infection. 13 developed TB disease.	No information provided.
Schaaf	1994-2000	South Africa	Regimen tailored to index patient's DST	41 contacts treated for TB infection. 2 developed TB disease.	Some GI AE reported.
Seddon	2010-2012	South Africa	Ofx, EMB and high dose INH	186 children treated for TB infection; 2 developed TB disease.	7 children developed serious AE;
Trieu	2005	USA	Mfx and PZA	50 HIV positive adults treated for TB infection; None developed TB disease with the same strain as index case.	3 stopped early due to AE.
Williams	2006-2010	UK	Various drug combinations	8 children treated for TB infection. None	No information provided.

				developed TB disease.	

5) Summary

Approximately 1 million children develop TB each year accounting for 10% of the disease incidence. The mortality rate in children with TB is 25%, with 96% of the deaths occurring in children not on treatment. Childhood TB is difficult to diagnose and causes a large burden of disease. Better diagnostic tools are urgently needed to help healthcare providers to easily diagnose childhood TB, especially in low-resource settings where access and capability of diagnosis is limited.

DR-TB disease is difficult to treat with a treatment success rate of 50% or lower, hence it is imperative that strategies to prevent development of disease in the high-risk group be employed, including use of preventive treatment in exposed contacts.

B. SIGNIFICANCE AND OVERVIEW OF THE AIMS

TB is the leading infectious cause of death globally and the 9th leading cause overall. It results in approximately 10 million new cases and 1.7 million deaths annually. Pediatric TB accounts for 1 million cases a year, with 250,000 children dying of the disease.[1] Most of the deaths occur in children less than 5 years and in those not on treatment.[60] Pediatric TB is difficult to diagnose as the sputum biospecimen of choice for diagnosing pulmonary TB is often not available because children are often unable to expectorate sputum. Also, the extent of extrapulmonary disease is higher in this age group. Hence, most of the diagnosis is based on algorithm and clinical judgement. This results in under- and over-diagnosis.[57,66,67,69] Better diagnostic tests and algorithms are needed to improve the diagnosis of pediatric TB and to reduce the morbidity and mortality from the disease. Also, better diagnostics would help rule out active disease in those children exposed to TB who can then be started on preventive therapy.

Host biomarker approaches including T-cell activation markers and gene profiling have shown promise in diagnosing active TB disease in children, but require specialized equipment and training and are expensive.[83-87] An approach that has generated interest recently is the use of monocyte-lymphocyte ratio (MLR). The absolute number of monocytes or lymphocytes in peripheral blood or their ratio has been shown to predict the risk of TB disease in patients with HIV and TB coinfection and in children born to HIV-infected mothers.[88-93] In a cohort of HIV-infected Kenyan children, MLR of greater than 0.378 was diagnostic of confirmed TB with a sensitivity of 79% and specificity of 77%.[95] Attractive features of MLR that can render it a feasible tool in low resource settings include use of blood instead of sputum, availability and affordability of differential blood count in most laboratories, and easy calculation of the ratio. Further evaluation is needed to validate these findings, including in HIV negative children and to see if combining clinical and demographic characteristics with MLR improves sensitivity and specificity for diagnosis.

Treatment for DR-TB is complex and long, lasting for over 20 months with treatment success rates of up to 50%.[1] The treatment is quite expensive and can cost over USD 26,000 for XDR-TB not accounting for patient-incurred cost.[108,109] This represents a large burden on the health system especially in countries where DR-TB is prevalent. Therefore, prevention interventions are essential to reduce morbidity and mortality from DR-TB.

Household contacts of DR-TB patients and especially children are at an increased risk of developing active TB disease. They are prime targets for preventive interventions. One hurdle is the lack of an effective intervention unlike for DS-TB.[20] No clinical trial data are available to guide the approach to preventive treatment. A few observational studies have shown efficacy of a levofloxacin-based regimen in preventing development of DR-TB disease in household contacts, further evaluation is needed of the effectiveness of preventive treatment especially in in a high-burden, low resource setting with low prevalence of HIV .[25,113-118,120,123-126] A better understanding of the effectiveness of preventive treatment will help guide policy makers and public health authorities approach to preventive treatment and help target contacts at highest risk of development of the disease.

Observational studies regarding use of levofloxacin-based preventive treatment have also shown concerns regarding development of adverse events during treatment with 10-50% of the contacts developing gastrointestinal, hepatic and musculoskeletal signs or symptoms and 4-5% of the contacts discontinuing treatment due to these.[25,113-118,120,123-126] Management of adverse events and treatment completion are essential for an effective preventive treatment plan to prevent development of DR-TB disease, hence, a better understanding of the individual level factors associated with these adverse events is required. This can help programs identify individuals on preventive treatment at risk of developing adverse events and can target and plan for resources accordingly including psychological counseling and provision of ancillary drugs for management of adverse events.

This dissertation will aim to (a) validate MLR as a diagnostic test in a large multi-country cohort of children and create a risk score with MLR to improve the sensitivity and specificity of diagnosis of pediatric TB, (b) evaluate the effectiveness of providing preventive treatment for DR-TB infection to household contacts including children exposed to a DR-TB patient using data from a cohort study from Pakistan, and (c) identify risk factors associated with the development of adverse events in household contacts receiving DR-TB preventive treatment using data from a cohort study from Pakistan.

C. SPECIFIC AIMS

- **Aim 1a: To evaluate the use of monocyte-lymphocyte ratio as a tool for diagnosis of TB in children.** *Hypothesis: Elevated monocyte-lymphocyte ratio will improve diagnostic accuracy of TB disease.*

- **Aim 1b: To construct a risk score with monocyte-lymphocyte ratio and clinical symptoms suggestive of TB to improve diagnosis of TB in children.** *Hypothesis: Adding clinical symptoms with monocyte-lymphocyte ratio in a risk score will increase the sensitivity and specificity of diagnosis.*

- **Aim 2: To evaluate the effectiveness of a fluoroquinolone-based two drug treatment vs no treatment to prevent drug-resistant (DR) TB disease in household contacts in Karachi, Pakistan.** *Hypothesis: Risk of future DR-TB disease will be reduced by provision of preventive therapy.*

- **Aim 3: To identify risk factors for adverse events of different fluoroquinolone-based preventive treatment regimen using data from Karachi, Pakistan.** *Hypothesis: Higher number of adverse events will be observed in ethionamide containing regimens and in males over 5 years of age.*

CHAPTER 2

Use of a blood-based bio-marker and risk score for the diagnosis of tuberculosis disease in children – a multi-country study.

Abstract

Background

One million children develop TB annually with 250,000 dying from the disease. More than 80% of the deaths occur in children younger than 5 years old and nearly all of them have not received TB treatment. One of the main reasons for children not being on treatment is under-diagnosis due to poor performance of available diagnostic tools in children.

Methods

We pooled individual level data from pediatric cohorts from Vietnam, Cameroon and South Africa to validate the use of monocyte-lymphocyte ratio (MLR) ≥0.378 for diagnosis of active TB disease in children. The combined cohort included both HIV positive and negative children. Using multivariable logistic regression with ROC curve analysis and adding more children from a cohort in Kenya to the dataset, we created an internally validated risk score with MLR and other clinical, demographical and laboratory predictors for diagnosis of active TB disease.

Results

Using the MLR value of 0.378, 145 (24.5%) of 592 children were classified as having active TB. Comparing against the culture/Xpert MTB/RIF results, this MLR value had a sensitivity of 32.8% and specificity of 77.8%. A logistic regression model using MLR ≥0.297, age ≥ 4 years, TST ≥5 mm, HIV positivity, chest x-ray consistent with TB, contact history with a TB patient, fever for greater than or equal to 2 weeks and failure to thrive predicted active TB disease with an area under the receiver operating characteristic curve of 0.84. Risk score developed using this model had a sensitivity of 65.7% and specificity of 86.9% at a cutoff of 9 points and a sensitivity of 90.5% and specificity of 61.5% at a cutoff of 5 points.

Conclusion

With its discriminatory performance, our score should enable clinicians to rapidly initiate children with presumptive/suspected TB disease on anti-tuberculous treatment without requiring microbiological confirmation.

Background

In 2017 an estimated one million children developed active TB and 250,000 died of the disease.[1] Over 80% of the deaths occur in children less than five years of age with 96% occurring in children not receiving treatment.[60]

Diagnosis of active TB disease in children is complex. Childhood TB is associated with pauci-bacillary disease with lesser bacilli expectorated in the sputum or gastric aspirate samples. This results in lower sensitivity of microbiological diagnosis. There are also challenges associated with sputum collection.[57,132] Because of these issues, only 15-50% of pediatric TB patients have *M. tuberculosis* confirmed by culture.[66]

In contexts where immediate microbiologic confirmation is not possible, current TB diagnosis in children requires an algorithmic approach with reliance on clinical presentation, contact history with a patient with active TB disease, evidence of *M. tuberculosis* infection (TST or IGRA positive), and chest radiography. This approach has many limitations. Children often have non-specific symptoms, which overlap with other diseases prevalent in low resource settings. Also, all required investigations may not always be available. This can result in over or under-diagnosis.[67] A number of different structured clinical approaches based on clinical judgement exist for diagnosis of childhood TB but most of these have not been validated against a gold standard. Also, as these approaches were developed in a hospital setting, they may not be valuable in community settings.[67] A study by Hatherill *et al* comparing nine such structured approaches showed poor concordance with significant variation (6.9-89.2%) for the frequency of diagnosis of childhood TB depending on the system used.[68]

With these challenges in place, need for a non-sputum based diagnostic tool is widely recognized. Testing of stool and nasopharyngeal aspirates on Xpert MTB/RIF assay have shown high specificity but sensitivity has been lacking in children. Approaches using host biomarkers and gene transcription profiling have

shown promise.[83] But these biomarker and gene transcriptional based approaches require specialized equipment and training and are generally costly, making them unavailable in most high TB burden settings. One blood-based bio-marker approach makes use of monocyte-lymphocyte ratio (MLR = monocyte count/lymphocyte count). The absolute number of monocytes or lymphocytes in peripheral blood or their ratio has been shown to predict the risk of TB disease in HIV-infected and HIV-exposed uninfected infants within two years of life and in children born to HIV-infected mothers.[88-93] Attractive features of MLR that can render it a feasible tool in low resource settings include: 1) easier collection of sample, 2) availability of differential blood count in most laboratories globally, and 3) easy calculation of the ratio.

A study by La Manna et al showed that an MLR ≥0.285 has a sensitivity of 91% and specificity of 93% to differentiate active TB patients from healthy controls in adults without HIV. Only a single study has assessed use of MLR in children for TB diagnosis. In a cohort of HIV-infected Kenyan children, MLR ≥0.378 was associated with active TB disease with a sensitivity of 79% and specificity of 77%.[95]

The objective of this study is to validate the findings from the Kenya study in a multi-country cohort of both HIV infected and uninfected children. We also sought to construct a risk score with MLR, demographic, clinical and laboratory features to increase the sensitivity and specificity of diagnosis.

Methods

Study Design

This study combined data from two different pediatric cohort studies from Vietnam & Cameroon and South Africa to validate the utility of MLR as a diagnostic tool for pediatric TB.[79,133,134] Data from the previous Kenya pediatric MLR study[95] was included to construct a risk score with demographic, clinical and laboratory features along with MLR to increase the sensitivity and specificity of diagnosis.

Study Population and Enrollment

Vietnam and Cameroon: As part of ANRS 12229 PAANTHER 01 study, 111 HIV infected children from Vietnam and 125 HIV infected children from Cameroon aged 13 years and less were enrolled between April 2011 and August 2013. These children had suspicion of intrathoracic TB based on at least one of the following: persistent cough; persistent fever; failure to thrive (defined as recent deviation in the growth curve or a weight-for-age Z score <−2 standard deviation); failure of broad spectrum antibiotics for a pulmonary infection; or suggestive chest radiograph anomaly. Children receiving TB treatment in the past two years were excluded. All children underwent a physical examination and chest x-ray. A baseline blood sample was also collected. Bacteriological samples were collected for Xpert MTB/RIF and AFB culture: two gastric aspirates for children <10 years old; and three expectorated sputum samples for children ≥10 years old. Further details of the cohort are reported elsewhere.[133]

South Africa: As part of Innovative strategies to improve the diagnosis of intrathoracic tuberculosis in children study, 427 children (HIV infected and uninfected) aged 12 years and less were enrolled between March 2012 and November 2017. These children had suspicion of intrathoracic TB based on at least one of

the following: persistent cough for two weeks or greater; failure to thrive (defined as recent deviation in the growth curve or a weight-for-age Z score <−2 standard deviation); persistent unexplained lethargy or reduced playfulness; or cough of any duration with one or more of the following: documented exposure to a TB case, positive TST or suggestive chest radiograph. In infants 0-60 days old, unresponsive neonatal pneumonia or unexplained hepatosplenomegaly or sepsis-like illness were also conditions for inclusion. Children only suspected of extrapulmonary TB and those having received >1 dose of anti-TB medication before collection of a respiratory sample were excluded. All children underwent a complete physical examination and chest x-ray. A baseline blood sample was collected for approximately half the children admitted at the hospital. Bacteriological samples consisting of two gastric aspirates/sputum samples and two induced sputum samples were collected for Xpert MTB/RIF and AFB culture. Further details of the cohort are reported elsewhere.[79,134]

Kenya: As part of Pediatric Urgent Start of HAART (PUSH) study,183 treatment naïve HIV-infected children were randomized between April 2013 and May 2015. Children were eligible for enrollment if they were 12 years or less, had confirmed HIV infection, were ART-naïve, were eligible for ART per national Kenyan and World Health Organization (WHO) guidelines, had caregivers who planned to live within the study catchment area for six months, and caregivers were able and willing to give informed consent for enrollment. Children with suspected or confirmed central nervous system (CNS) infection as ascertained by medical history and physical examination were excluded. Complete medical history, physical examination and chest x-ray was performed at enrollment and blood samples collected for complete blood counts. Two sputum or gastric aspirate samples for direct smear microscopy and AFB culture, one sputum or gastric aspirate for Xpert MTB/RIF, one stool Xpert MTB/RIF, and Alere Determine™ urinary lipoarabinomannan (LAM) antigen test were collected. Further details of the cohort are reported elsewhere.[135] Of these 183 children, 23 children were excluded from the previous MLR analysis for the following reasons: 1 for HIV-negative status, 1 for concurrent CNS infection, 13 for TB treatment 14 days

before or after enrollment, and 8 for failing to receive microbiological confirmation testing.[95] We included these 160 children in our risk score analysis.

Sample Size

The sample size required to validate MLR ≥0.378 for diagnosis of confirmed TB was determined to be 480 children with an expected sensitivity and specificity of 75%, precision/margin of error of 10%, prevalence of microbiologically confirmed TB of 15% and 95% confidence level.

The sample size required to estimate the diagnostic accuracy (AUC) of risk score for diagnosis of confirmed TB was determined to be 94 confirmed TB cases and 94 non-cases (total n=188) for AUC of 0.8 with precision/margin of error of 0.07 and 95% confidence level.[136]

Analysis

To validate the findings from Kenya, data from the Vietnam & Cameroon and South Africa cohorts were pooled to create a combined data set and MLR was calculated using differential blood count for monocytes and lymphocytes at initial diagnostic visit. All children were classified as having confirmed TB, unconfirmed TB or unlikely TB using NIH's the Clinical Case Definitions for pediatric TB 2015.[66] MLR value of ≥0.378 was used to classify all participants as having active TB disease while those below that value were classified as not having active TB disease. Microbiologically confirmed TB, either on AFB culture or Xpert MTB/RIF was used as reference to calculate the sensitivity and specificity of MLR for TB diagnosis.

To construct a risk score, data from the Kenya cohort was combined with the above dataset. Multivariable logistic regression was performed with AFB culture or Xpert MTB/RIF confirmed TB as the outcome. For ease of use in clinical practice, we converted all continuous predictor variables into dichotomous variables using univariate ROC curve analysis with the optimal cutoff determined based on the maximum value of Youden's index, J (J = sensitivity + specificity - 1). Model selection using Akaike's Information Criteria (AIC) was used to select the final, parsimonious model where MLR, age, gender, TB symptoms and laboratory/radiological data were included as explanatory variables. AIC is an estimator that provides the relative quality of various statistical models and allows for the selection of the most suitable set of predictor variables for the final model. We used bootstrap resampling (1000 samples) for internal validation and to obtain a value accounting for model optimism.[137,138] Log odds values from the final model were normalized by dividing them by their respective standard error (SE) and rounding off to the nearest integer. These integer values were considered the risk score for these specific variables and a cumulative risk score for each subject was calculated by summing these up. A ROC curve analysis was carried out to find the optimal cutoff for the risk score using the maximum value of Youden's index, J.

Data were analyzed using Stata version 15 (StataCorp, College Station, Texas).

Ethical Approval

The ANRS 12229 PAANTHER 01 study (Vietnam and Cameroon) was approved by national ethics committees of Vietnam and Cameroon. Innovative strategies to improve the diagnosis of intrathoracic tuberculosis in children study (South Africa) was approved by the Stellenbosch University Institutional Review Board. PUSH study (Kenya) was approved by Kenyatta National Hospital/University of Nairobi

Ethics Research Committee, Kenya Pharmacy and Poisons Board, and the University of Washington Institutional Review Board.

This study was approved by the Institutional Review Board of Emory University.

Results

Cohort Characteristics

Of the 663 children present in the combined dataset from Vietnam, Cameroon and South Africa, we excluded 62 children from the analysis because they did not have the blood samples collected within 7 days of starting TB treatment (Supplementary Figure 2-1). All 62 children were from South Africa and they did not differ demographically and clinically from the remaining 365 children from the country that remained in the analytical dataset. Nine children from Vietnam and Cameroon did not have results for the blood counts recorded (Supplementary Figure 2-1).

The median age of the combined cohorts was 1.9 years (IQR: 0.9 – 5.3) with 300 (50%) children being male. The median age of children from the South Africa cohort was younger (median: 1.3 years; IQR:0.7 – 2.5) as compared to the cohort from Vietnam and Cameroon (median: 5.5 years; IQR: 1.7 – 9.5). The overall median WBC count was 10.9 giga/L (IQR: 7.3 – 15.2) with children from South Africa having a higher count (median: 12.9 giga/L; IQR: 9.5 – 17.0) compared with children from Vietnam and Cameroon (median: 7.3 giga/L; IQR: 5.1 – 11.2). The overall median MLR was 0.22 (IQR: 0.14 – 0.38). Other characteristics of the cohort are summarized in Table 2-1.

Overall, 128 (21%) children met the criteria for confirmed TB, 183 (31%) had unconfirmed TB and 290 (48%) were unlikely to have TB. The median MLR amongst those with confirmed TB was 0.29 (IQR:0.17 – 0.53). Children with unconfirmed TB (median: 0.21; IQR: 0.12 – 0.36) had similar MLRs as compared to children with unlikely TB (median: 0.21; IQR: 0.12 – 0.35).

Diagnostic utility of MLR ≥0.378

Using the MLR value of ≥0.378, 145 (25%) children were classified as having active TB. Comparing against the culture/Xpert MTB/RIF results, this MLR value had a sensitivity of 33% (95%CI: 25 – 42), specificity of 78% (95%CI: 74 – 82), PPV of 29% (95%CI: 22 – 37) and NPV of 81% (95%CI: 77 – 84) (Table 2-2). Sensitivity, specificity, PPV and NPV values were similar when the analysis was repeated stratified by country (Table 2-2). The best cutoff using Youden's J was 0.297 resulting from a sensitivity of 47% (95%CI:39 – 55) and specificity of 67% (95%CI: 63 – 71).

Deriving a risk score for diagnosis of TB

One hundred and sixty children from the Kenya cohort were added to the overall dataset resulting in a total of 761 eligible children (supplementary Figure 2-1) of which 141 (19%) had confirmed TB, 250 (33%) had unconfirmed TB and 370 (49%) were unlikely to have TB. Other characteristics were similar to the overall cohort characteristics described above (Table 2-3). Of these 761 children, 580 had data available for all the variables included in the model building process. These children did not notably differ demographically and clinically from the ones excluded (Supplementary Table 2-1).

Using ROC curve analysis with Youden's J, the best MLR cutoff was ≥0.297 with sensitivity of 51% (95%CI: 43 – 60) and specificity of 68% (95%CI: 64 – 72). Similarly, for age the best cutoff to classify children was ≥4 years.

Using AIC values, the best model to predict active TB disease had the following variables: MLR ≥0.297, age ≥ 4 years, TST ≥5 mm, HIV positivity, chest x-ray consistent with TB (CXR), contact history with a

TB patient, fever for greater than or equal to 2 weeks and failure to thrive with an area under the curve (AUC) of 84.3% (Figure 2-1a). Model optimism was estimated to be 1.9%. Other symptoms and demographic characteristics that were considered included sex, cough for greater than or equal to 2 weeks and reduced playfulness.

Table 2-4 summarizes the coefficients for the final logistic regression model and the corresponding risk scores. The highest risk score was for TST ≥5mm (6 points) followed by CXR (5 points) while HIV positivity resulted in a negative score (-4 points). The median cumulative risk score was 4 (IQR: 1 – 8) with the area under the curve (AUC) being 84.1%. Model optimism was estimated to be 0.1%. The best cutoff for diagnosis of active TB disease using Youden's J was 9 points (Figure 2-1b). This resulted in a sensitivity of 66% (95%CI: 56 – 75), specificity of 87 % (95%CI:84 – 90), PPV of 53% (95%CI: 44 – 62%) and NPV of 92% (95%CI: 89 – 94). Sensitivity and specificity for a cutoff of 5 points was 91% and 62%, respectively. Table 2-5 summarizes sensitivities and specificities associated with other potential cutoff values. Performance of the risk score across countries was very similar for the cutoff of 5 points (Table 2-5).

Discussion

Using a multi-country cohort of children with suspected TB, we sought to validate the previous finding of MLR value of ≥0.378 for diagnosis of active TB disease and derive a risk score with MLR, demographic, clinical and laboratory features to increase the sensitivity and specificity of diagnosis.

Although an MLR value of ≥0.378 has previously performed well for diagnosis of active TB disease in HIV-infected children, that threshold's sensitivity was substantially lower in these cohorts (33% vs 79%).[95] We found the best cutoff of MLR for diagnosis of active TB was ≥0.297. This value is similar to what La Manna et al found in their study involving adults, although our sensitivity and the specificity was much lower.[89] One possible explanation for the higher threshold in Chaudry el al's study is that the study only involved HIV positive children while our study has both HIV positive and negative children. HIV infection results in decrease in lymphocytes and may thereby increase the MLR value.

We found that by itself, the sensitivity of MLR for diagnosing active TB disease is quite similar to other non-sputum based tests but the specificity is lower. Studies evaluating stool Xpert MTB/RIF have reported test sensitivities between 32% and 81% and specificities between 99% and 100% as compared to the gold standard of culture-positive respiratory specimens.[78-80,139,140] Nasopharyngeal aspirate Xpert MTB/RIF assays also had similar diagnostic performance in children (sensitivity: 39%–65% and specificity: 98%–99%).[141,142] A meta-analysis of C-reactive protein for diagnosis of TB in adults showed pooled sensitivity to be 93% (95%CI 88 – 98) and specificity to be 60% (95%CI 40 – 75) for diagnosis of active TB in outpatient setting.[143]

We developed a risk score for diagnosis of active TB disease in children that included MLR to improve the sensitivity and specificity of diagnosis. This score is intended to provide clinicians in resource-limited high–tuberculosis burden settings with a decision-making tool to initiate anti-tuberculous treatment. We found

the best cutoff for the score to be 9 points using Youden's J, which resulted in sensitivity of 66% and specificity of 87%. Given the consequences of missed diagnosis, some clinicians might be more inclined to use a lower value to increase the sensitivity of diagnosis.[60] We found that a score of 5 points resulted in a sensitivity of 91% and specificity of 61%, which is similar to what Marcy et al found with their predictive score.[144]

The two biggest contributors to our score were TST and CXR findings. TST availability and implementation including requiring repeated visits is a challenge in resource-limited settings.[145,146] Perhaps, use of IGRAs as a test of infection can overcome this, though the higher cost of IGRAs may be prohibitive.[38] Availability of quality CXR and requisite expertise to read them may not always be readily available in resource-limited settings.[147-150] This limitation may be offset in the future with the availability of digital x-ray and scoring systems, which have now been validated in pediatric population.[151,152]

Limitations of our study include inability to stratify our analysis based on severity of the disease as this information was not available to us. Severe disease, especially TB, might increase the MLR resulting in a higher threshold value, a result that was seen in the Kenyan cohort. Having a lower threshold, as we have found in our study would result in increased sensitivity and decreased specificity in such settings. As we combined data from three different cohorts for the development of the risk score, difference in recruitment strategies may bias the results. MLR and the risk score performed similarly when stratified by cohorts, giving us confidence that our results are robust. Last, we had missing predictor data on 24% of the final cohort when creating the risk score especially for CXR and TST.

Strengths of our risk score include data from a multi-country cohort with both HIV positive and negative children ensuring better external validation and generalizability. Data for these cohorts were collected prospectively. Predictors in our score include parameters that are known to be associated with TB disease in children.[66] We used methods recommended for diagnostic prediction models to create our score and

internally validate it using bootstrap resampling similar to what Marcy and colleagues did.[144] This is in contrast to most previous pediatric TB diagnostic scores and algorithms, which have been based on expert opinions and have not been validated.[67,153] Additionally, our score does not rely on sputum-based testing including Xpert MTB/RIF assay. Although availability of Xpert MTB/RIF assay is increasing globally, access in resource-limited settings is still challenging with clinical staff training, program guidelines, electrical supply, transportation and cartridge availability as possible barriers.[154-156] Even when the test is available, children may not be able to provide the requisite sputum sample.[57] Hence, a scoring system that does not rely on microbiological testing may have more clinical value.

Conclusions

With its discriminatory performance, our score should enable clinicians to rapidly initiate children with presumptive/suspected TB disease on anti-tuberculous treatment without requiring microbiological confirmation. This would reduce morbidity and mortality in children with TB. Further studies to external validate the risk score and assess its clinical usefulness are needed.

Table 2-1: Baseline demographics of the cohort used for validating MLR ≥0.378 for diagnosis of confirmed TB

Baseline characteristics	Total N=601	Vietnam N=111	Cameroon N=125	South Africa N=365
Median Age in years (IQR)	1.9 (0.9 – 5.3)	5.3 (1.6 – 8.8)	6.2 (1.8 – 9.4)	1.3 (0.7 – 2.5)
Male (n, %)	300 (50)	59 (53)	55 (44)	186 (51)
TB				
• Confirmed TB (n, %)	128 (21)	18 (16)	22 (18)	88 (24.)
• Unconfirmed TB (n, %)	183 (31)	42 (38)	39 (31)	102 (28)
Cough>2 weeks (n, %)	343 (57)	89 (80)	105 (85)	149 (41)
Fever>2 weeks (n, %)	133 (22)	47 (42)	68 (55)	18 (4.9)
Failure to Thrive (n, %)	286 (48)	42 (38)	80 (64)	164 (45)
Reduced playfulness (n, %)	193 (32)	21 (19)	94 (76)	78 (21)
Contact History (n, %)	147 (25)	10 (9.0)	5 (4.0)	132 (36)
TST>5mm (n, %), N	99 (21), 475	13 (12),105	9 (9.5), 95	77 (28), 275

CXR suggestive of TB as per NIH (n, %), N	283 (50), 568	70 (65), 107	82 (71), 116	131 (38), 345
Baseline Median WBC Count in giga/L (IQR), n	10.9 (7.3 – 15.2), 592	7.6 (5.0 – 11.2), 107	7.3 (5.4 – 11.3), 120	12.9 (9.5 – 17.0), 365
Baseline Median Monocyte Count in giga/L (IQR), n	7.4 (5.0 – 11.0), 592	5.0 (7.0 – 10.0), 107	10.0 (8.0 – 15.0), 120	6.5 (4.7 – 9.3), 365
Baseline Median Lymphocyte Count in giga/L (IQR), n	38.6 (25.0 – 54.4), 592	40.0 (27.0 – 54.0), 107	37.0 (23.0 – 54.5), 120	38.4 (24.9 – 54.4), 365
Baseline Median MLR (IQR), n	0.22 (0.14 – 0.38), 592	0.18 (0.12 – 0.30), 107	0.32 (0.20 – 0.43), 120	0.21 (0.13 – 0.37), 365
Median MLR if Confirmed TB (IQR), n	0.29 (0.17 – 0.53), 128	0.29 (0.19 – 0.38), 18	0.34 (0.30 – 0.60), 22	0.23 (0.16 – 0.52), 88
Median MLR if Unconfirmed TB (IQR), n	0.21 (0.12 – 0.36), 181	0.18 (0.12 – 0.33), 40	0.29 (0.20 – 0.40), 39	0.20 (0.11 – 0.34), 102
Median MLR if Unlikely TB (IQR), n	0.21 (0.13 – 0.35), 283	0.15 (0.11 – 0.24), 49	0.26 (0.17 – 0.44), 59	0.21 (0.12 – 0.36), 175

Table 2-2: Sensitivity, Specificity, PPV and NPV for MLR ≥0.378

	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
Complete Cohort	33 (25 – 42)	78 (74 – 82)	29 (22 – 37)	81 (77 – 84)
Vietnam	28 (9.7 – 54)	90 (82 – 95)	36 (13 – 65)	86 (77 – 92)
Cameroon	41 (21 – 64)	67 (57 – 77)	22 (11 – 38)	84 (74 – 91)
South Africa	32 (22 – 43)	78 (72 – 82)	31 (22 – 42)	78 (73 – 83)
HIV positive	36 (23 – 51)	80 (74 – 85)	28 (18 – 41)	85 (79 – 89)
HIV negative	31 (21 – 42)	76 (70 – 82)	30 (20 – 41)	77 (71 – 82)

Table 2-3: Baseline demographics of the cohort used for risk score derivation

Baseline characteristics	Total N=761	Vietnam N=111	Cameroon N=125	South Africa N=365	Kenya N=160
Median Age in years (IQR)	1.9 (0.9 – 5.3)	5.3 (1.6 – 8.8)	6.2 (1.8 – 9.4)	1.3 (0.7 – 2.5)	1.9 (0.83 – 5.2)
Male (n, %)	387 (51)	59 (53)	55 (44)	186 (51)	87 (54)
HIV	443 (58)	111 (100)	125 (100)	47 (13)	160 (100)
TB					
• Confirmed TB (n, %)	141 (19)	18 (16)	22 (18)	88 (24)	13 (8.1)
• Unconfirmed TB (n, %)	250 (33)	42 (38)	39 (31)	102 (28)	67 (42)
Cough>2 weeks (n, %)	381 (50)	89 (80)	105 (85)	149 (41)	38 (24)
Fever>2 weeks (n, %)	162 (21)	47 (42)	68 (55)	18 (4.9)	26 (18)
Failure to Thrive (n, %)	358 (47)	42 (38)	80 (64)	164 (45)	72 (45)
Reduced playfulness (n, %)	304 (40)	21 (19)	94 (76)	78 (21)	111 (69)
Contact History (n, %)	168 (22)	10 (9.0)	5 (4.0)	132 (36)	21 (13)

TST>5mm (n, %), N	107 (17), 619	13 (12),105	9 (9.5), 95	77 (28), 275	8 (5.6), 144
CXR suggestive of TB as per NIH (n, %), N	365 (52), 707	70 (65), 107	82 (71), 116	131 (38), 345	82 (59), 139
Baseline Median WBC Count in giga/L (IQR), n	10.8 (7.3 – 15.2), 752	7.6 (5.0 – 11.2), 107	7.3 (5.4 – 11.3), 120	12.9 (9.5 – 17.0), 365	10.8 (7.3 – 14.9), 160
Baseline Median Monocyte Count in giga/L (IQR), n	8.0 (5.2 – 12.6), 750	5.0 (7.0 – 10.0), 107	10.0 (8.0 – 15.0), 120	6.5 (4.7 – 9.3), 365	11.5 (7.5 – 16.4), 158
Baseline Median Lymphocyte Count in giga/L (IQR), n	42.7 (27.0 – 56.7), 751	40.0 (27.0 – 54.0), 107	37.0 (23.0 – 54.5), 120	38.4 (24.9 – 54.4), 365	50.9 (40.6 – 60.8), 159
Baseline Median MLR (IQR), n	0.22 (0.14 – 0.38), 750	0.18 (0.12 – 0.30), 107	0.32 (0.20 – 0.43), 120	0.21 (0.13 – 0.37), 365	0.21 (0.15 – 0.40), 158
Median MLR if Confirmed TB (IQR), n	0.30 (0.18 – 0.57), 141	0.29 (0.19 – 0.38), 18	0.34 (0.30 – 0.60), 22	0.23 (0.16 – 0.52), 88	0.41 (0.38 – 0.67), 13
Median MLR if Unconfirmed TB (IQR), n	0.21 (0.13 – 0.35), 241	0.18 (0.12 – 0.33), 40	0.29 (0.20 – 0.40), 39	0.20 (0.11 – 0.34), 102	0.21 (0.15 – 0.35), 66

Median MLR if Unlikely TB (IQR), n	0.21 (0.14 – 0.35), 362	0.15 (0.11 – 0.24), 49	0.26 (0.17 – 0.44), 59	0.21 (0.12 – 0.36), 175	0.21 (0.14 – 0.39), 79

Table 2-4: Coefficients from logistic regression model and corresponding risk score for selected variables for predicting confirmed TB

Variable	Coeff (from logit)	SE	Coeff/SE	Score
MLR ≥0.297	0.774	0.262	2.95	3
TST+	1.760	0.290	6.07	6
CXR suggestive	1.458	0.292	4.99	5
Contact History	0.980	0.306	3.20	3
HIV+	-1.231	0.348	-3.53	-4
Fever ≥2wks	1.051	0.330	3.18	3
Age ≥4 years	0.914	0.307	2.98	3
Failure to thrive	0.365	0.259	1.41	1

Final Model: logit (Confirmed TB) = $\beta_0 + \beta_1$ MLR ≥0.297 + β_2 TST + β_3 CXR + β_4 Contact History + β_5 HIV + β_6 Fever ≥2wks + β_7 Age ≥4yrs + β_8 Failure to thrive

Table 2-5: Sensitivities and specificities of different risk score cutoffs

	Overall		Vietnam		Cameroon		South Africa		Kenya	
Risk Score	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
4	91	50	83	44	94	38	95	50	78	62
5**	91	62	78	61	91	43	95	62	78	74
6	78	70	44	68	75	60	90	66	67	87
7	74	74	44	68	63	61	87	75	67	87
8	72	80	44	82	63	67	87	78	44	91
9*	66	87	22	90	57	84	84	82	44	97
10	60	91	22	90	57	84	76	91	33	97

* Best cutoff based on Youden's J

** Cutoff to consider because of clinical reasons

Figure 2-1a: ROC curve for the model logit (Confirmed TB) = β_0 + β_1 MLR ≥0.297 + β_2 TST + β_3 CXR + β_4 Contact History + β_5 HIV + β_6 Fever ≥2wks + β_7 Age ≥4yrs + β_8 Failure to thrive

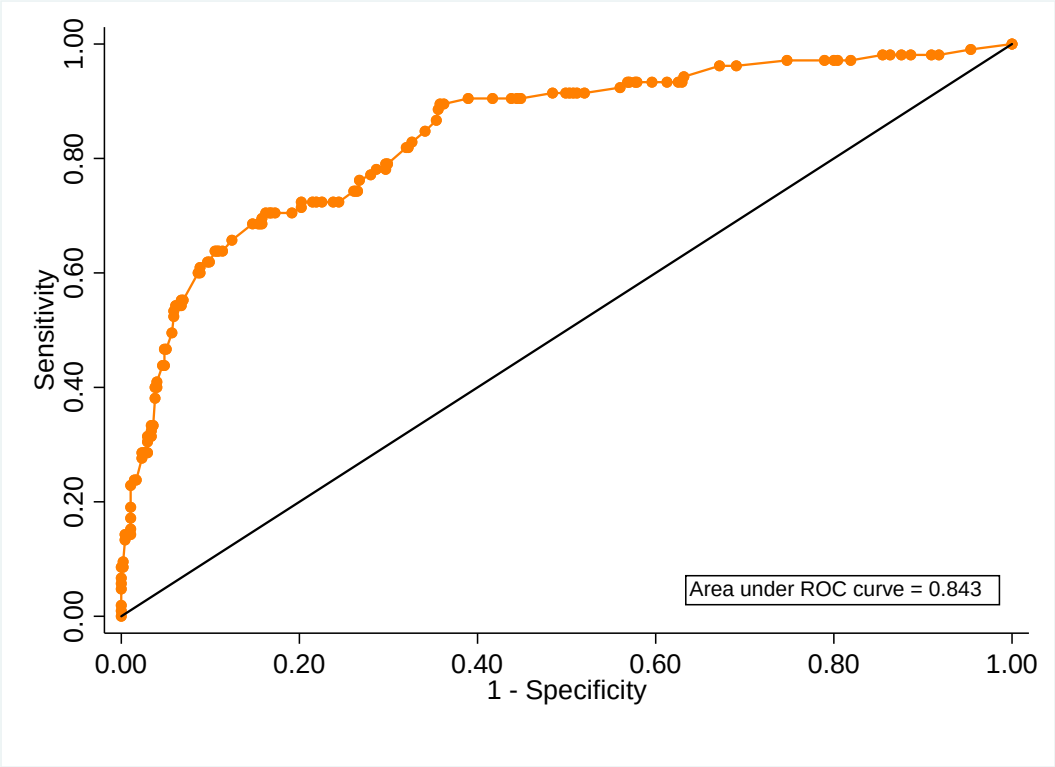
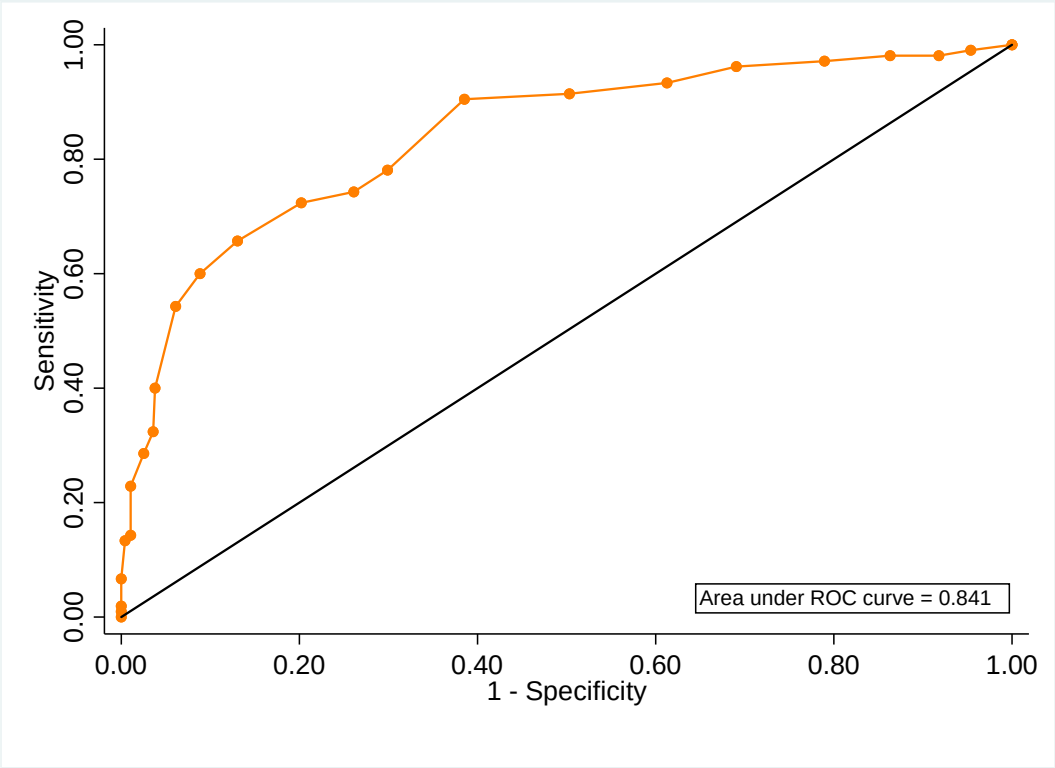
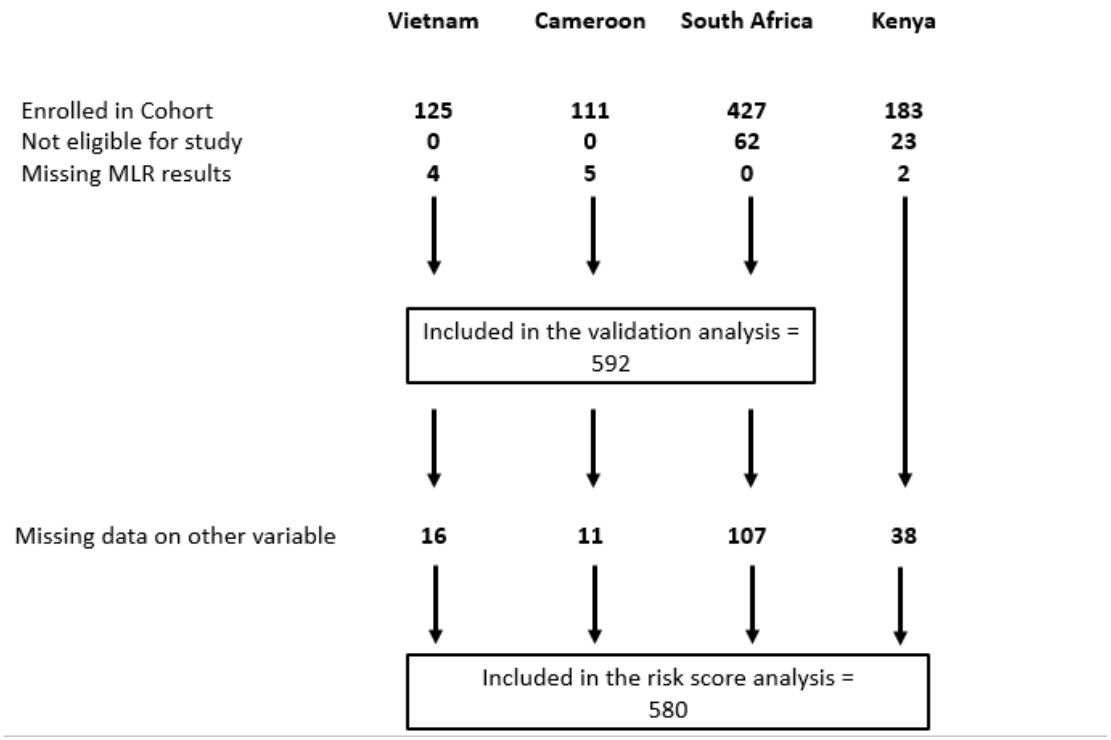


Figure 2-1b: ROC curve for the model logit (Confirmed TB) = $\beta_0 + \beta_1$ Risk score



Supplementary Figure 2-1: Details of children included in the analysis



Supplementary Table 2-1: Comparison of those included vs those excluded from risk score derivation

Baseline characteristics	Total N=761	Complete data N=580	Missing data N=181
Median Age in years (IQR)	1.9 (0.9 – 5.3)	1.9 (0.9 – 5.3)	1.8 (0.8 – 5.8)
Male (n, %)	387 (51)	293 (51)	94 (52)
HIV	443 (58)	357 (62)	86 (48)
TB			
• Confirmed TB (n, %)	141 (19)	105 (18)	36 (20)
• Unconfirmed TB (n, %)	250 (33)	195 (34)	55 (30)
Cough>2 weeks (n, %)	381 (50)	299 (52)	82 (46), 180
Fever>2 weeks (n, %)	162 (21)	130 (22)	32 (18), 180
Failure to Thrive (n, %)	358 (47)	277 (48)	81 (45)
Reduced playfulness (n, %)	304 (40)	222 (38)	82 (46), 180
Contact History (n, %)	168 (22)	131 (23)	37 (21), 178
TST>5mm (n, %), N	107 (17), 619	102 (18)	5 (13), 39

CXR suggestive of TB as per NIH (n, %), N	365 (52), 707	301 (52)	64 (50), 127
Baseline Median WBC Count in giga/L (IQR), n	10.8 (7.3 – 15.2), 752	10.8 (7.3 – 15.2)	10.9 (7.4 – 15.0), 172
Baseline Median Monocyte Count in giga/L (IQR), n	8.0 (5.2 – 12.6), 750	8.0 (5.0 – 12.1)	8.0 (5.8 – 14.8), 170
Baseline Median Lymphocyte Count in giga/L (IQR), n	42.7 (27.0 – 56.7), 751	43.3 (27.1 – 56.6)	38.6 (26.0 – 58.0), 171
Baseline Median MLR (IQR), n	0.22 (0.14 – 0.38), 750	0.21 (0.13 – 0.35)	0.27 (0.16 – 0.54), 170
Median MLR if Confirmed TB (IQR), n	0.30 (0.18 – 0.57), 141	0.30 (0.16 – 0.41), 105	0.42 (0.19 – 0.65), 36
Median MLR if Unconfirmed TB (IQR), n	0.21 (0.13 – 0.35), 247	0.20 (0.12 – 0.34), 195	0.28 (0.15 – 0.42), 52
Median MLR if Unlikely TB (IQR), n	0.21 (0.14 – 0.35), 362	0.20 (0.13 – 0.34), 280	0.25 (0.14 – 0.52), 82

CHAPTER 3

TB Preventive Therapy for individuals exposed to drug-resistant tuberculosis: feasibility and safety of a community-based delivery of fluoroquinolone-containing preventive regimen

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While not formally part of the approved aims of this dissertation, the results presented here are pertinent to understanding the design of the DR-TB preventive treatment study, the recruitment of study subjects and the nature of the target population. It is reproduced here with permission from *Clinical Infectious Diseases*]

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Summary

This study evaluated the use of fluoroquinolone-based preventive treatment for drug-resistant TB exposure in a community-based setting in Karachi, Pakistan. We found the treatment to be operationally feasible and safe in a programmatic setting in a high-burden country.

Abstract

Background

Observational studies have demonstrated the effectiveness of a fluoroquinolone-based regimen to treat individuals exposed to or presumed to be infected with drug-resistant (DR)-TB. We sought to assess the feasibility of this approach in an urban setting in South Asia.

Methods

From February 2016 until March 2017, all household contacts of DR-TB patients enrolled at The Indus Hospital were screened for TB symptoms at home. Children 0-17 years, symptomatic adults and those with an immunocompromising condition (HIV, diabetes or malnutrition) were evaluated for TB disease. Contacts diagnosed with TB disease were started on treatment. Contacts without TB disease (i) younger than 5 years; (ii) between 5 and 17 years old with either a positive TST or an immunocompromising condition; or (iii) 18 years and older with an immunocompromising conditionwere offered six month treatment with a fluoroquinolone.

Results

One hundred households with 800 contacts were enrolled: 353 (44·1%) individuals age 17 years or younger with a median age of 19 years (IQR: 10-32); 423 (52·9%) were males. In total, 737 (92·1%) individuals were screened, of which eight were already on treatment for TB (1·1%), and another three (0·4%) contacts were diagnosed with TB disease and started on treatment. Of 215 eligible for infection treatment, 172 (80·0%) contacts initiated and 121 (70·3%) completed treatment. No TB disease nor significant adverse events were observed during 12 months of follow up in any group**.**

Conclusions

Fluoroquinolone-based treatment for contacts with presumed DR-TB infection is feasible and well tolerated in a high TB burden setting.

Keywords: drug-resistant tuberculosis infection; preventive therapy; fluoroquinolone; household contacts

Background

In 2017, TB killed 1.3 million people worldwide, making it the biggest infectious killer of adults, and the leading cause of death for people living with HIV.[1] Almost 558,000 cases of TB out of 10 million new cases were due to drug-resistant TB (DR-TB), making this disease one of the highest contributors to the burden of global antimicrobial resistance.[1,157] Most DR-TB disease results from infection with drug-resistant strains of *Mycobacterium tuberculosis* rather than drug resistance acquired during treatment.[100,101,158] Among household contacts of those sick with DR-TB, 47·2 % (95% CI: 30·0-61·4%) are infected.[27] Of persons infected with TB, 10-20% develop TB disease.[23] In children under 5 years who are exposed at home to DR-TB, 6 to 24% become sick themselves.[25,159] The current global cure rate for people with disease caused by DR-TB is 55%.[1]

Preventing the progression of TB infection to disease is a fundamental part of a comprehensive epidemic control strategy for TB elimination,[40,62] and the cornerstone of the Zero TB Initiative, a strategy aimed at TB elimination.[160,161] As with drug-susceptible TB, this is achieved for those infected with strains of DR-TB by screening and treating close contacts of patients with DR-TB for disease and infection.[20,117] Such an approach has been used to successfully stop ongoing transmission of DR-TB in the Federated States of Micronesia, US, UK, and South Africa , with high effectiveness and low toxicity.[25,114-116,118] While no clinical trials have evaluated regimens specifically for presumed DR-TB infection, the existing body of observational data supports the efficacy of fluoroquinolone-based regimens,[117,162] and two clinical trials are underway that use levofloxacin to treat children exposed to multidrug-resistant TB.[127] A significant body of evidence has shown fluoroquinolones to be safe in infants and children with TB,[116,130] with the American Academy of Pediatrics[163] and the WHO[128] supporting their use. However, programmatic experience with fluoroquinolone-based treatment for presumed DR-TB infection in high-burden and resource-limited settings is limited.

In 2018, the World Health Organization recommended treatment of infection for individuals exposed to DR-TB who are at high risk of developing TB disease.[20] While this important policy change created an opportunity to use tried-and-tested strategies to stop the TB epidemic in low- and middle-income countries, there has been a lag in the inclusion of regimens to treat presumed DR-TB infection, even in high-risk contacts such as household members, in the guidelines of most national TB programs.[131] In places like Karachi, Pakistan, this has resulted in situations where infected close contacts of DR-TB patients have progressed to disease, underlining ongoing transmission and need for prevention.[159] Drawing from the successful experiences identified above, we undertook a prospective cohort study to assess the feasibility and safety of delivering treatment for presumed DR-TB infection (DR-TB Preventive Therapy) in a programmatic context in Karachi, Pakistan, a model for other Asian cities to adapt.

Methods

Setting and Study Design

We implemented this prospective cohort study as an extension of a community-based DR-TB treatment program at The Indus Hospital, Karachi, Pakistan. Karachi is the largest city in Pakistan with a population of 20 million and 16,339 cases of TB reported in 2017. The Indus Hospital is one of three public sector clinical sites for DR-TB treatment in the city. The annual number of DR-TB cases reported in Karachi is approximately 400. For the study protocol— including procedures for ruling out disease, regimen selection, and follow-up— we adapted procedures outlined in a freely available handbook that we co-authored.[164]

Study Population and Enrollment

From 1st February 2016 to 9th March 2017, we prospectively enrolled the household contacts of consecutive (index) patients initiating treatment for DR-TB disease who (1) did not have extensively drug-resistant (XDR) TB, defined as resistant to isoniazid, rifampin, any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin) (2) lived in Karachi, Pakistan, and (3) consented to participate in the study. If the index TB patient's isolate was resistant to a fluoroquinolone on drug susceptibility testing (DST) but not resistant to any of the second-line injectables, the household contacts were still eligible for the study.

At the time of starting the index patient's treatment, study staff completed a household registry form to enumerate all individuals living in the patient's household, defined as those sleeping under the same roof. The index patient and any family member accompanying him or her were counseled on the importance of a TB evaluation for all household members. A community health worker assigned to this study undertook a household visit to consent and verbally screen all household members for TB symptoms within 2-4 weeks

of index patient's enrollment in the DR-TB program. All children 0-17 years of age, symptomatic adults, adults with self-reported diabetes or HIV, and adults with malnutrition (BMI < 18.5 kg/m^2, measured at the household visit) were asked to come to the TB clinic to rule out TB disease (Figure 1). Household members were not eligible to participate in this study if they were already receiving treatment for TB disease. All household members who came to the TB clinic were seen by a TB-trained study physician and underwent a complete history and physical examination. They further underwent a chest xray and sputum examination by Xpert MTB/RIF assay if able to produce sputum. For children 5-17 years of age, a tuberculin skin test (TST) was placed and read 24-72 hours later.

Intervention

Household members diagnosed with TB disease were enrolled in the TB treatment program at The Indus Hospital. Those free of TB disease after complete TB evaluation were offered DR-TB preventive therapy if they met any of the following criteria: (1) younger than 5 years old; (2) 5-17 years old with a positive TST, diabetes, HIV, or malnutrition (weight for age less than 3rd percentile); or (3) ≥18 years old with diabetes, HIV, or malnutrition (BMI 18.5 kg/m^2). Infection treatment was provided using one of the following 6-month, 2-drug regimens:

1. Levofloxacin (15–20 mg/kg for children 5 years and younger; 7.5–10 mg/kg for individuals older than 5 years; max dose: 1000 mg/day) and ethambutol (15-25mg/kg; max dose: 2000 mg/day)

2. Levofloxacin (15–20 mg/kg for children 5 years and younger; 7.5–10 mg/kg for individuals older than 5 years; max dose: 1000 mg/day) and ethionamide (15-20 mg/kg; max dose: 750 mg/day)

3. Moxifloxacin (7.5-10 mg/kg; max dose: 400 mg/day) and ethambutol (15-25mg/kg; max dose: 2000 mg/day)

4. Moxifloxacin (7.5-10 mg/kg; max dose: 400 mg/day) and ethionamide (15-20 mg/kg; max dose: 750 mg/day)

Levofloxacin was the fluoroquinolone of choice to start treatment as per expert consensus.[14] Moxifloxacin based regimens were given to individuals whose index patients had a TB strain resistant to levofloxacin. Ethambutol was the companion drug of choice unless it was not available in the right dosing form where it was replaced by ethionamide. Table 3-1 shows the complete dosing schedule.

Subjects were started on DR-TB preventive therapy and assessed every 2 months at the clinic for a total of 6 months. In the months between clinic visits, a community health worker visited each household to monitor treatment adherence and adverse events. Infection treatment at home was administered and supervised by volunteer treatment supporters, many of whom were family members. Volunteer treatment supporters and study workers (community health workers) were trained to recognize signs and symptoms of adverse events using a standardized checklist. Subjects who reported any adverse events were immediately referred to the TB clinic for evaluation. Study physicians assessed adverse events at each clinical visit as well. Each subject was offered PKR 600 (approx. USD 6) for each clinic visit including the evaluation visit, to reduce the financial burden on the household from associated travel cost. At each visit, subjects were counseled about the infection treatment and importance of completion.

During the 6 months that followed the completion of the 6-month regimen, subjects were followed up every 2 months at home or by phone to monitor for development of TB symptoms. All subjects not receiving

infection treatment were followed up every 2 months to monitor for development of TB symptoms for 12 months.

Analysis

To assess the feasibility of delivering preventive treatment, we constructed a cascade of care tracing the flow of household contacts through each of four steps of the cascade: (1) evaluated for TB disease and eligibility, (2) prescribed preventive treatment, (3) initiated preventive treatment, and (4) completed preventive treatment.[165,166] We calculated the proportion of participants completing each step. We then calculated the cumulative probability of a contact completing infection treatment by multiplying the proportions completing each of the major steps of the cascade as conditional probabilities. Chi Square tests were used to compare treatment completion between the three age-groups and between contacts receiving ethionamide and ethambutol as companion drugs. To assess safety, we calculated the proportion of contacts who developed adverse events amongst those receiving preventive treatment.

Ethical Approval

This study was approved by the Institutional Review Boards (IRB) of Interactive Research and Development (IRD) (OHRP Registration No. 00005148) and Harvard Medical School (OHRP Registration No. 00000298).

Role of funding source

The funder of this study had no role in study design, data collection, data analysis, data interpretation, writing of the report, or in the decision to submit for publication. The authors had full access to the data and made the decision to publish the manuscript.

Results

We enrolled 100 DR-TB index patients in the study, with 800 household contacts. Of these 800 individuals, 353 (44·1%) were 17 years of age or younger with a median age of 19 years (IQR: 10-32), and 423 (53·1%) were male (Table 3-2). Eight (1·0%) were already receiving treatment for TB disease at the time of study enrollment, of which five (62·5%) were receiving DR-TB disease treatment.

Of the 792 remaining contacts, we verbally screened 737 (93·1%), of which 402 (54·5%) contacts met criteria for further evaluation (Figure 3-1), and we evaluated 326 (81·1%). We diagnosed three (0·9%) with TB disease. Among the 323 in whom TB disease was ruled out, 215 (66·6%) were eligible for infection treatment based on the study criteria. Of these, 76 (35·3%) were eligible based on age (under 5 years of age) and 133 (61·9%) were malnourished; the rest either had a positive TST result or diabetes (Figure 3-2).

Of the 215 contacts eligible for infection treatment, 172 (80·0%) started DR-TB preventive therapy. Of these, 102 (59·3%) received levofloxacin and ethambutol, 54 (31·4%) received levofloxacin and ethionamide, 11 (6·4%) received moxifloxacin and ethambutol, and 5 (2·9%) received moxifloxacin and ethionamide. The proportion who completed treatment if infection treatment was started was 70·3%

(range: 67·4 to 75·4% across the three age groups) (Figure 3-2). The probability of completing treatment if DR-TB preventive therapy was prescribed was 56·3%. The cumulative probability of cascade completion was similar across the three age groups (Chi Sqr p-value= 0·15) (Figure 3-3). Ninety-nine percent of the cohort completed six months of follow up, with 96 percent completing 12 months of follow up, and no TB disease was observed in any household contact.

Thirty-six (20·9%) contacts reported at least one adverse event during infection treatment and 19 (11·1%) reported more than one adverse event. Table 3-3 shows the adverse event profile by companion drug. Individuals treated with ethionamide reported significantly more adverse events (n=20, 33·4%) compared

to those treated with ethambutol (n=16, 14·2%) (Chi Sqr p-value<0·05). The adverse event profile by system amongst those who completed treatment and who did not complete treatment was not statistically different. Eleven (30·6%) of the 36 contacts experiencing adverse events discontinued treatment. This was not statistically different between those treated with ethionamide (n=7, 35·0%) compared to those treated with ethambutol (n=4, 25·0%) (Chi Sqr p-value=0·52).

Discussion

Our study found that provision of infection treatment to household contacts of DR-TB patients in a programmatic setting in Karachi, Pakistan is both feasible and safe. No TB disease was observed in household contacts during the 12 months of follow-up. To our knowledge, this is the largest study to provide DR-TB preventive therapy to confirmed or presumed-infected contacts of DR-TB patients in a low-income, high-burden setting. Moreover, this was the first large Asian city to undertake a comprehensive strategy to TB elimination as part of the Zero TB Initiative. Our study found the overall completion rate to be 71% amongst those who initiated treatment in comparison to the study from the Federated States of Micronesia which achieved a completion rate of 89% amongst those who initiated treatment.[114] One of the strengths of our study is the prospective design and dedicated follow up staff, with 96% of the cohort completing 12 months of the follow up. Our study provides timely evidence of the operational feasibility and tolerability of infection treatment.

We identified on average 8 household contacts per index patient and were able to evaluate a very high percentage of these contacts. We only found 11 contacts with TB disease with a prevalence of 1·4%, which is lower than reported in the literature.[167] Over half the household contacts sick with TB disease had DR-TB. The cumulative probability of a contact completing the prevention cascade was 47%; this is higher than completion observed with contact management efforts in other studies globally.[168]

The adverse events observed were rare, adding to the evidence that fluoroquinolone-based infection treatment is safe. The observation that one in five individuals in our cohort experienced an adverse event was consistent with previous reports on provision of infection treatment to DR-TB contacts, which have reported 10-50% of contacts experiencing adverse events.[25,114-118] We found that the fluoroquinolone and ethambutol combination was associated with a lower risk of adverse events compared to fluoroquinolone

and ethionamide. Thus, we recommend using ethambutol, where possible, if a program decides to use a companion drug with either levofloxacin or moxifloxacin in the treatment of presumed DR-TB infection.

A major limitation of our study is that we were not able to evaluate all the contacts, which could have contributed to the low baseline prevalence for household contacts with TB disease. For example, the majority of adults in the household were only screened by symptoms, an approach that has been shown to have a sensitivity of about 38%.[169] As there was shortage of TST, we had to prioritize infection testing in contacts 5-17 years old and hence were not able to conduct infection testing for the whole cohort and quantify the prevalence of TB infection. Other limitations include reliance on contacts or family members for adherence reporting, which may have resulted in over-reporting. However, adherence was crosschecked on home visits through pill counts by the health workers. We also lacked detailed information on contagiousness of index TB patients, although most were culture positive at time of diagnosis. Finally, younger children may not always report symptoms and there is a possibility of missing TB disease.

In this study, 80% of the eligible household contacts initiated treatment and 70.3% of that group completed treatment. The reasons given by contacts who refused treatment or did not complete treatment were the long duration of preventive treatment with daily pills and not having the disease. There is a need for shorter preventive regimens to be better able to convince individuals exposed to TB to initiate preventive treatment. Numerous factors have been identified in the literature that contribute to low completion rates and overall cumulative probability of a contact completing each step of the prevention cascade including factors related to health systems infrastructure and resources, knowledge gaps and lack of health education of index cases, contacts and health care workers, attitude and perception about need of preventive treatment, stigma associated with TB, access to care, competing priorities of the family, and treatment related challenges.[166] This study addressed some of these including counseling of the index patients and their household members by an experienced psychologist, household screening visits, provision of free medications, DOT support

and travel reimbursements for clinical visits. We also had a strong monitoring component for adverse events with DOT workers trained to recognize signs of adverse events, phone calls by the study staff and bi-monthly clinical visits. These likely contributed to the high overall cumulative probability of a contact completing each step of the prevention cascade observed in the study.

While it reports TB outcomes to the public sector, The Indus Hospital is a private sector site with strong programmatic controls that likely contributed to high completion rates at each step of the cascade. Further studies are needed to see how well these results can be replicated in other settings, including public sector sites providing care for DR-TB.

Conclusions

We found that the treatment of presumed DR-TB infection in household contacts was operationally feasible and safe. No TB disease was observed in household contacts during the 12 months of follow-up. Our results can guide national policy for Pakistan and for other high TB-burden countries for treatment of DR-TB contacts.

Authors' contribution

HH, FA and MCB conceptualized the study and wrote the protocol; AAM, JF, SS, MJ, ZB, and FJ implemented the study and collected data under supervision from HH, NS, FA, RF, AJK, SK and MCB; AAM, JF, SS, CMY, MCB and HH performed and reviewed the analysis; AAM, CMY, and HH wrote the initial draft of the manuscript. All authors helped interpret the findings, read and approved the final version of the manuscript.

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Declaration of interests

FA is the Chair of the WHO's Child and Adolescent TB Working Group. FA and MCB are part of the writing group that wrote the 2015 consensus recommendations for the use of presumed DR-TB infection treatment in household contacts. CMY reports grants from Janssen Global Public Health, outside the submitted work. All other authors declare no conflict of interest.

Table 3-1: Dosing chart for prescribing preventive treatment to household contacts

Drug	Age	Dose	Frequency	Duration
Levofloxacin	<5 years	15-20mg/kg/day	In 2 divided doses	Daily for 6 months
	5 - <15 years	7.5-10mg/kg/day	Once daily	
	15 years and older	750-1000 mg/day	Once daily	
Moxifloxacin	<15 years	7.5-10mg/kg/day	Once daily	Daily for 6 months
	15 years and older	400 mg/day	Once daily	
Ethambutol	<15 years	15-25 mg/kg/day	Once daily	Daily for 6 months
	15 years and	600-1200mg/day	Once daily	

	older			
Ethionamide	<15 years	15-20mg/kg/day	In 2 divided doses	Daily for 6 months
	15 years and older	500-750mg/day	In 2 divided doses	

Table 3-2: Baseline demographics and clinical characteristics of DR-TB household contacts (N=800)

	<5 years **n (%) or median [IQR]**	**5 – 17 years** **n (%) or median [IQR]**	**>17 years** **n (%) or median [IQR]**	**Total** **n (%) or median [IQR]**
	N=94	*N=258*	*N= 448*	*N= 800*
Age in years	2 [0.8 – 3·0]	11 [7·0 – 15·0]	30 [23·0 – 43·0]	19 [10·0 – 32·0]
Sex (Male)	50 (53·2)	134 (51·9)	241 (53·8)	425 (53·1)
Contacts on TB disease treatment	0 (0)	1 (0·4)	7 (1·6)	8 (1·0)
	N=77	*N=214*	*N= 333*	*N= 624*
BMI (kg/m^2)	14·7 [13·7 – 16·9]	15·0 [13·6 – 17·0]	22·5 [18·7 – 26·7]	18·1 [14·8 – 24·0]
	N=78	*N=215*	*N= 333*	*N= 626*
Weight for Age Z-score	-1·6 [-3·3 – -0·1]	-1·9 [-2·9 – -0·9]	N/A	N/A
Presence of symptoms	*N= 88*	*N= 238*	*N= 411*	*N= 737*

Cough	1 (1·1)	4 (1·7)	0 (0)	5 (0·7)
Fever	3 (3·4)	0 (0)	1 (0·2)	4 (0·5)
Weight loss	1 (1·1)	0 (0)	0 (0)	1 (0·1)
Additional TB risk factors	*N= 88*	*N= 238*	*N= 411*	*N= 737*
History of TB disease	0 (0)	8 (3·3)	1(0·2)	9 (1·2)
TST => 5mm	1 (1·1)	5 (2·1)	0 (0)	5 (0·7)
Index Case resistant to FQ	10 (11·4)	50 (21·0)	78 (19·0)	138 (18·7)

Table 3-3: Adverse events profile experienced by individuals treated for presumed DR-TB infection, by companion drug received (N= 172)

Adverse Event†	Regimen included ethambutol, n (%) N= 113	Regimen included ethionamide, n (%) N= 59	Total who received infection treatment regimen, n (%) N= 172
Gastrointestinal	5 (4·4)	14 (23·7)	19 (11·1)
Jaundice	1 (0·9)	0 (0)	1 (0·6)
Generalized	9 (8·0)	15 (25·4)	24 (14·0)
Rheumatic	3 (2·7)	1 (1·7)	4 (2·3)
Disturbance in menstrual cycle	0 (0)	1 (1·7)	1 (0·6)
Renal	1 (0·9)	0 (0)	1 (0·6)

† Not mutually exclusive

Figure 3-1: Screening and prevention treatment initiation algorithm for household contacts

Abbreviations: BMI, body mass index; CXR, chest X-ray; HIV, human immunodeficiency virus; MTB, Mycobacterium tuberculosis; TB, tuberculosis; Tx, treatment

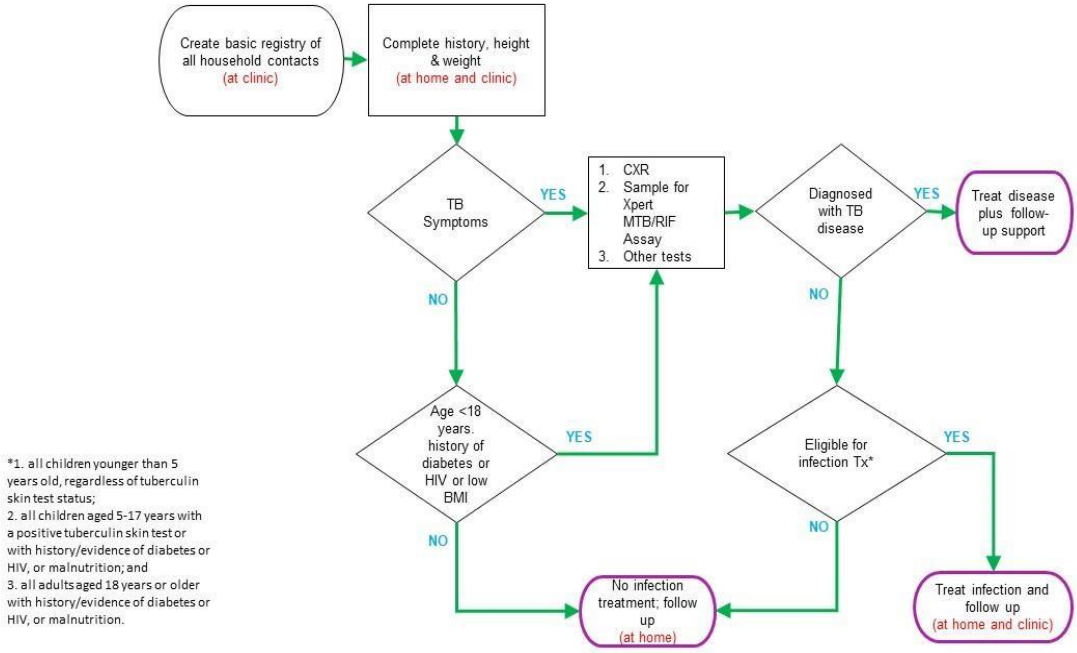


Figure 3-2: Prevention cascade for drug-resistant tuberculosis household contacts (N = 800)

Abbreviations: PT, preventive treatment; TST, tuberculin skin test

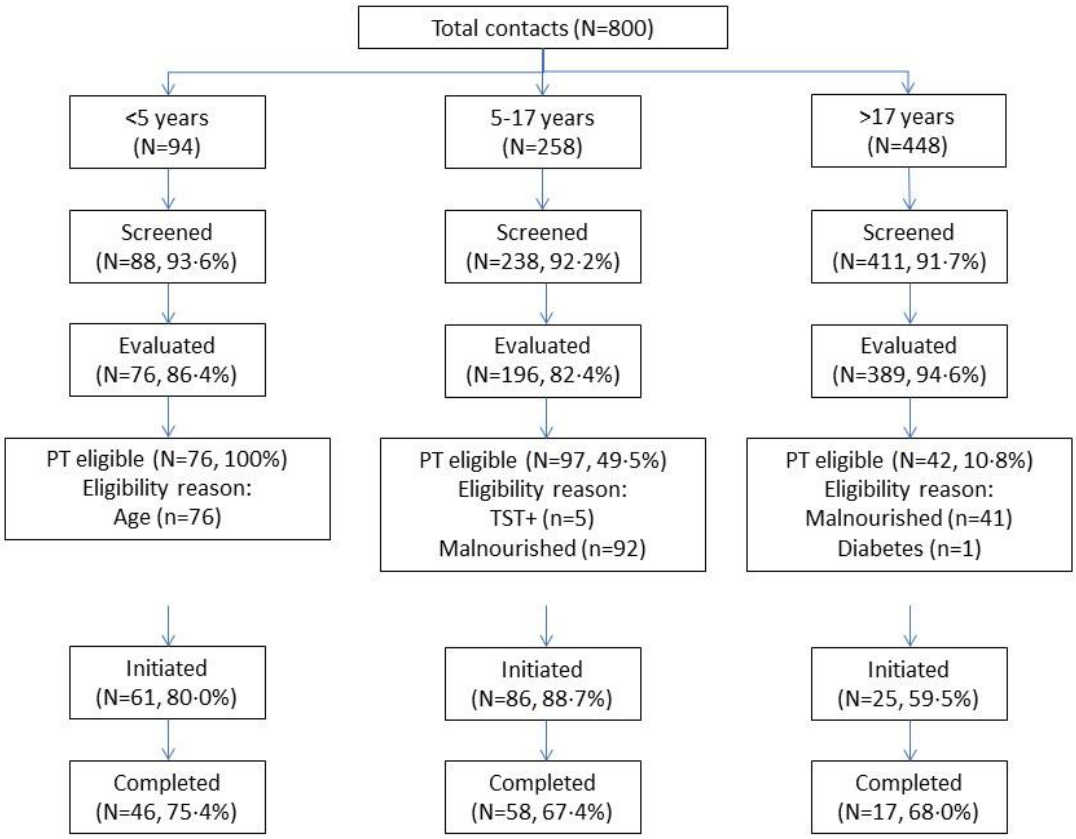
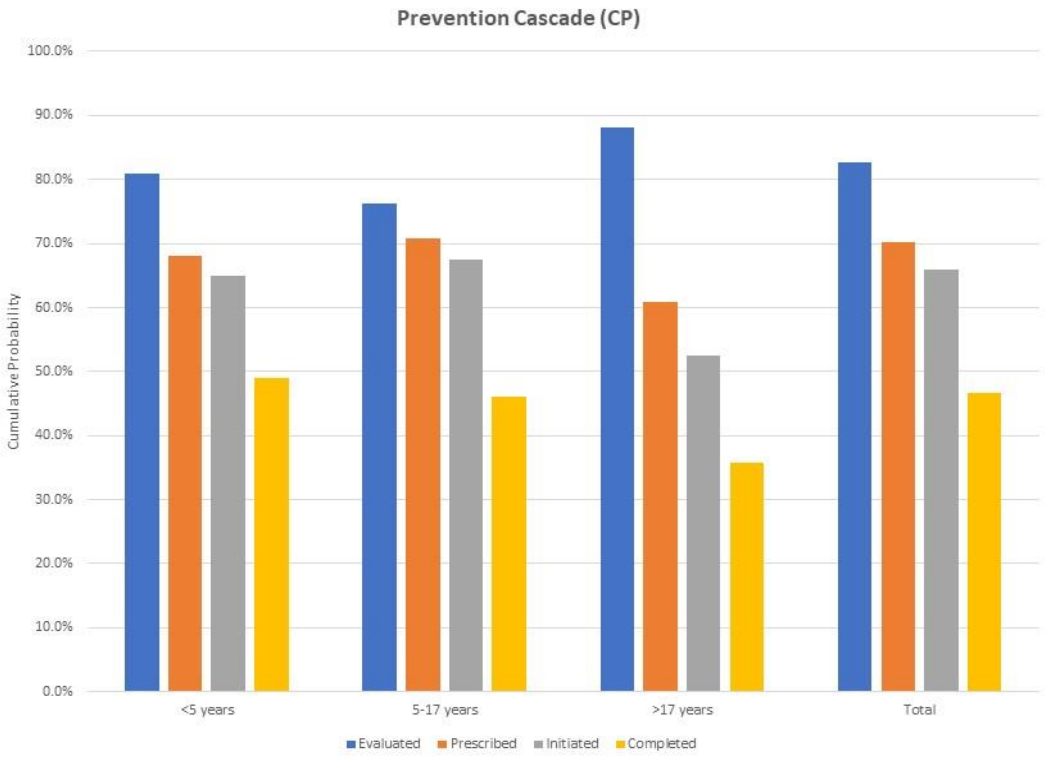


Figure 3-3: Cumulative probabilities for evaluation, prescription, uptake, and completion of treatment for presumed drug-resistant tuberculosis infection treatment by age group (N = 792)
Abbreviation: CP, cumulative probability



CHAPTER 4

Effectiveness of fluoroquinolone-based preventive treatment for persons exposed at home to drug-resistant tuberculosis

Abstract

Background

Effectiveness of a fluoroquinolone-based regimen to treat individuals exposed to, or presumed to be infected with, drug-resistant (DR) TB has been demonstrated in some settings. We sought to assess the effectiveness of a fluoroquinolone-based two drug regimen in a high-burden, low resource setting in South Asia with low prevalence of HIV.

Methods

Between February 2016 and March 2017 at The Indus Hospital in Karachi, Pakistan, 172 household contacts of DR-TB patients were started on a six-month preventive treatment with a fluoroquinolone-based two drug regimen. We assessed effectiveness in this cohort by comparing the rate and risk of TB disease occurrence over two years of follow up to the rates and risks reported in the literature.

Results

Of the 172 individuals, two developed TB disease over 336 person-years (p-y) of follow up**.** The incidence rate observed in our cohort was 595 per 100,000 p-y and cumulative incidence was 0.12. Compared to rates reported in the literature, the incidence rate ratio (IRR) ranged between 0.29 (95%CI: 0.04-1.3) and 0.50 (95%CI: 0.06-2.8) with a pooled estimate of 0.35 (95%CI: 0.14 – 0.87). The risk ratio (RR) ranged between 0.11 (95%CI: 0.03-0.43) and 0.85 (95%CI: 0.21-3.4) with a pooled estimate of 0.28 (95%CI: 0.15 – 0.53).

Conclusions

Fluoroquinolone-based treatment for presumed DR-TB infection reduces progression to TB disease and should be considered for high-risk household contacts of DR-TB patients.

Background

TB is the leading infectious cause of death globally and the ninth leading cause overall. It results in approximately 10 million new cases and 1.7 million deaths annually. Approximately, 650,000 TB patients annually have drug-resistant (DR) TB.[1]

Treatment for DR-TB is complex and long with average treatment success at a dismal 55%.[1] Treatment is often costly and represents a large burden on health systems, especially in countries where DR-TB is prevalent.[108,109] Therefore, prevention interventions are essential to reduce disease and deaths from DR-TB. Household contacts of DR-TB patients are at high risk of developing TB disease[27] and hence, are prime targets for preventive interventions. Currently available preventive therapies would not be expected to protect persons exposed to DR-TB as the presumed infecting TB organism is resistant to these drugs.

One hurdle is the paucity of evidence about effective preventive regimens in DR-TB specifically, in contrast to the abundant evidence available for preventive therapy in drug-sensitive TB.[20] There are no data from randomized controlled trials available to guide the approach to DR-TB preventive treatment, but observational studies from Federated States of Micronesia, US, UK and South Africa have shown efficacy of fluoroquinolone-based preventive treatment in adults and children.[25,113-118] The largest study with a comparison arm from Federated States of Micronesia described 119 household contacts infected with TB, of which 104 were started on preventive treatment with a fluoroquinolone-based regimen for 12 months. None of the contacts initiated on preventive treatment developed TB disease over three years of follow up while, of the 15 contacts that refused treatment, 20% developed DR-TB.[114]

In another observational cohort, 186 children infected with DR-TB strains presumed susceptible to ofloxacin in Western Cape, South Africa, were started on a three-drug preventive treatment regimen. Of these, six children developed incident TB disease in 219 patient-years of follow up. Nine of the children

started on therapy were HIV positive, of which two developed TB disease. The study did not have a comparison arm.[116] A meta-analysis by Marks et al of published observational studies concluded preventive treatment to be 90% effective with a very wide range of 9 to 99% effectiveness reported.[162]

Most studies of DR-TB preventive treatment have been conducted in either high-resource settings or in settings with a high-burden of HIV; they also have limited sample size and no comparison arm. Hence, there remains a need to evaluate the effectiveness of DR-TB preventive treatment in other settings. In Karachi, Pakistan, a high TB burden and low HIV prevalence setting, we sought to evaluate the effectiveness of providing fluoroquinolone-based two-drug preventive treatment to high-risk household contacts of DR-TB patients.

Methods

Setting, study design, and population

From February 2016 to March 2017, we prospectively enrolled household contacts of 100 consecutive (index) patients initiating treatment for culture confirmed DR-TB disease at The Indus Hospital in Karachi, Pakistan. Patients whose isolates were resistant to a fluoroquinolone on drug susceptibility testing but not resistant to any of the second-line injectables were eligible for the study. Thus, we had confirmation that none of these index patients had extensively drug-resistant (XDR) TB, defined as resistant to isoniazid, rifampin, any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin). Full details of the cohort are reported elsewhere.[170]

Briefly, the study population consisted of all children and adults sleeping in the same house as the index patients at time of DR-TB diagnosis. At the baseline contact investigation, all household members were evaluated for TB disease clinically including chest x-ray and sputum testing if they were able to produce sputum. We excluded household members already on TB treatment at time of this evaluation (n=8) or those diagnosed with TB treatment at this evaluation (n=3) (co-prevalent TB patients). Preventive treatment with fluoroquinolone-based two-drug regimen for six months was offered to the remaining household contacts if they were: (1) 0-4 years old; (2) 5-17 years old with either a positive tuberculin skin test or evidence of immunocompromising condition such as diabetes, HIV and/or malnutrition; and (3) 18 years and older with evidence of an immunocompromising condition such as diabetes, HIV and/or malnutrition (BMI $<18.5 kg/m^2$). Contacts who did not meet these criteria were not prescribed preventive treatment, but were followed for the development of active TB disease.

All household contacts were evaluated for TB disease through symptom screen every two months for up to 24 months. If an individual had a positive symptom screen at any visit, he or she was evaluated further clinically, including chest x-ray and sputum testing if able to produce sputum.

Preventive treatment was provided using one of the following six-month, two-drug regimens:

Regimen	**Drug 1, dose**	**Drug 2, dose**
LFX and EMB	Levofloxacin <5 yrs: 15-20 mg/kg >5 yrs: 7.5-10 mg/kg *max dose: 1000 mg/day*	Ethambutol 15-25 mg/kg *max dose: 2000 mg/kg*
LFX and ETO	Levofloxacin <5 yrs: 15-20 mg/kg >5 yrs: 7.5-10 mg/kg *max dose: 1000 mg/day*	Ethionamide 15-20 mg/kg *max dose: 750 mg/kg*
MFX and EMB	Moxifloxacin 7.5-10 mg/kg *max dose: 400 mg/day*	Ethambutol 15-25 mg/kg *max dose: 2000 mg/kg*
MFX and ETO	Moxifloxacin	Ethionamide

	7.5-10 mg/kg	15-20 mg/kg
	max dose: 400 mg/day	*max dose: 750 mg/kg*

Moxifloxacin based regimens were given to individuals whose index patients had a TB strain resistant to levofloxacin. Ethambutol was the companion drug of choice unless it was not available in the correct dosing form.

Contacts started on preventive treatment were clinically evaluated every two months for six months by a study physician. Between clinic visits, a study worker visited each household monthly to assess occurrence of symptoms of TB disease and adverse events and to monitor treatment adherence. After the completion of the six-month preventive treatment, participants were followed up every two months at home or by phone to monitor for occurrence of TB symptoms. Contacts not receiving preventive treatment were followed up every two months at home or by phone through symptom screen to monitor for occurrence of TB symptoms until the end of the study period. Any contact with TB symptoms was referred to The Indus Hospital clinic for further evaluation.

Analysis

The primary outcome of interest was the effectiveness of preventive treatment in contacts who received preventive treatment defined as disease-free survival two years from the time of known contact with the DR-TB patient.

We searched the published literature to find systematic reviews and meta-analysis of studies of the incidence of TB disease in contacts after exposure to a TB patient. We found two such studies.[27,167] We then searched for studies that were conducted after these meta-analyses were published. We did not restrict the search to studies that evaluated TB incidence in persons exposed to drug-resistant TB disease. We used the definition of an incident case and tuberculin skin test (TST) positive as defined by each study.

From the identified studies, we extracted the incidence of TB disease amongst household contacts by age, year post exposure, TST positive/high-risk classification and preventive treatment status if provided.[167,171-176] Available data from each study are summarized in Table 4-1.

We calculated the observed incidence rate in contacts who took preventive treatment by dividing the number of persons who developed TB disease by the person-years (p-y) accumulated over two years. Cumulative incidence of TB disease over two years in these individuals was calculated by dividing the number of persons who developed TB disease by the total number of persons who took preventive treatment. We applied the incidence rates extracted from the literature (Table 4-1) to our cohort to calculate the expected number of persons who would have developed TB disease within two years of exposure to a DR-TB patient in the absence of preventive treatment. We calculated the expected incidence rate by dividing the expected number of persons who would have developed TB disease by the total p-y accumulated in our cohort over two years. To assess the effectiveness of preventive treatment, we then compared the expected incidence rate and cumulative incidence of TB disease from the studies in Table 4-1 against the observed incidence rate and cumulative incidence in our cohort. Incidence rate ratio (IRR), risk ratio (RR), incidence rate difference (IRD) and risk difference (RD) were used for comparison, depending on the available data. Number needed to treat (NNT) to prevent one case of TB disease was calculated as total number of persons receiving preventive treatment divided by the number of TB cases

averted. Number of TB cases averted was calculated by subtracting the observed number of TB cases from the expected number of TB cases.

We generated pooled estimates of IRR and RR using inverse variance weighting with random effects for the effectiveness of preventive treatment that are robust to a range of different assumptions.

We evaluated the validity of the pooled estimation method from the random effects model by a simulation study with 10,000 replications using a Poisson distribution for the incidence rate and a Binomial distribution for the risk for each study.

Data were analyzed using Stata version 15 (StataCorp, College Station, Texas) and SAS software version 9.4 (SAS Institute Inc., Cary, NC).

Ethical Approval

This study was approved by the Institutional Review Boards (IRB) of Interactive Research and Development (IRD), Harvard Medical School, and Emory University.

Role of Funding Source

The funder of this study had no role in study design, data collection, data analysis, data interpretation, writing of the report, or in the decision to submit for publication. The authors had full access to the data and made the decision to publish the manuscript.

Results

Of the 800 household contacts enrolled, 11 had co-prevalent TB disease at time of contact investigation. The remaining 789 subjects contributed 1512 person-years of follow-up over the study period. Two hundred and fifteen subjects with a median age of 7 years (IQR: 3 – 16), median BMI of 14.8 kg/m^2 (IQR: 13.4 – 16.9) and 52% males met the study criteria and were offered preventive treatment; of these, 172 accepted the treatment and contributed 336 person-years. Preventive treatment was refused by 43 individuals who were eligible for treatment, but they remained under follow up. The 43 individuals who did not start treatment were older, with a median age of 16 years (IQR: 3 – 22), compared to those who started preventive treatment, with a median age of 7 years (IQR: 3 – 15). There were no other notable differences between these two groups (Table 4-2). Eighty one percent of the whole cohort (91% of those who were started on preventive treatment) completed two years of follow up.

Over two years of follow up, there were two subjects diagnosed with TB disease in the cohort. Both had received preventive treatment resulting in a TB incidence rate of 595 per 100,000 p-y and cumulative incidence of 0.12. Table 4-3 summarizes the characteristics of the two subjects who developed TB disease in the follow up period.

Using the age and year of follow up stratified incidence rates from a DR-TB household cohort from Peru,[171,172] we calculated the expected incidence of TB disease in the group that received preventive treatment in Karachi. Had no preventive treatment been given, we would have expected 4.7 patients with TB disease, based on the 336 p-y accumulated by our cohort (incidence rate: 1399 per 100,000 p-y). We observed only two patients with TB over the two years of follow up with an IRR comparing observed to expected number of TB cases being 0.40 (95%CI: 0.05-2.0) and IRD being -804/100,000 p-y (95%CI: (-)2314 – 706). Number needed to treat (NNT) to avert one TB disease case was 64.

To demonstrate the range of IRR and IRD, we performed the same exercise using TB disease incidence rates from two other studies and a meta-analysis.[167,173,174] Table 4-4 summarizes the findings from this analysis. Using rates from Reichler et al[173] and Martin-Sanchez et al[174] led to equivalent results; expected number of TB cases: 6.6 and IRR: 0.29 (95%CI: 0.04-1.3). Using rates from Fox et al's meta-analysis of TB disease incidence in household contacts of TB patients,[167] we calculated the IRR to be 0.50 (95%CI: 0.06-2.8) and IRD to be -566/100,000 p-y (95%CI: (-)1983 – 852). The pooled estimate for IRR was 0.35 (95%CI: 0.14 – 0.87) (Figure 4-1a). Using the simulation study, the median IRR was 0.42 (2.5^{th} – 97.5^{th} percentile: 0.18 – 0.79).

We found six studies that estimated the risk of TB disease in household contacts exposed to a TB patient in the absence of preventive treatment, including the four studies used for incidence rate calculations above.[167,171-176] Using risk figures from these studies, we estimated the risk ratio for preventive treatment to be between 0.11 (95%CI: 0.03-0.43) and 0.85 (95%CI: 0.21-3.4), and RD between -9.8/100 persons (95%CI: (-)13.1 – (-)6.5) and -0.2/100 persons (95%CI: (-)1.8 – 1.4) (Table 4-4). The pooled estimate for RR was 0.28 (95%CI: 0.15 – 0.53) (Figure 4-1b). Using the simulation study, the median RR was 0.36 (2.5 – 97.5 percentile: 0.17 – 0.68).

Discussion

In our cohort of 172 persons exposed at home to DR-TB who then received preventive treatment with a fluoroquinolone-based regimen, we observed just two patients with TB disease over two years of follow up. Using the rates from a study of DR-TB households from Lima, Peru[172], we would have expected to observe almost five TB patients over the same follow up period among the 172 started on treatment. Thus, by providing preventive treatment, we averted almost three TB patients with an effectiveness of 60%.

Household contacts are a combination of several populations with different risks based on biological susceptibility. Saunders et al in a study from Peru developed a risk score to predict which individuals would develop TB disease after being exposed at home to TB and showed that 90% of the TB cases arose among individuals at high or medium risk over 10 years. Two of the risk factors for TB disease in that study included low body-mass index (BMI) and age, with the score predicting the risk for TB disease independent of the TB infection status.[176] Other studies have also documented increased risk for TB disease in children less than five years and people with low BMI. The Indus Hospital provided preventive treatment to the contacts at known high risk for TB disease based on their demographics and clinical presentation, with 35% of those started on preventive treatment younger than five years of age. Hence, the 60% effectiveness is likely an underestimate of the true effectiveness as the rates used for calculating expected patients came from the whole household cohort and not from contacts deemed at highest risk of incident TB disease. Some of the children in the comparison cohort also received IPT, which may have lowered their risk of TB disease.

Using TB incidence rates from two studies from the US[173] and Spain[174] and a meta-analysis by Fox et al,[167] the range of the number of cases averted lies between two and five patients with an effectiveness between 50% and 71%. The meta-analysis also included contacts who were prescribed preventive treatment and did not differentiate between those at higher and lower risk of incident TB disease, which probably resulted in

lower overall incidence rate. The other two studies measured TB incidence rate over five years of follow up but the highest risk of incident TB disease is within the first two years after exposure. Thus, applying the rate measured over five years to a cohort followed for two years may result in underestimation of expected number of incident TB cases. The pooled estimate of effectiveness of the preventive treatment in the Karachi cohort compared to all four studies was 65%.

Using the pooled RR, we estimated the effectiveness of preventive treatment to be 72%. This estimate is comparable to the effectiveness that we found using incidence rate data from other cohorts and gives more confidence in interpretation of our results. Notably, these studies also suffered from some of the limitations highlighted above.

Our results are also consistent with the TB risk reduction reported with use of isoniazid preventive treatment (IPT) for drug-susceptible TB (RR: 0.40; 95%CI: 0.31 – 0.52).[177] Marks et al in their meta-analysis of published observational studies that provided preventive treatment for DR-TB exposure estimated a risk reduction of 90%, with a wide range from 9 to 99% as most studies had small sample sizes.[162]

One key limitation of our study was reliance on symptom screen at home and asking household members whether someone in the household was diagnosed with TB or started on TB treatment during the follow up period, since the parent study was designed to evaluate operational feasibility of providing preventive treatment and was not designed as an effectiveness study, which explains these design features.[170] This limitation could have led to an ascertainment bias. We do not, however, expect our estimates would be significantly biased with this approach. The reason is that, in the same population between 2008 and 2011, Amanullah et al conducted a household cohort study using a similar approach and found a high TB disease incidence of 5.4% among children in the first year after exposure to a DR-TB patient.[159] Using rates from low- to medium-burden TB countries like Peru to compare the rates from this study, which is in a high-burden TB country, may also have led to an underestimation of effect of preventive treatment.

Strengths of our study include the prospective design with over 91% follow up retention at two years and a high completion rate of preventive treatment.[170] Our results were robust to a range of different assumptions and showed similar decrease in TB disease incidence after provision of preventive treatment as other observational studies.

Conclusion

In a high TB burden setting with low HIV prevalence, we found preventive treatment reduced the risk of TB disease in persons exposed at home to DR-TB with RR being 0.35. This result adds to the growing evidence base for effectiveness of preventive treatment for DR-TB and is consistent with evidence that a fluoroquinolone-based two-drug regimen is one option that can be used.

Table 4-1: Details of studies from which data were extracted for analysis

	Becerra et al 2013[172]	**Fox et al 2013[167]**	**Reichler et al 2019[173]**	**Martin-Sanchez et al 2019[174]**	**Sloot et al 2014[175]**	**Saunders et al 2017[176]**
Incidence Rate/Risk	Incidence Rate and Risk	Incidence Rate and Risk	Incidence Rate and Risk	Incidence Rate and Risk	Risk	Risk
IR/Risk by PT status	No PT for DR-TB exposure	No	Yes	Yes	Yes	No
IR/Risk by age and year of follow up	Yes	Not by age but by year of follow up	No	No cases in children	No	No
IR/Risk by risk group	No	No	Yes	Yes	No	Yes
IR/Risk reported	<15 yrs, Yr 1: 2079/100,000 p-y	Yr 1: 1478/100,000 p-y	Rate: 1951/100,000 p-y	Rate: 1970/100,000 p-y	2 yr risk in TST positive contacts	2.5 yr risk for medium to high

	<15 yrs, Yr 2: 315/100,000 p-y 15+ yrs, Yr 1: 2610/100,000 p-y	Yr 2: 831/100,000 p-y Risk: 898/65,935 (1.4%)	Risk: TST positive contacts without PT: 49/446 (11.0%)	Risk: TST positive contacts without PT: 6/72 (8.3%)	without PT: 9/372 (2.4%)	risk contacts in validation cohort: 57/1335 (4.3%)
	<15 yrs, Yr 2: 1309/100,000 p-y Risk: 163/4515 (3.6%)					
Other Limitations	Some children received IPT.		P-y accumulated over 5 years.	No cases in children less than 15 years. P-y accumulated over 5.3 years.	Definition of incidence >6 months	HHCs >15 years.

Abbreviations: IR: Incidence rate; PT: Preventive treatment; DR-TB: drug-resistant TB; Yr: Year; P-y: Person-years; TST: Tuberculin skin test; HHC: Household contacts

Table 4-2: Demographics and clinical characteristics of DR-TB household contacts who were free of TB disease at baseline

	Total* **n (%) or median [IQR]**	**On Tx** **n (%) or median [IQR]**	**Did not start Tx** **n (%) or median [IQR]**	**Not eligible for Tx** **n (%) or median [IQR]**
	N= 789	*N=172*	*N=43*	*N=574*
Age in years	19 [10 – 32]	7 [3 – 15]	16 [3 – 22]	24 [15 – 36]
Age Categories				
<15 years	283 (36)	128 (74)	21 (49)	134 (23)
>= 15 years	506 (64)	44 (26)	22 (51)	440 (77)
Sex (Male)	423 (54)	91 (53)	20 (47)	312 (54)
	N= 616	*N=171*	*N=42*	*N=403*
BMI (kg/m^2)	18.1 [14.8 – 24.0]	14.8 [13.4 – 16.9]	15.2 [13.4 – 16.9]	21.6 [17.1 – 26.0]
Presence of symptoms	*N=737*	*N=172*	*N=43*	*N=522*

Cough	10 (1)	3 (2)	2 (5)	5 (1)
Fever	7 (1)	1 (1)	3 (7)	3 (1)
Weight loss	12 (2)	1 (1)	2 (5)	9 (2)
Additional TB risk factors	*N=737*	*N=172*	*N=43*	*N=522*
History of TB	9 (1)	0 (0)	0 (0)	9 (2)
TST => 5mm	6/136 (4)	6/64 (9)	0/11 (0)	0/61 (0)
Index Case resistant to FQ	138 (19)	16 (9)	11 (26)	111 (21)
Developed TB disease during follow up	2 (0.3)	2 (1)	0 (0)	0 (0)

* Excluding 3 contacts found with TB disease and 8 already on disease treatment at time of screening

**Chi2 for categorical variables and Wilcoxon Rank Sum for continuous variables

Table 4-3: Characteristics of household contacts who developed incident TB disease

S. No.	Age	Gender	Months on PT	Regimen	Total household members on PT	Time to TB development	Type of TB	TB Tx outcome	Comments
1	19 years	Female	5 months	Levofloxacin 750 mg and Ethionamide 500 mg	5	16 months	RR-TB	Lost to follow up	Variable compliance with PT due to adverse events. 3 members of household had TB disease.

2	15 years	Female	6 months	Levofloxacin 750 mg and Ethionamide 500 mg	6	19 months	DS-TB (culture negative)	Completed treatment	2 members of the family on concurrent TB treatment and failing treatment.

Table 4-4: Effectiveness of TB preventive treatment in published studies

	Becerra et al 2013[172]	**Fox et al 2013[167]**	**Reichler et al 2019[173]**	**Martin-Sanchez et al 2019[174]**	**Sloot et al 2014[175]**	**Saunders et al 2017[176]**
No. of expected cases	4.7	3.9	6.6	6.6	4.2	7.4
Expected IR per 100,000 p-y	1509	1161	1965	1965	N/A	N/A
Expected Risk	3.6% at 2 years	1.4% at 2 years	10.3% at 2 years 11.0% at 5 years	5.0% at 2 years 11.2% at 5 years	2.4% at 2 years	4.3% at 2.5 years
IRR/RR (95% CI)	IRR: 0.40 (0.05-2.0) RR: 0.32 (0.08 – 1.3)	IRR: 0.50 (0.06-2.8) RR: 0.85 (0.21 – 3.4)	IRR: 0.29 (0.04-1.3) RR: 0.11 (0.03 – 0.43)	IRR: 0.29 (0.04-1.3) RR: 0.14 (0.03 – 0.68)	RR: 0.48 (0.10-2.2)	RR: 0.27 (0.07-1.1)
Incidence rate diff per 100,000 p-y (95%CI)/Risk	IRD: -804 ((-)2314 – 706)	IRD: -566 p-y ((-)1983 – 852)	IRD: -1369 ((-)3080 – 342)	IRD: -1369 ((-)3080 – 342)	RD: -1.3 ((-)3.5 – 0.98)	RD: -3.1 ((-)5.0 – (-)1.2)

diff per 100 persons (95%CI)	RD: -2.5 ((-)4.1 – (-)0.76)	RD: -0.2 ((-)1.8 – 1.4)	RD: -9.8 ((-)13.1 – (-)6.5)	RD: -7.2 ((-)13.8 – (-)0.59)		
NNT	64 (using IR)	91 (using IR)	37 (using IR)	37 (using IR)	78	32
Preventive Fraction in exposed	57.5%	48.7%	69.5%	69.7%	51.9%	72.8%

Abbreviations: IR: Incidence rate; PT: Preventive treatment; DR-TB: drug-resistant TB; Yr: Year; P-y: Person-years; TST: Tuberculin skin test; HHC: Household contacts

Figure 4-1a: Incidence rate ratios for effectiveness of preventive treatment using data from published studies and a summary measure

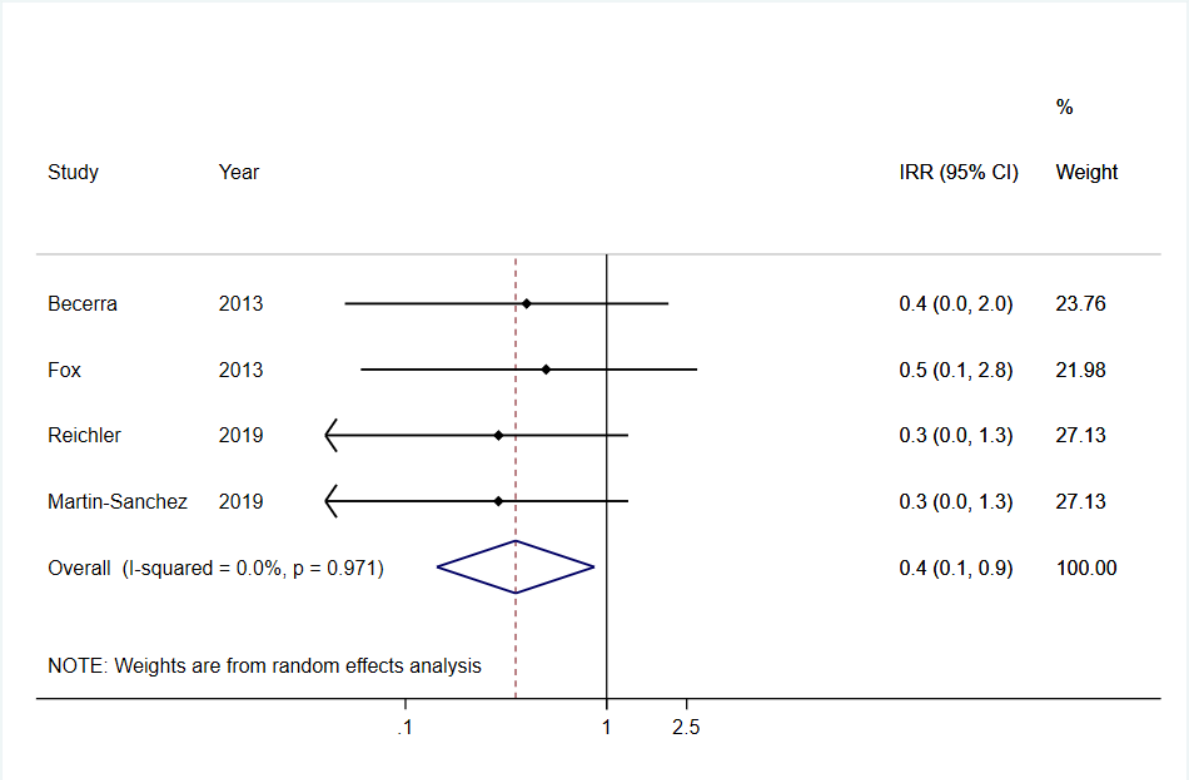
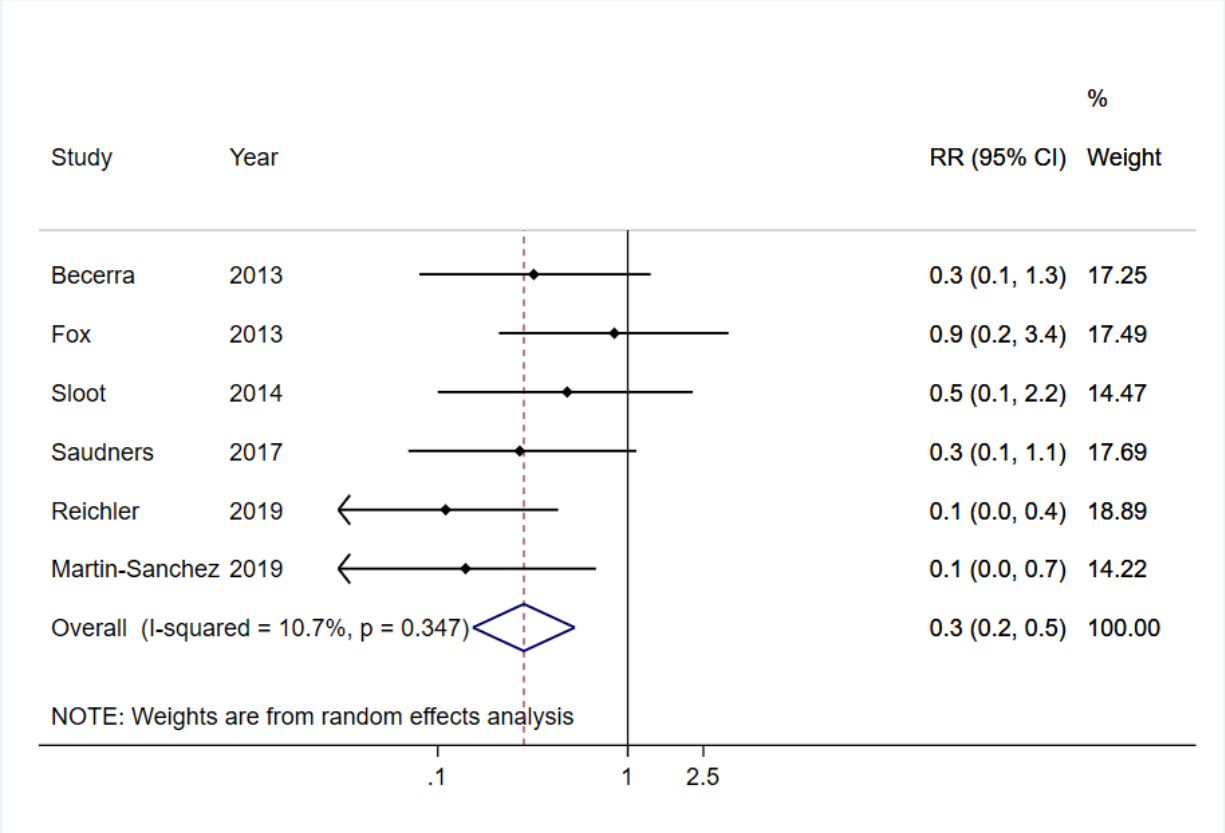


Figure 4-1b: Risk ratios for effectiveness of preventive treatment using data from published studies and a summary measure



CHAPTER 5

Risk factors for adverse events in household contacts prescribed preventive treatment for drug-resistant TB exposure

Abstract

Background

Completion of TB preventive treatment is important to optimize efficacy; treatment-related adverse events sometimes result in discontinuation. This study aims to describe the occurrence of adverse events and their associated risk factors during a 6-month 2-drug fluoroquinolone-based preventive treatment intervention for household contacts of drug-resistant TB patients in Karachi, Pakistan.

Methods

The primary outcome was development of any clinical adverse event during preventive treatment. Adverse events were categorized using the adverse events grading tables of the National Institute of Allergy and Infectious Diseases' Division of AIDS and National Institute of Health's Common Terminology Criteria for Adverse Events. Time to event analysis with Kaplan-Meier curves and Cox proportional hazards models accounting for recurrence were used to analyze associated risk factors for adverse events.

Results

Of the 172 household contacts started on preventive treatment, 36 (21%) developed 64 adverse events during 812.9 months of treatment. The incidence of adverse events over 6 months of treatment was 7.9 per 100 person-months (p-m); 16 per 100 p-m with ethionamide and 4.4 per 100 p-m with ethambutol. Of the 64 clinical adverse events recorded, 53 (83%) were grade 1 and 11 were grade 2. There was no grade 3 or 4 adverse event. In multivariable analysis, the risk of adverse events was higher in contacts prescribed ethionamide as compared to ethambutol as the companion drug adjusting for age, sex and BMI (aHR: 2.1 [95% CI: 1.2-3.6]). Overall, there was no notable difference in treatment completion amongst the contacts who experienced an adverse event and those who did not (cOR: 1.1 [95% CI: 0.52-2.5]).

Conclusion

A fluoroquinolone-based preventive treatment regimen for DR-TB exposure is well tolerated with no grade 3 or 4 adverse events observed. Regimens with ethionamide are more likely to result in grade 1 or 2 adverse events, which may merit clinical counseling, but patients' completion was not affected.

Background

In 2017, 10.4 million people developed TB disease of whom 1.3 million died; almost 558,000 cases of TB globally were due to drug-resistant (DR-) TB.[1] Among household contacts of DR-TB patients, almost half are infected.[27]

Approximately 10-20% of those infected with TB progress to active TB disease from re-activation of latent TB infection (LTBI) over their life time.[23,26] As such, household contact tracing and preventive treatment for exposed contacts have long been key elements of TB control.[27,28] Preventive treatment for TB can reduce incidence of TB amongst contacts by 60 to 90%[127] but currently available preventive treatment would not be effective in contacts exposed to DR-TB as the organism is resistant to these TB drugs.

Observational studies have suggested that fluoroquinolone-based preventive treatment may be effective in preventing DR-TB; WHO recommends treating DR-TB infection in high risk contacts although the quality of available evidence is considered quite low, because observational studies are given lower quality score and experimental studies.[25,113-120,122-126,20] There is concern about development of adverse events because some preventive treatment regimens for DR-TB make use of second-line TB drugs that have been shown to be toxic in DR-TB patients.[1] However adverse events have not been systematically documented across studies of preventive treatment. Bamrah et al study from Federated States of Micronesia showed 53% of the contacts experiencing some adverse event with only 4% stopping preventive treatment.[114] A systematic review and meta-analysis found that a mean of 19% of TB contacts started on preventive treatment eventually discontinued treatment because of adverse events.[162]

Completion of TB preventive treatment is important to optimize efficacy of preventing progression to TB disease with data showing that compared to people who complete preventive treatment, those who do not complete treatment are at 5 times the risk for developing TB disease (HR: 5.4; 95%CI: 2.1-14).[178] However,

it can be challenging to persuade persons who do not feel sick to take preventive treatment when it may result in an adverse event.[162]

Studies providing preventive treatment to persons exposed to DR-TB have shown that fluoroquinolone with ethambutol and fluoroquinolone with ethionamide regimens are effective in preventing active TB disease. The rate of adverse events using fluoroquinolone with ethambutol is lower (16%) as compared to fluoroquinolone with ethionamide regimen (58%).[162] However, the adverse event profiles and individual level factors associated with them have not been well described or compared. Better understanding of these can help programs more promptly identify individuals on preventive treatment at risk of developing adverse events and plan for resources accordingly, including psychological counseling and provision of ancillary drugs for management of adverse events.

Here we compare the adverse events profiles between two preventive treatment regimens: fluoroquinolone with ethambutol and fluoroquinolone with ethionamide. These regimens were offered to eligible household contacts of DR-TB patients in Karachi, Pakistan. We also examine the association between development of adverse events and treatment discontinuation.

Methods

Setting and Study Population

This study utilized data collected from a household contacts cohort established in Karachi, Pakistan, to evaluate the feasibility, safety and effectiveness of TB preventive therapy for individuals exposed to DR-TB (defined as resistance to either isoniazid and/or rifampicin). Details of the cohort are reported elsewhere.[170] Briefly, 800 household contacts, defined as those sleeping under the same roof identified at time of treatment initiation, of 100 consecutive (index) patients initiating treatment for culture-confirmed DR-TB disease at The Indus Hospital were evaluated. Contacts were eligible for the study if the index patient (i) did not have extensively drug-resistant (XDR) TB, defined as resistant to isoniazid, rifampin, any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin); (ii) lived in Karachi, Pakistan; and (iii) consented to participate in the study. If the index TB patient's isolate was resistant to a fluoroquinolone on drug susceptibility testing but not resistant to any of the second-line injectables, the household contacts were still eligible for the study. TB disease free contacts (i) younger than 5 years; (ii) between 5 and 17 years old with a positive tuberculin skin test (TST), diabetes, HIV, or malnutrition (weight for age less than 3rd percentile); or (iii) 18 years and older with diabetes, HIV, or malnutrition (BMI <18.5 kg/m2) were eligible to start a 6-month fluoroquinolone-based preventive treatment regimen based on expert consensus.[117] Levofloxacin was the fluoroquinolone of choice unless the index patient's TB strain was resistant to it on culture. In such cases, moxifloxacin was prescribed. Ethambutol was the companion drug of choice unless it was not available in the right dosing form due to drug supply chain disruption, in which case it was replaced by ethionamide.

Overall 215 household contacts were eligible to receive a fluoroquinolone-based TB preventive treatment of which 172 were started on one of the following six-month, two-drug combinations:

1. Levofloxacin (15–20 mg/kg for children 5 years and younger; 7.5–10 mg/kg for individuals older than 5 years; max dose: 1000 mg/day) and ethambutol (15-25mg/kg; max dose: 2000 mg/day)

2. Levofloxacin (15–20 mg/kg for children 5 years and younger; 7.5–10 mg/kg for individuals older than 5 years; max dose: 1000 mg/day) and ethionamide (15-20 mg/kg; max dose: 750 mg/day)

3. Moxifloxacin (7.5-10 mg/kg; max dose: 400 mg/day) and ethambutol (15-25mg/kg; max dose: 2000 mg/day)

4. Moxifloxacin (7.5-10 mg/kg; max dose: 400 mg/day) and ethionamide (15-20 mg/kg; max dose: 750 mg/day)

A clinical psychologist called these contacts 15 days after treatment initiation to monitor for treatment adherence and adverse events. Contacts were then followed up in clinic every two months and evaluated by a study doctor for the duration of treatment. In between clinic visits, a study health worker visited the household to monitor treatment adherence and adverse events monthly. All contacts completing at least five months of treatment were considered to have completed treatment.

Adverse Events

At follow-up visits either at clinic or home, patients or parents/caregivers were interviewed concerning the occurrence of adverse events using a structured questionnaire. These adverse events were classified retrospectively by the study team using the adverse events grading tables of the National Institute of Allergy and Infectious Diseases' Division of AIDS (DAIDS). As cough, increased frequency of urination and

disturbed menstruation are not categorized in this classification, we used National Institute of Health's Common Terminology Criteria for Adverse Events (CTCAE) to classify these three types of events.

Analysis

The primary outcome of interest was the development of any clinical adverse event during preventive treatment at any follow up visit. Descriptive analysis was performed with frequency counts reported for each adverse event. For each contact on treatment, the total number of adverse events over the 6-month course of preventive treatment was calculated. The incidence of adverse events was calculated by dividing the sum of all adverse events in all contacts on treatment by the total person-months of follow-up measured and expressed as events per 100 person-months of follow-up. We also calculated the rate of adverse events in each of the six months of treatment.

Different covariates were examined between those who developed adverse events versus those who did not develop adverse events using Chi square or Fisher's exact test for dichotomous, and t-test or nonparametric Wilcoxon tests for continuous variables.

Time to event analysis with Kaplan-Meier curves and Cox Proportional Hazards modeling was used to analyze associated risk factors for adverse events. Participants were censored at time of treatment completion or discontinuation of treatment. As adverse events are a recurrent outcome, we used Prentice, Williams and Peterson with total time to event (PWP-TT) extension to the Cox Proportional Hazard model for analysis to account for all adverse events. This model analyzes ordered multiple events by stratification with only those persons with an event in the previous stratum at risk. All participants are at risk of an event in the first stratum.[179]

We repeated the multivariable analysis using just gastrointestinal, respiratory and dermatological adverse events as a sensitivity analysis as these are more objectively reported. We completed this sensitivity analysis to address the concern that very young children may not always be able to express some of the subjective symptoms recorded as adverse events.

We constructed a matched analysis with matching with respect to analysis time, age category and gender to analyze the effect of adverse event on treatment discontinuation. Each contact discontinuing treatment was matched with three contacts who completed treatment for this analysis.

Data were analyzed using Stata version 15 (StataCorp, College Station, Texas) and SAS version 9.4 (SAS Institute Inc., Cary, NC).

Ethical Approval

The parent study was approved by the Institutional Review Boards (IRB) of Interactive Research and Development (IRD) and Harvard Medical School. The adverse event analysis presented in this manuscript was also approved by the Institutional Review Board (IRB) of Emory University.

Role of Funding Source

The funder of this study had no role in study design, data collection, data analysis, data interpretation, writing of the report, or in the decision to submit for publication. The authors had full access to the data and made the decision to publish the manuscript.

Results

Of the 215 household contacts who were eligible to start preventive treatment, 172 (80%) started preventive treatment with a median age of 7 years (IQR: 3-15) and 53% males (table 5-1). Contacts who started preventive treatment were younger compared to those who did not start preventive treatment (median age: 16 years; IQR:3-22). Otherwise these contacts did not differ from each other with respect to demographics and clinical features.

Of the 172 household contacts started on preventive treatment, 113 (65%) received ethambutol as a companion drug and the rest received ethionamide (table 1). Thirty-six (21%) of the 172 contacts developed 64 adverse events during 813 months of treatment with a median of two adverse events per contact experiencing an adverse event. The incidence of adverse events over six months of treatment was 7.9 events per 100 person-months.

Of the 36 contacts experiencing an adverse event, 22 (61%) experienced the first adverse event within first month of treatment. Almost all contacts had experienced their first adverse event by 3 months of treatment (figure 5-1).

The most common adverse event was vertigo/dizziness with 19 (11%) of the contacts experiencing it (table 5-2) while the most common system involved was gastrointestinal with 21 (12%) contacts experiencing at least one gastrointestinal adverse event (e.g., vomiting, nausea). Of the 64 clinical adverse events recorded, 53 (83%) were grade 1 while 11 were grade 2. There was no grade 3 or 4 adverse event observed during treatment (table 5-2).

Household contacts who received ethionamide with a fluoroquinolone had an incidence rate of 16 adverse events per 100 person-months while those who received ethambutol with a fluoroquinolone had an

incidence rate of 4.4 adverse events per 100 person-months (IRR: 3.7, 95% CI: 2.2-6.3). Figure 5-2 shows the probability of an adverse event by companion drug during treatment.

In bi-variate Cox Proportional Hazards analysis accounting for recurrence (PWP-TT model), risk of adverse event was two-fold higher among contacts prescribed ethionamide as compared to ethambutol as the companion drug (HR: 2.2, 95% CI: 1.2-3.8) (table 5-3). Similarly, older children and adults were at a higher risk for adverse events as compared to children <5 years of age (5-9 years HR: 2.7, 95% CI: 1.1-6.5; 10-19 years HR: 3.9, 95% CI: 1.8-8.6; > 19 years HR: 4.1, 95% CI: 1.7-9.7) (table 5-3). In multivariable analysis, the risk remained higher for the group exposed to ethionamide as compared to ethambutol after adjusting for age, sex and BMI (aHR: 2.1, 95% CI: 1.1-3.9) (table 5-3).

To assess interaction between age and companion drug, we performed an age-stratified analysis. The hazard ratios for adverse events for ethionamide as compared to ethambutol were as follows: <5 years HR: 2.4 [95% CI: 0.54-11]; 5-9 years HR: 5.2 [95% CI: 2.1-13]; 10-19 years HR: 4.5 [95% CI: 1.1-18]; > 19 years HR: 0.16 [95% CI: 0.03-0.80]). We were unable to account for this interaction in our multivariable analysis because of collinearity.

We repeated the multivariable analysis using just gastrointestinal, respiratory and dermatological adverse events as a sensitivity analysis. Of the 64 total adverse events, only 23 were included in this model. In this analysis, the HR for adverse events in contacts prescribed ethionamide was 1.7 (95% CI: 0.62-4.6). Age-stratified hazard ratios for ethionamide as compared to ethambutol were as follows: <5 years HR: 3.1 [95% CI: 0.26-37]; 5-9 years HR: 4.7 [95% CI: 1.2-19]; 10-19 years HR: 3.3 [95% CI: 0.28-36]. There were no observable adverse events with ethionamide in the age group over 19 years.

There were 11 (31%) contacts experiencing adverse events and 40 (29%) contacts not experiencing an adverse event who did not complete treatment. Overall, there was a near null difference in treatment

completion among the contacts who experienced an adverse event and those that did not (cOR: 1.1, 95% CI: 0.52-2.5 (figure 5-3).

Discussion

There was no grade 3 or 4 event or any adverse event requiring hospitalization during 6 months of preventive treatment. The incidence of adverse events was rare, with 7.9 instances occurring in 100 person-months of follow up. In contacts who experienced any adverse events, the first instance of an adverse event occurred within the first month of treatment 61% of the time and almost all adverse events occurred within 3 months of treatment initiation, suggesting that these events occur early during the treatment. We found lower rate of adverse events than found in studies from Federated States of Micronesia and South Africa.[114,116] As compared to other studies, the proportion of contacts on a fluoroquinolone-based preventive treatment experiencing adverse events in our study was also lower (21% vs 33%).[162]

Subjects in our study received either ethambutol or ethionamide as the companion drug along with a fluoroquinolone during six months of treatment. Contacts who received ethionamide had an almost two-fold increase in risk of an adverse event as compared to those who received ethambutol. This risk decreased slightly after adjusting for age, sex and BMI. Ethionamide is generally not well tolerated and our results are consistent with the reports of adverse events observed with ethionamide use for DR-TB treatment showing gastrointestinal adverse events in 50% of the patients and neuropsychotoxic effects in 25-30% of the patients.[180] A meta-analysis on preventive treatment of DR-TB found fluoroquinolone with ethionamide to be the most effective regimen for preventing active TB disease development, while the combination of a fluoroquinolone and ethambutol is the most cost-effective treatment option after consideration of both adverse events and treatment discontinuation.[162]

On age-stratified analysis, adults (>19 years) were more likely to have an adverse event when prescribed ethambutol in contrast to children, suggesting an interaction between age and companion drug. We were unable to account for this interaction in our multivariable analysis because of collinearity. Because of the limited availability of ethambutol in the right dosage form, most adults were prescribed ethionamide in our

study, which may have led to age and drug variables being collinear. Further research is needed to parse this association further.

Our results show that younger children tolerate preventive treatment better than older children and adults, with 10% of children under five years experiencing an adverse event and 37% of contacts over 19 years experiencing an adverse event. The literature suggests that children generally tolerate DR-TB treatment better than adults and our results are consistent with this observation.[181,182] This is an important finding as children under five years are at an increased risk of developing active TB disease after exposure and should receive preventive treatment.[20,63] There is a possibility of ascertainment bias with younger children not being able to communicate more subjective adverse events like dizziness. On sensitivity analysis with just gastrointestinal, respiratory and dermatological adverse events, the directionality and magnitude of the effect was maintained, although precision was necessarily diminished by restricting to half the total events.

We observed no association between treatment discontinuation and adverse events. Contacts who experienced any adverse event were as likely to complete treatment as those who did not experience an adverse event. Reports from other studies have shown that 1-4% of the contacts of a fluoroquinolone-based treatment (excluding regimens with pyrazinamide) discontinue treatment because of adverse events and our results follow similarly.[162] This may be because there were no serious adverse events in our study and also the treatment program had a strong counseling component in which contacts and their parents/guardians were counselled before starting treatment and during each follow up visit along with phone counseling by a certified psychologist. This counseling may have helped contacts experiencing an adverse event to continue treatment.

Our study had several limitations. Although contacts were systematically followed up clinically, there was no systematic laboratory testing with that component left to clinical discretion. Hence, we are only able to report clinical adverse events. We did not test for ocular toxicity in our study, an adverse event observed

with ethambutol and therefore there may be under-reporting of adverse events of ethambutol. Clinical adverse events relied on self-reporting with possible variations in cultural attributes among families and ascertainment in younger children.

In younger children there is an increased likelihood of underreporting of adverse events resulting in misclassification. This may bias the comparison between regimens to show no difference between the regimens. To assess this, we performed a sensitivity analysis using only observable adverse events that also showed increased risk of adverse events in household contacts receiving ethionamide as compared to ethambutol.

Strengths of our study include a prospective design and excellent follow up with over 70% of the contacts on treatment completing the full course of treatment.

Overall, we found there to be no serious adverse event (grade 3 or 4) associated with preventive treatment for DR-TB exposure using a fluoroquinolone-based two-drug regimen with better tolerability in younger children, a group at high risk for TB disease following exposure. Our results can guide programs looking to implement preventive treatment for DR-TB exposure.

Table 5-1: Demographics and clinical characteristics of DR-TB contacts enrolled on preventive treatment at The Indus Hospital

	On Tx n (%, col) or median [IQR]]	No AE n (%, col) or median [IQR]	With AE n (%, col) or median [IQR]
	N=172	*N=136*	*N=36*
Age in years	7 [3 – 15]	5.5 [2.5 – 12]	11.5 [6 – 17.5]
Age categories			
<5 years	61 (35)	55 (40)	6 (17)
5-9 years	44 (26)	35 (26)	9 (25)
-19 years	48 (28)	34 (25)	14 (39)
9 years	19 (11)	12 (9)	7 (19)
Sex (Male)	91 (53)	72 (53)	19 (53)
	N=171	*N=135*	*N=36*
BMI (kg/m^2)	14.8 [13.4 – 16.9]	14.7 [13.4 – 16.9]	15.2 [13.3 – 16.7]

Presence of symptoms			
Cough	3 (2)	3 (2)	0 (0)
Fever	1 (1)	1 (1)	0 (0)
Weight loss	1 (1)	0 (0)	1 (3)
Additional TB risk factors			
History of TB	0 (0.0)	0 (0)	0 (0)
TST => 5mm	6/64 (9)	5/50 (10)	1/14 (7)
Index Case resistant to FQ	16 (9)	12 (9)	4 (11)
Regimen given			
Levofloxacin/Ethambutol	102 (59)	90 (66)	12 (33)
Levofloxacin/Ethionamide	54 (31)	34 (25)	20 (56)
Moxifloxacin/Ethambutol	11 (6)	7 (5)	4 (11)
Moxifloxacin/Ethionamide	5 (3)	5 (4)	0 (0)

*Chi2/Fischer Exact test for categorical variables and Wilcoxon Rank Sum test for continuous variables

Table 5-2: Severity of reported adverse events*

Adverse event	Ethambutol (n=28)		Ethionamide (n=36)		
	Grade 1, n (row %) n=24	Grade 2, n (row %) n=4	Grade 1, n (row %) n=29	Grade 2, n (row %) n=7	Total AEs, n (col %) N= 64
Vertigo/dizziness	8 (42)	0 (0)	11 (58)	0 (0)	19 (30)
Vomiting	4 (36)	0 (0)	6 (55)	1 (9)	11 (17)
Anxiety	0 (0)	2 (20)	2 (20)	6 (60)	10 (16)
Nausea	4 (50)	0 (0)	4 (50)	0 (0)	8 (13)
Arthralgia	3 (100)	0 (0)	0 (0)	0 (0)	3 (4)
Pain	2 (67)	0 (0)	1 (33)	0 (0)	3 (4)
Abdominal distention	1 (100)	0 (0)	0 (0)	0	1 (2)
Bloating	0 (0)	0 (0)	1 (100)	0 (0)	1 (2)
Cough	1 (100)	0 (0)	0 (0)	0 (0)	1 (2)
Disturbed menstruation	0 (0)	0 (0)	1 (100)	0 (0)	1 (2)
Fatigue	1 (100)	0 (0)	0 (0)	0 (0)	1 (2)
Headache	0 (0)	0 (0)	1 (100)	0 (0)	1 (2)
Insomnia	0 (0)	0 (0)	1 (100)	0 (0)	1 (2)

Myalgia	0 (0)	0 (0)	1 (100)	0 (0)	1 (2)
Polyuria	0 (0)	1 (100)	0 (0)	0 (0)	1 (2)
Yellow discoloration of skin	0 (0)	1 (100)	0 (0)	0 (0)	1 (2)

* There was no grade 3 or 4 adverse event

Table 5-3: Risk of recurrent adverse event

	UNADJUSTED		ADJUSTED	
	HR	95% CI	HR	95% CI
Ethionamide	2.2	1.2 - 3.8	2.1	1.1 - 3.9
Age <5 years		Ref		
Age 5-9 years	2.7	1.1 - 6.5	2.9	1.2 - 7.1
Age 10-19 years	3.9	1.8 - 8.6	3.2	1.5 - 6.9
Age >19 years	4.1	1.7 - 9.7	4.3	1.7 - 11
Sex (male)	0.91	0.56 - 1.5	0.72	0.44 - 1.2
BMI	1.0	0.95 - 1.0	0.99	0.92 - 1.1

Figure 5-1: Time to first adverse event in months by regimen

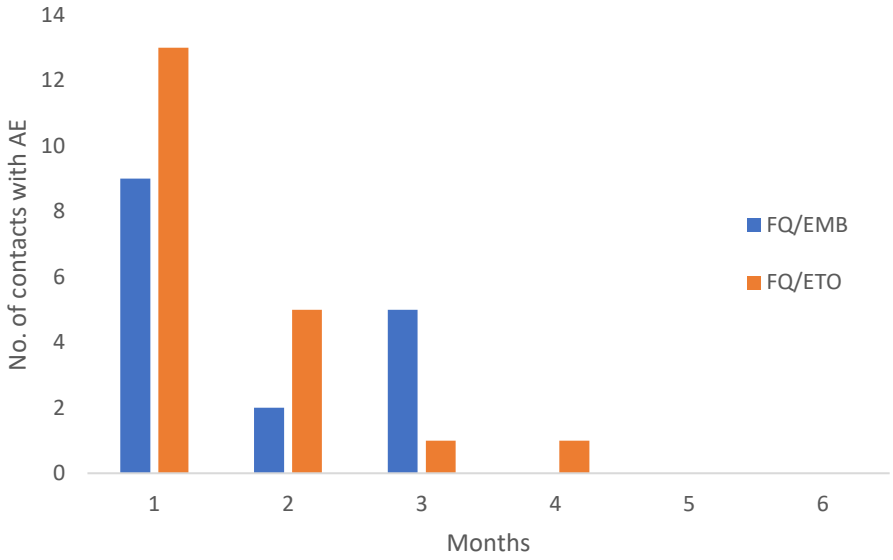


Figure 5-2: Time to first adverse event by companion drug

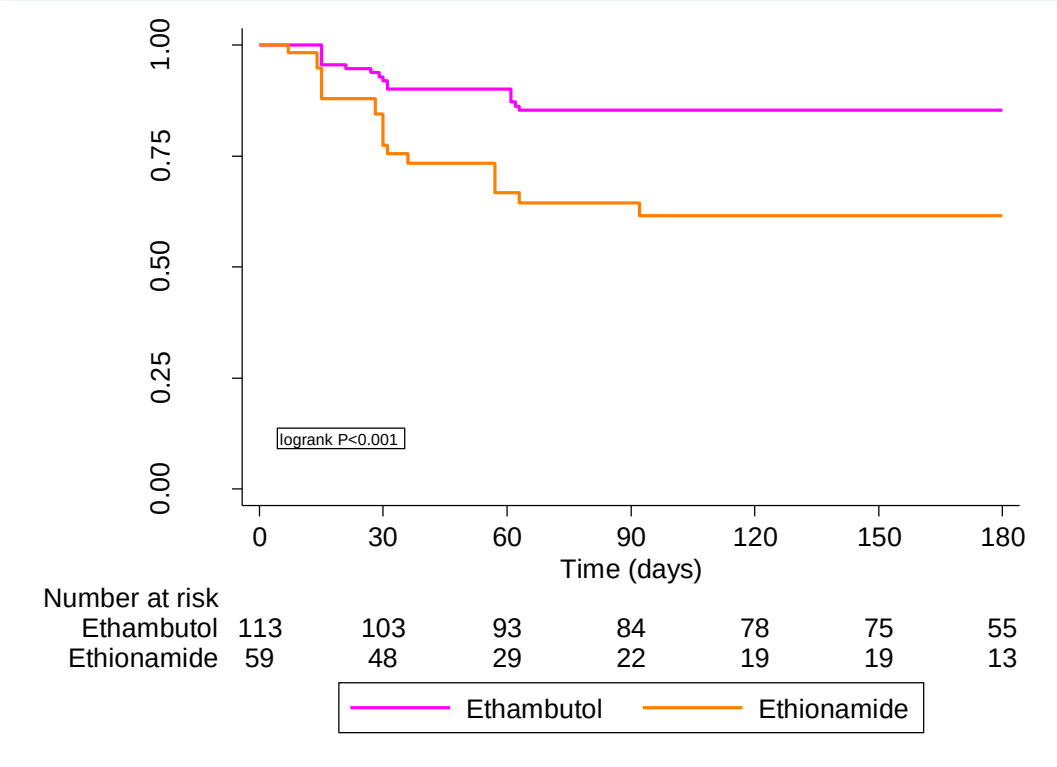
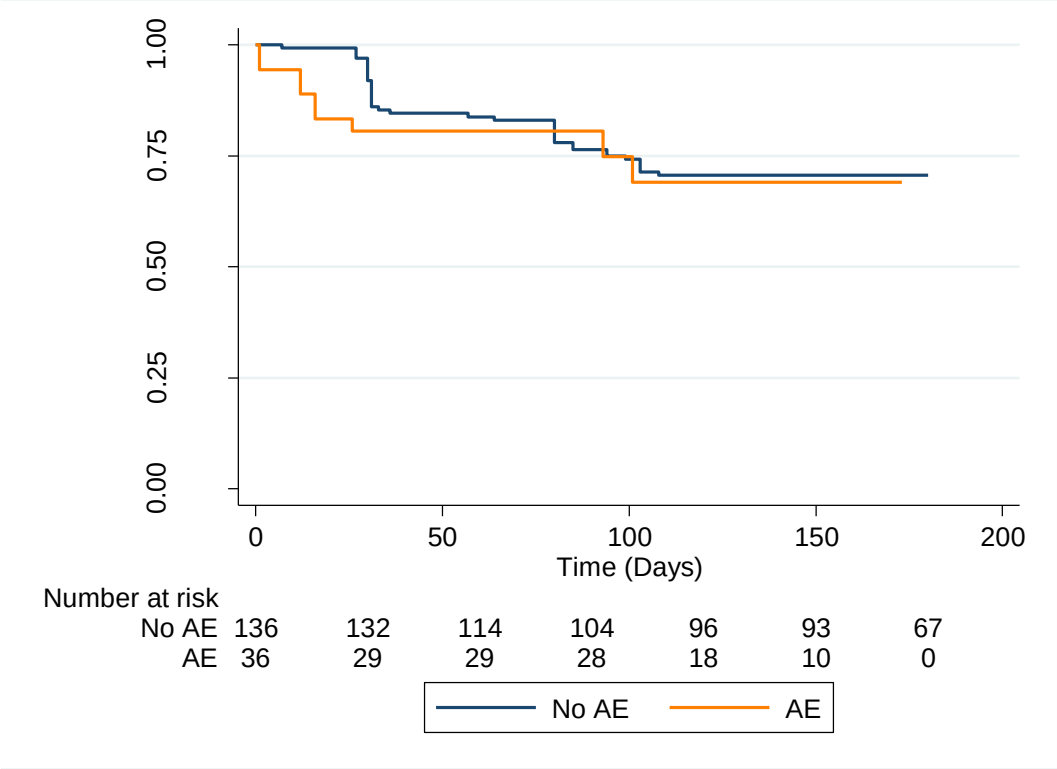


Figure 5-3: Probability of treatment completion by adverse event status*



Log rank p-value: 0.707

*Time scale for the graph is as follows: start of treatment for no adverse event group; time of adverse event occurrence for adverse event group

CHAPTER 6

Research summary, public health implications and future directions

This dissertation aimed to:

1) validate the use of monocyte-lymphocyte ratio (MLR) for diagnosis of active TB disease in children and create a risk score to improve diagnosis;

2) evaluate the effectiveness of fluoroquinolone-based treatment for preventing progression to active TB disease in household contacts of drug-resistant TB patients; and

3) evaluate risk factors for development of adverse events in household contacts receiving preventive treatment.

Summary of Findings

Study 1 aimed to validate the use of MLR value of ≥0.378 for the diagnosis of active TB disease in children. A previous study from Kenya reported a sensitivity of 77% for this value.[95] We were unable to validate the findings from the Kenya study. The sensitivity for this value was 33% in our multi-country cohort that included both HIV positive and negative children. Instead, we were able to create an internally validated risk score that included MLR as a variable for diagnosis of confirmed TB in children. The area under the curve (AUC) for this risk score was 84.1% with a cutoff being 9 points with a sensitivity of 66% (95%CI: 56 – 75), specificity of 87 % (95%CI:84

– 90). Clinicians may want to use a lower cutoff (5 points), which will result in a higher sensitivity and more false positives (lower specificity). Given the serious consequences of missed diagnosis this trade off may be clinically reasonable. We believe that the use of this risk score will improve the diagnosis of active TB in children globally, decreasing the reliance on sputum-based testing.

Study 2 and 3 evaluated the effectiveness of fluoroquinolone-based preventive treatment for drug-resistant (DR) TB exposure and associated adverse events. We found that preventive treatment decreased the incidence of active TB disease in high-risk household contacts by 65% over two years, as compared with historical controls. We also found the preventive treatment to be safe with no grade 3 or 4 adverse events over 6-months of treatment and incidence of grade 1 or 2 adverse event (7.9 per 100 person-months) lower than previously reported. To our knowledge, this work is the first of its kind from South Asia, a high-burden TB setting with a low prevalence of HIV and adds to the growing body of literature that has found preventive treatment for DR-TB to be as effective as that for drug-sensitive TB. Our study is also the first to have systematically evaluated the incidence of adverse events associated with different fluoroquinolone-based treatment regimens used for preventive treatment. We believe that our work can help guide National TB Programs in formulating effective policies with regards to DR-TB prevention.

Study Limitations

Important research limitations should be kept in perspective. In our multi-country cohort for aim 1, we combined three cohorts with different recruitment and data collection strategies. The laboratory investigations and their reporting may have differed between countries as well. This can lead to misclassification and may introduce error. We also had missing predictor data on 24% of the final cohort, especially on chest x-rays (CXR) and tuberculin skin test (TST), two key

predictors of the risk score. The children who were excluded due to missing data did not differ demographically from those who were included in the analysis.

For study 2, we did not have a comparison arm in the cohort because of the way the cohort was assembled. Hence, we could not make a direct comparison and had to rely on extrapolating data from other cohorts in different settings. This extrapolation could introduce bias as the exact counterfactual conditions were not met and we were not able to adjust for different risk factors because of data availability. We believe that this bias would underestimate the effectiveness that we observed with the actual effectiveness being higher. One other key limitation of our study was reliance on symptom screen at home and asking household members whether someone in the household was diagnosed with TB or started on TB treatment during the follow up period, since the parent study was designed to evaluate operational feasibility of providing preventive treatment and was not designed as an effectiveness study. This might have resulted in missed cases of TB resulting in overestimation of the effectiveness.

For study 3, there was no systematic laboratory testing for adverse events with that component left to clinical discretion. Hence, we are only able to report clinically reported adverse events. This restriction could have resulted in underestimation of the rate of adverse events. We did not test for ocular toxicity in our study— an adverse event observed with ethambutol in other studies— and therefore there may be under-reporting of adverse events of ethambutol. Clinical adverse events also relied on self-reporting with possible variations in cultural attributes among families and ascertainment in younger children. We were also unable to account for interaction between age and companion drug in our multivariable statistical model due to collinearity.

Study Strengths

Despite the limitations reported above, this dissertation had several strengths. They are described below.

For study 1, we were able to include data from a multi-country cohort, with both HIV positive and negative children, ensuring better external validation and generalizability of our risk score. Data for these cohorts were collected prospectively and under research settings resulting in better quality. We used a recommended method for model building and internal validation in contrast to most previous pediatric TB diagnostic scores and algorithms, which have been based on expert opinions and not been validated.[67,153] Predictors in our score include parameters that have been known to be associated with TB disease in children and do not include sputum-based testing.[66]

Study 2 is the first of its kind to our knowledge from South Asia. Our results from study 2 were robust to a range of different assumptions and showed similar effectiveness. Additionally, our study had a prospective follow up with excellent retention (91%) at two years and high treatment completion rate.[170]

Study 3 is unique as it systematically describes and compared adverse events from two different fluoroquinolone-based regimens for preventive treatment in household contacts exposed to DR-TB. We had a prospective design and excellent follow up with over 70% of the contacts on treatment completing the full course of treatment.[170]

Public Health Significance

Difficulty in diagnosis of pediatric TB results in under-diagnosis and delayed diagnosis, leading to increased morbidity and mortality in young children. Of the 250,000 deaths annually from the disease in children, 96% occur in children not on treatment, mostly due to missed diagnosis.[1,60] Most of the missed diagnoses and deaths occur in low resource settings where establishing a microbiological diagnosis confronts logistical challenges.[57,132] Hence, a better diagnostic tool may result in faster diagnosis with improved outcomes. Using a risk score approach, which includes MLR, could help improve diagnosis and rapidly start sick children on TB disease treatment. Ruling out active TB disease will also improve use of preventive treatment in contacts exposed to TB where some clinicians are hesitant in providing preventive treatment without being able to rule out disease unequivocally.

One of the most effective means of preventing TB disease is to treat TB infection. With a dismal 55% treatment success rate of current treatment regimens, long duration of treatment and morbidity caused by adverse events, it is important to prevent progression to DR-TB disease by treating DR-TB infection.[1] Household contacts of DR-TB patients are at a higher risk of developing active TB disease.[27] Providing preventive treatment to high-risk household contacts can decrease progression to active TB disease by 65%. This better understanding of the effectiveness of preventive treatment will help guide policymakers and public health authorities to formulate and implement effective prevention guidelines.

Management of adverse events and improving treatment completion are essential for an effective preventive treatment plan to prevent development of DR-TB disease.[162,178] A better understanding of the factors associated with adverse events will help programs promptly identify individuals who are at risk of developing adverse events on preventive treatment. Treatment programs can then

tailor efforts by targeting those at increased risk of adverse events for enhanced care and plan accordingly for resources including psychological counseling and provision of ancillary drugs for management of adverse events. A better understanding of regimen-associated adverse events will also help policy makers and programs decide on the safest available option for preventive treatment.

Considering the global burden of TB and the morbidity and mortality from the disease especially in children, this work has a potential to have significant impact on how TB care is provided especially in resource limited settings.

Future Directions

Further studies are needed to confirm and replicate the findings from this dissertation work. This includes studies to externally validate the risk score for diagnosis of active TB in children and assess its clinical usefulness. A feasibility study piloting this risk score in a limited resource setting would provide useful data to assess the impact on diagnosis of TB and the limitations of still having to rely on TST and CXR to calculate the score. Studies substituting IGRA results for TST will also be useful given the operational barriers associated with TST. Impact assessment of using this risk score for TB diagnosis and subsequent preventive treatment prescriptions for children without TB diagnosis are also needed to evaluate whether there was any increase in prescription practices or not.

To help national programs and policy makers in formulating preventive treatment guidelines further, we are already conducting a feasibility of implementing preventive treatment for DR-TB exposure in a rural setting in Pakistan to validate the treatment completion rates from our earlier

study. We also plan to conduct a cost-effectiveness analysis to assess the cost-effectiveness of DR-TB preventive treatment.

More studies are required to evaluate laboratory associated adverse events of preventive treatment for DR-TB exposure. Two clinical trials, V-QUIN and TB CHAMP, are evaluating levofloxacin based preventive treatment and will provide more data for these adverse events.[127] There is also a need to better understand the role that age plays in adverse events development with different preventive treatment regimens (age by companion drug interaction). This understanding will help clinicians and programs choose the safest and most effective possible options for their target population.

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