The Pediatric Infectious Disease Journal Publish Ahead of Print

DOI: 10.1097/INF.0000000000002131

Immunologic-Based Diagnosis of Latent Tuberculosis among Children Less Than 5 Years of Age Exposed and Unexposed to Tuberculosis in Tanzania: Implications for Tuberculosis Infection Screening

Khadija Said, MD, PhD[1,2,3♠], Jerry Hella, MD, MSc[1,2,3], Mwajabu Ruzegea, Ms[1], Rajesh Solanki, MD, MMed[4], Magreth Chiryamkubi[5], Francis Mhimbira, MD, PhD [1,2,3], Nicole Ritz, MD, PhD[3,6], Christian Schindler, PhD[2,3], Anna M. Mandalakas, MD, PhD[7], Karim Manji, MBBS, MMED[8], Marcel Tanner, PhD, MPH[2,3], Jürg Utzinger, PhD[2,3], Lukas Fenner, MD, MSc[9♠]

1 Ifakara Health Institute, Bagamoyo Research and Training Centre, Bagamoyo, Tanzania, **2** Swiss Tropical and Public Health Institute, Basel, Switzerland, **3** University of Basel, Basel, Switzerland, **4** Temeke Municipal Council Hospital, Dar es Salaam, Tanzania, **5** National Tuberculosis and Leprosy Control Program, Dar es Salaam, Tanzania, **6** Basel University Children's Hospital, Basel, Switzerland, **7** The Global Tuberculosis Program, Texas Children's Hospital, Baylor College of Medicine, Houston, United States of America, **8** Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, **9** Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland

♠ KS: +255 686 997 491, ksaid@ihi.or.tz; and LF: +41 79 242 2066, lukas.fenner@ispm.unibe.ch

Abbreviated Title/Running head: Tuberculosis Screening in Young Children

Funding: This work was supported by funding from the Rudolf Geigy Foundation (Basel, Switzerland). KS is a beneficiary of the Swiss Government Excellence Scholarship for Foreign Scholars and Artists (ESKAS; Bern, Switzerland).

Acknowledgments

We thank all parents/caregivers who allowed their children to participate in this study. We are grateful to the Temeke municipality, Temeke hospital, district and regional TB coordinators of the Temeke district, as well as the National TB and Leprosy Control Program in Tanzania for their support.

Authors' contribution

KS, JH, FM, NR, AM, KM, MT, JU, LF designed the study. KS, JH, MR, RS, MC, FM, collected the data. KS, CS did the analysis. KS wrote the manuscript. JH, FM, NR, CS, AM, KM, MT, JU, LF reviewed the manuscript. All the authors authorized the submission and publication of the manuscript.

Author information

KS has recently obtained her PhD from the Swiss Tropical and Public Health institute and the University of Basel in Basel, Switzerland.

Abstract

Background: Childhood tuberculosis (TB) is acquired following exposure to an infectious TB case, often within the household. We prospectively screened children 6-59 months of age, exposed and unexposed to an infectious TB case within the same household, for latent tuberculosis infection (LTBI), in Dar es Salaam, Tanzania.

Methods: We collected medical data and clinical specimens (to evaluate for helminths, TB and HIV coinfections) and performed physical examinations at enrollment and at 3-month and 6-months follow-up surveys. LTBI was assessed using QuantiFERON (QFT) at enrollment and at 3 months.

Results: In total, 301 children had complete data records (186 with TB exposure and 115 without known TB exposure). The median age of children was 26 months (range 6-58); 52% were females, and 4 were HIV-positive. Eight children (3%) developed TB during the 6-month follow-up. We found equal proportions of children with LTBI among those with and without exposure: 20% (38/186) *vs.* 20% (23/115) QFT-positive, and 2% (4/186) *vs.* 4% (5/115) indeterminate QFT. QFT conversion rate was 7% (22 children) and reversion 8% (25 children). Of the TB-exposed children, 72% initiated isoniazid preventive therapy (IPT), but 61% of parents/caregivers of children with unknown TB exposure and positive QFT refused IPT.

Conclusions: In this high burden TB setting, TB exposure from sources other than the household was equally important as household exposure. Nearly one third of eligible children did not receive IPT. Evaluation for LTBI in children remains an important strategy for controlling TB, but should not be limited to children with documented TB exposure.

Key words: Children, Interferon-gamma release assay, Latent tuberculosis infection, Screening, Isoniazid preventive therapy.

INTRODUCTION

Tuberculosis (TB) continues to claim the lives of infected children younger than 5 years, and has been identified among the top 10 causes of death in this age group.[1] Young children typically acquire *Mycobacterium tuberculosis* infection from an infectious adult with pulmonary TB within the same household. Children with latent tuberculosis infection (LTBI) are at high risk of progressing to active TB disease within 12 months after infection;[2] and the risk is highest among children whose mothers are index cases.[3, 4] Contact tracing and isoniazid preventive treatment (IPT) are recommended by the World Health Organization (WHO) to prevent active TB disease among high-risk populations, including under-5 year-old children. However, IPT uptake is a challenge; only 7% of children eligible for IPT received medication in 2015 worldwide.[5]

Despite the WHO's emphasis on providing TB preventive therapy, contact tracing remains a largely neglected part of TB control in high-burden countries due to lack of financial and human resources, coupled with the high costs associated with diagnostics and contact tracing itself.[6] High-income countries mostly use interferon-gamma release assay (IGRA) for TB contact tracing, which has high specificity for detecting *M. tuberculosis* infection but limited sensitivity, particularly in children.[7] Most TB programs in low-income countries and high TB-incidence rates use tuberculin skin test (TST) instead of IGRA, such as the ELISpot-based test (T-SPOT.TB) and the enzyme-linked immunosorbent assay (ELISA)-based test QuantiFERON-TB Gold (QFT), that are restricted to research use only in these settings.[8-10] Due to the limitations of IGRAs for the diagnosis of LTBI in children in high TB incidence countries, a quantified exposure score was recently proposed to serve as a surrogate measure of *M. tuberculosis* infection, specifically in TB-exposed children.[8, 11]

In Tanzania, the National TB and Leprosy Control Program (NTLP) reported that childhood TB cases constituted 10% of the country's TB cases in 2004–2012.[12] To interrupt

transmission of TB and combat the childhood TB epidemic in the country, since 2013, the program has been working to improve childhood TB case detection and reporting. However, the yield of infection screening, particularly among children without documented TB exposure, and the implications for screening strategies in routine settings has not yet been investigated.[13] We conducted a prospective study among children under the age of 5 years exposed to infectious pulmonary TB cases in their households and controls from neighboring households, to assess the prevalence of LTBI among under-5 year-old children in a high TB burden setting in Tanzania, using immunodiagnostic tests and a quantified TB exposure score.

MATERIAL AND METHODS

Study Setting and Design

Temeke is one of three districts in Dar es Salaam, the economic capital of Tanzania, with an estimated 1.4 million residents. The under-five mortality in Dar es Salaam is 72 per 100,000.[14] In 2014, TB prevalence in adults was 270 per 100,000 population in Temeke.[15] HIV prevalence among pregnant mothers in the district is estimated to be 6%.[16] The NTLP conducts routine contact tracing of young children by counselling smear-positive adult TB cases to bring children under the age of 5 years from their households for symptom-based TB screening and IPT if not found to have disease.

We conducted a prospective study in Temeke district among TB-exposed children, with the overarching aim of exploring TB/helminth co-infection, as previously described.[17] To recruit controls, we invited children from neighboring households according to standard operating procedures (Supplemental Digital Content 1, http://links.lww.com/INF/D201 standard operating procedure),[18] and after asking if there was an adult on TB treatment or suspected of having TB in the household. Children were referred to the Reproductive and Child Health (RCH) Clinic at Temeke Municipal Hospital (where children under the age of 5

years are typically seen) for enrollment and follow-up visits at 3 and 6 months after enrollment (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/D201).

Study Population and Sample Size Consideration

We invited 398 parents/caregivers of children 6–59 months of age, between October 2015 and September 2016. Children were included if: (i) their parents/caregiver provided written informed consent; (ii) they were 6–59 months of age at the time of enrollment; (iii) they resided within Temeke district and would continue to do so in the next 6 months after enrollment; and (iv) they lived with a smear-positive adult pulmonary TB case, as diagnosed by sputum smear microscopy and culture[19] (for case children) or came from households without documented TB case (for control children) (Text, Supplemental Digital Content 2, http://links.lww.com/INF/D202). Children with known TB exposure were recruited within a month of the index case starting TB treatment.

We aimed for a sample size of 300 children 6–59 months of age, with 150 TB-exposed and 150 TB-unexposed children. This sample size was estimated to provide a precision of 5% in estimating TB infection prevalence, with an error probability of 5%.

Study Procedures

At Enrollment: The study clinician collected demographic and socioeconomic information and, using a standardized case report form (CRF), evaluated children for the following TB signs and symptoms: (i) current cough of any duration; (ii) abnormal fatigue or reduced playfulness; (iii) documented weight loss or failure to thrive in the past 3 months; (iv) axillary temperature >37.5 °C; (v) visible cervical mass (>2 x 2 cm); (vi) gibbus (sharp angular spine deformity); and (vii) other signs suggestive of possible extra-pulmonary TB. The clinician also collected histories on feeding practices, prior illnesses (including TB), previous use of IPT and other medications, and recorded vaccination history (including BCG vaccination), which was verified on the RCH card. All children had chest radiographs taken.

Chest X-rays were interpreted by two independent radiologists; in case of discrepancies, a third radiologist was asked to resolve the discrepancies. Trained study nurses recorded anthropometric measurements, including height and weight, and collected venous blood for quantiferon (QFT), full blood counts (FBC), HIV testing, and malaria screening (see also "Laboratory Procedures" below). The nurses also performed sputum induction the morning after enrollment and collected induced sputum samples for Xpert MTB/RIF (Cepheid; Sunnyvale, CA, USA).[20, 21]

Parents/caregivers were given two pre-labeled containers and were instructed to bring urine and stool samples from their children the following day for screening of helminth infection. Additionally, each participant was provided with a plastic pocket that had an adhesive tape (50 x 20 mm) and a pre-labeled glass slide and asked to submit the slide with anal adhesive tape for *Enterobius vermicularis* examination.[22]

At 3-Month and 6-Month Follow-Up Visits*:* Children were clinically evaluated for TB and other medical conditions. We collected blood samples for QFT analysis at the 3-month visit and for FBC at the 6-month visit. Clinical management was determined based on case presentation.

TB Exposure Score**:** We used a TB exposure score chart, adopted from South Africa, to assess TB exposure.[11] The chart consists of 10 questions: (i) is the child's mother the index case?; (ii) is the child's primary caregiver the index case?; (iii) does the child sleep on the same bed as the index case?; (iv) does the child sleep in the same room as the index case?; (v) is the index case coughing?; (vi) is the index case a pulmonary TB case?; (vii) does the index case have smear positive TB?; (viii) does the index case live in the household with the child?; (ix) does the index case see the child every day?; and (x) is there more than one TB case in the household? Control participants were not assessed for TB exposure.

***Screening for, and Diagnosing Active TB Disease*:** We used a comprehensive symptom-based TB tool to screen for active TB disease at each visit (Table, Supplemental Digital Content 3, http://links.lww.com/INF/D203). At baseline, in addition to symptom-based screening, we performed chest X-rays and Xpert MTB/RIF for every child within 3 days after enrollment (see also "Laboratory procedures" below). At follow-up visits, we used the symptom-based screening tool; X-ray and/or Xpert MTB/RIF were ordered only if there was an indication. Confirmation and final diagnosis were based on clinical evaluation, chest X-ray findings and Xpert MTB/RIF, according to Graham et al.[23]

Children known to have TB exposure but without active disease were started on IPT (20 mg/kg), in accordance with the Tanzanian NTLP treatment guideline.[12] Parents/caregivers of children with positive QFT results but without documentation of exposure to an infectious TB case were counselled and advised to start their children on IPT. Children diagnosed with TB were referred to NTLP for TB treatment. All children diagnosed with medical conditions were clinically managed in accordance with Tanzanian national guidelines.

Laboratory Procedures

***Microbiologic Investigations**:* We performed Xpert MTB/RIF (Cepheid; Sunnyvale, CA, USA) on the induced sputum samples[20] to diagnose active TB disease in the laboratory at Temeke district hospital. The laboratory is subject to continuous quality control monitoring by the Central Tuberculosis Reference Laboratory (Dar es Salaam, Tanzania).

QuantiFERON -TB Gold (QFT): We used venous blood to measure *M. tuberculosis*-specific T-cell responses, measuring levels of interferon-gamma released in whole blood in response to stimulation with the *M. tuberculosis*-specific antigens. The blood was collected in three QFT tubes (Quantiferon-TB Gold, Cellestis; Carnegie, Victoria, Australia): TB-Ag (coated with MTB specific antigens), Mitogen (positive control coated with phytohaemaggluttin), and Nil (negative control coated with saline). The blood was

transferred to the laboratory within 1 hour and incubated at 37ºC for 16–18 hours. After incubation, the samples were centrifuged and supernatants stored at -80º C pending further processing by ELISA to detect interferon-γ (IFN-γ) response associated with *M. tuberculosis* infection. Results were interpreted according to the manufacturer's instructions.

***Other Blood Tests**:* HIV screening was done with Alere Determine HIV-1/2 (Alere; Holliston, MA, USA) among children ≥18 months of age, or PCR for children <18 months of age. Full blood cell counts were done using a MS4 Vet hematology analyzer (Diamond Diagnostics; Holliston, MA, USA).

***Helminth Investigations**:* Stool and urine samples were examined for helminth infections using the Kato-Katz, FLOTAC, Baermann, direct microscopy methods and a point-of-care circulating cathodic antigen (POC-CCA) urine cassette test for *Schistosoma mansoni* diagnosis, as previously described.[17, 22]

Definitions

We recorded clinical data directly onto tablet computers using the open-source software, open data kit (ODK; http://opendatakit.org/), and the data management tool, "odk_planner".[24] The TB exposure score was categorized into not likely to have TB infection (with a score of 1–6) or presumptive TB infection (score of ≥7). A composite QFT result was defined as positive QFT at either baseline or 3-month follow-up. A child was considered TB infected if the QFT result was positive. A child was considered to have active TB (probable or possible diagnosis) according to international consensus from an expert panel.[25] Chest radiograph features suggestive of TB included consolidation, cavities, and hilar lymphadenopathy. Children diagnosed with active TB disease 3 months after enrollment were defined as coprevalent TB cases, while children diagnosed with TB 3 months after enrollment were defined as incident TB cases.[8] Anemia was defined as hemoglobin (Hb) <11.0g/dl, as per WHO recommendations, further stratified into mild (Hb: 10-10.9 g/dl), moderate (Hb: 7.0-9.9

g/dl), and severe anemia (Hb: <7.0 g/dl).[26] Anthropometric z-scores were calculated using the 2006 WHO Growth Standards in Stata version 13.1 (Stata Corp. LLC; College Station, TX, USA), using the 'zscore06' command.[27] Helminth infection was defined as infection with any of the following helminth species: *Ascaris lumbricoides*, *E. vermicularis*, *Hymenolepis diminuta*, hookworm, *Schistosoma haematobium*, *S. mansoni*, *Strongyloides stercoralis*, and *Trichuris trichiura*.

Statistical Analysis

Frequencies and proportions were used to describe the children's demographic and clinical characteristics, both overall and stratified according to the presence or absence of documented TB exposure. QFT indeterminate results were excluded in the LTBI risk factor analysis. We performed mixed logistic regression analyses to identify risk factors for LTBI taking into account the matching of TB-exposed and unexposed children. These analyses were conducted with and without interaction terms between the respective risk factor variables and TB-exposure status. Additionally, we ran stratified analyses separating TB-exposed and unexposed children.

We generated an exposure scale based on the TB exposure score (0, no exposure; 1–4, minimal exposure; 5–6, medium exposure; and 7–10, maximum exposure). We constructed a core multivariable mixed logistic regression model for QFT-conversion and reversion between baseline and follow-up, comprising age, sex, and lymphocyte count, and alternatively added TB exposure, IPT during follow-up, and helminth infection as potential risk factors for QFT conversion and reversion. For children documented to have TB exposure, we also added TB exposure score and mother being index case as potential risk factors for QFT conversion and reversion. We also presented graphically the paired QFT results at enrolment and after 3 months, separately for QFT converters and reverters to illustrate the distribution of IFN-γ values during the two visits for children who converted to

QFT positive, reverted to QFT negative, and those who did not change. All analyses were performed in Stata version 13.1 (Stata Corporation; College Station, TX, USA).

Ethical Considerations

The study was approved by the Institutional Review Board of the Ifakara Health Institute (IHI; reference no. IHI/IRB/No: 12-2015), the Medical Research Coordinating Committee of the National Institute for Medical Research in Tanzania (NIMR; reference no. NIMR/HQ/R.8a/Vol. IX/2002), and by the Ethics Committee of the Northwestern and Central Switzerland (EKNZ; reference no. UBE-15/49). All children were recruited only after their parents/caregivers gave written informed consent.

RESULTS

Children's Baseline Characteristics

Of the 398 children 6-59 months of age invited to participate in the study, 325 parents/caregivers provided written informed consent, and hence, their children were enrolled. Clinical, demographic, and socioeconomic information were obtained from 316 children. Fifteen children did not provide blood samples for QFT analysis, thus, 301 children were included in the final analysis (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/D201).

Among the 301 child participants, 186 (62%) were exposed to adults with smear-positive pulmonary TB, and 115 (38%) were not known to have been exposed to TB. Overall, the median age was 26 months (range 6-58 months); 156 (52%) were girls and four were HIV-positive (Table, Supplemental Digital Content 4, http://links.lww.com/INF/D204). All children were BCG vaccinated based on vaccination documents. TB-exposed children were, on average, older than children without known TB exposure. Ninety-five (32%) children presented with signs and symptoms suggestive of TB (Fig., Supplemental Digital Content 5, http://links.lww.com/INF/D205).

A total of eight (2.7%) children developed active TB during follow-up (six in the exposed, two in the unexposed group). All TB cases were diagnosed based on a clinical case definition,[25] which included patient's history, chest radiography, positive response to anti-TB medication, and evidence of TB infection. Five children (1.7%) were considered co-prevalent TB cases (three exposed to infectious TB cases); four of them were <2 years, four were boys, and one was HIV-positive. Three children were considered incident TB cases; one was <2 years, and none was HIV positive. The proportion of children with active TB (prevalent and incident) in the exposed and non-exposed group was statistically not significantly different (3.2% *vs.* 1.7%, p=0.44). Children exposed to HIV-seropositive mothers were not more likely to have active TB compared with HIV non-exposed children (7% *vs.* 2%, p=0.15). All children with TB had mild or moderate anemia.

Prevalence of LTBI in TB Exposed and Unexposed Children

At enrollment, 15% (27/186) of TB exposed children were QFT-positive compared with 10% (12/115) of children without documented TB exposure (p=0.37). Indeterminate QFT results were also equally distributed between the two groups (9/115 [8%] *vs*. 9/186 [5%], p=0.37). At the 3-month follow-up visit (composite results), QFT was positive in 20% (61/301) children: 20% (38/186) of TB exposed *vs.* 20% (23/115) of TB unexposed (p=0.9), as shown in Table 1. The prevalence of indeterminate results was 3% (9/301).

Of those children diagnosed with active TB during follow-up, 10% (4/39) had a positive QFT result at enrolment. Among co-prevalent TB cases, 2/5 had positive QFT results, and 2/3 incident TB cases were QFT positive.

A fitted model, taking clustering into account, did not suggest any correlation between QFT-positive results (indicating LTBI) and the TB exposure score (odds ratio (OR) 1.06, 95% confidence interval (CI) 0.81-1.40, p=0.7). There was also no correlation between LTBI and the presence of a BCG scar; all children had a documented BCG vaccination.

Risk Factors for LTBI

We found that TB-exposed children from households with up to five members had a higher risk of having LTBI (defined as a positive QFT test result) compared with all other children (OR 3.57, 95% CI 1.54-7.69, p=0.003) (Table 2). All other risk factors such as age, sex, lymphocyte count, having a mother with TB, sleeping in the same bed as the index case, and household income showed no association. Among children without documented TB exposure, none of the risk factors showed significant association with LTBI.

QFT Conversion, Reversion, and Associated Patient Factors

Forty-seven (16%) children had different QFT results when retested at the 3-month visit: 22 (8%) children converted to QFT-positive at the 3-month follow-up, 11 (50%) of which had documented TB exposure. Twenty-five children (8%) reverted to negative QFT results at the 3-month follow-up. Considering variables such as sex, age category, average BCG scar size, lymphocyte counts, an index mother, isoniazid uptake, and infection with any helminth, we found none of the factors to be predictive for either QFT conversion or reversion (Table, Supplemental Digital Content 6, http://links.lww.com/INF/D206). Neither were coprevalent TB cases and incident cases associated with QFT conversion or reversion. Figure 1 shows the paired semiquantitative QFT results at enrolment and at the 3 months follow-up visit.

IPT Uptake among Exposed and Unexposed Children

By the six-month visit (end of follow-up), 72% (133/186) children exposed to an infectious TB case had started IPT (Table 1), and 28% (53/186) did not start IPT. Among the 53 who did not start IPT, 57% (30/53) parents/caregivers refused IPT, and 43% (21/53) were lost to follow-up. Among the 133 children who started IPT, 3% (4/133) developed active TB later on (two co-prevalent, two incident cases) and were started on anti-TB medications. Of the 53 exposed children who were not started on IPT, 6% (2/53) developed TB disease and started anti-TB medications (1 co-prevalent, 1 incident case).

Among 115 children with undocumented TB exposure, 20% (23/115) had an LTBI diagnosis based on QFT testing. Of these 23 children, one child started IPT, and one child developed TB later on (coprevalent case) and was started anti-TB medication; 61% (14/23) of parents/caregivers refused IPT; and 30% (7/23) were lost to follow-up.

DISCUSSION

We present findings from a prospective study of 186 TB-exposed children 6-59 months of age and 115 unexposed controls, who were followed-up for 6 months in the Temeke district in Tanzania's economic capital of Dar es Salaam, characterized by high TB notification rate. QFT diagnosed equal proportions of children with LTBI in the two groups. IPT uptake was low among TB-exposed children, and even lower among children with undocumented TB exposure.

We showed that, overall, TB infection among exposed children, less than 5 years-old, was low, but interestingly similar proportions of children in the two groups (TB-exposed and unexposed) were diagnosed with LTBI. The positive QFT results among children that did not have known exposure to TB is likely the result of community exposure to infectious TB cases. Previous studies have suggested that young children are more likely to be infected in domiciliary settings,[28-30] but due to the high prevalence of the disease in our setting, community exposure cannot be ruled out. Our findings show a higher proportion of LTBI among TB-unexposed children (20%) compared with other studies that assessed LTBI by QFT in under-5 year-old children (range 2.2-17.9%).[31-33] Previously documented reasons associated with LTBI among children include visiting other households and poor ventilation in the visited households.[30] However, our reported proportion of LTBI was considerably lower compared with other studies that report LTBI prevalence as high as 49%.[8] The lower LTBI prevalence in our study population may be due to a timely TB diagnosis in index cases, resulting in lower risk of transmission. Children from larger households were less likely to

have QFT-positive results, indicating lower transmission resulting from less exposure time to an infectious TB case.[30]

Our results suggest that IPT implementation in our study population remains a challenge. Indeed, about a third of the children eligible for IPT had not yet started treatment, as recommended by WHO in its roadmap for childhood TB.[34] It should be noted, however that a study in India reported a much lower uptake (22%), while more than half of the exposed children in our study were on medication.[35] High-income settings use IGRA for TB screening, while many under-resourced settings use symptomatic screening techniques to initiate TB preventive therapy, due to high cost of IGRA tests.[8, 10] The WHO recommends IPT in all children below the age of 5 years who are in contact with an infectious TB case or with proven TB infection after excluding active disease.[34, 36] IGRAs could be a useful tool to guide delivery of IPT in TB high burden settings, but screening strategy must consider transmission dynamics and population characteristic unique to each setting. This policy-practice gap that exists in high-burden settings needs to be addressed provided resources allow.

Contrary to other studies, our findings show a small proportion of children with indeterminate QFT results. The prevalence of indeterminate QFT results varies, especially among children.[32, 37-40] For instance, Italy reported a prevalence of 0.6%, while in South Africa, a prevalence of 2.5% was found. Compared with those two countries, our prevalence was considerably higher.[8, 32] Malnutrition, helminth infection, and HIV infection are reportedly associated with indeterminate QFT results.[38, 40] However, in our study population, the proportion of indeterminate results was low even after repeating the test at the 3-month follow-up visit and considering composite QFT results, which we used to define the final results. Contrary to other studies, we did not find any association between indeterminate QFT results and helminths.[38]

Overall, a low proportion of children had active TB disease at the end-of-follow-up visit. Despite the high risk of disease progression in young children after TB infection, the number of children developing active TB disease in our cohort was very low compared with those in Italy, where 5% of children developed active TB disease.[32] The majority of children diagnosed with active TB did not have radiologic findings consistent with active TB disease. This might be explained by a mild form of the disease diagnosed during presentation. However, in children, absence of radiologic findings can be explained by transient radiologic changes and the natural history of primary TB complex, which self-cures without intervention.[2] Due to the low number of incident cases, we did not find sufficient evidence for risk factors associated with active disease. Despite systematic and thorough screening of active TB disease, none of the TB cases, neither coprevalent nor incident TB cases, were microbacteriologically confirmed with Xpert MTB/RIF. Although this may be due to the paucibacillary nature of childhood TB, this finding may also reflect early diagnosis of the disease at presentation in our study.

Our study has strengths and limitations to be considered. We encountered a few challenges, mainly during enrollment, that are worth highlighting. Several parents/caregivers, especially those whose children had no history of TB exposure, were reluctant to subject their children for screening and were concerned with the sputum induction procedure despite intensive counseling. All children were systematically screened for TB, HIV, malaria, and helminth infection, as all of these diseases contribute to morbidity and mortality in this age-group.

In conclusion, our findings suggest that non-household transmission of TB occurs at significant rates and warrants consideration of screening among young children in high TB incidence countries. Similar to many other settings, IPT uptake among children exposed to an infectious TB case is far below the global target of 90%, as set by WHO. Contact tracing of

young children remains an important strategy to prevent active TB disease. To attain this, a road map to zero TB deaths among children emphasizes, among other issues, the collective responsibility of the health system, greater awareness among healthcare providers, and increased childhood TB screening.[34] Integrating routine pediatric TB screening with child health clinics will enhance awareness to the community and advocate for early screening among young children suspected to have TB.[41-43] In TB-endemic settings where TB exposure in the community is high, strategies to evaluate TB infection should not be limited to child contacts only.

REFERENCES

1. Dodd PJ, Yuen CM, Sismanidis C, Seddon JA, Jenkins HE. The global burden of tuberculosis mortality in children: a mathematical modelling study. *Lancet Glob Health.* 2017;5(9):e898-e906.

2. Marais BJ, Gie RP, Schaaf HS, et al. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis.* 2004;8(4):392-402.

3. Beyers N, Gie RP, Schaaf HS, et al. A prospective evaluation of children under the age of 5 years living in the same household as adults with recently diagnosed pulmonary tuberculosis. *Int J Tuberc Lung Dis.* Feb 1997;1(1):38-43.

4. Fox GJ, Barry SE, Britton WJ, Marks GB. Contact investigation for tuberculosis: a systematic review and meta-analysis. *Eur Respir J.* 2013;41(1):140-156.

5. World Health Organization. Global tuberculosis report 2016. *WHO.* 2016.

6. Oliveira CD, Melo AC, Oliveira LR, Froede EL, Camargos P. Control measures to trace </= 15-year-old contacts of index cases of active pulmonary tuberculosis. *J Bras Pneumol.* 2015;41(5):449-453.

7. Ritz N, Curtis N. Novel concepts in the epidemiology, diagnosis and prevention of childhood tuberculosis. *Swiss Medical Weekly.* 2014;144:w14000.

8. Mandalakas AM, Kirchner HL, Walzl G, et al. Optimizing the detection of recent tuberculosis infection in children in a high tuberculosis-HIV burden setting. *Am J Respir Crit Care Med.* 2015;191(7):820-830.

9. Rose MV, Kimaro G, Nissen TN, et al. QuantiFERON(R)-TB gold in-tube performance for diagnosing active tuberculosis in children and adults in a high burden setting. *PLoS One.* 2012;7(7):e37851.

10. Shah M, Kasambira TS, Adrian PV, Madhi SA, Martinson NA, Dorman SE. Longitudinal analysis of QuantiFERON-TB Gold In-Tube in children with adult household tuberculosis contact in South Africa: a prospective cohort study. *PLoS One.* 2011;6(10):e26787.

11. Mandalakas AM, Kirchner HL, Lombard C, et al. Well-quantified tuberculosis exposure is a reliable surrogate measure of tuberculosis infection. *Int J Tuberc Lung Dis.* 2012;16(8):1033-1039.

12. NTLP, MoHSW. National Guidelines for the Management of Tuberculosis in Children (1st edition). *National Tuberculosis and Leprosy Programme and Ministry of Health and Social Welfare.* 2012. http://ntlp.go.tz

13. Lobato MN, Mohle-Boetani JC, Royce SE. Missed opportunities for preventing tuberculosis among children younger than five years of age. *Pediatrics.* 2000;106(6):E75.

14. National Bureau of Statistics, Office of Chief Government Statistician. Mortality and Health. 2015. https://www.nbs.go.tz/nbs/takwimu/census2012/Mortality_and_Health_Monograph.pdf

15. NTLP, MoHSW. National Tuberculosis and Leprosy 2014 Annual Report. Dar es Salaam: Ministry of Health and Social Welfare. 2015. http://ntlp.go.tz

16. National Bureau of Statistics, Regional Commissioner's Office. *Dar es Salaam Region Socio-economic Profile* 2014. http://www.dsm.go.tz/kurasa/habari_nyingine/DSM%20Regional%20Profile.pdf

17. Said K, Hella J, Knopp S, et al. Schistosoma, other helminth infections, and associated risk factors in preschool-aged children in urban Tanzania. *PLoS Negl Trop Dis.* 2017;11(11):e0006017.

18. Grimes DA, Schulz KF. Compared to what? Finding controls for case-control studies. *Lancet.* 2005;365:1429-1433.

19. Mhimbira F, Hella J, Said K, et al. Prevalence and clinical relevance of helminth co-infections among tuberculosis patients in urban Tanzania. *PLoS Negl Trop Dis.* 2017;11(2):e0005342.

20. Sabi I, Kabyemera R, Mshana SE, et al. Pulmonary TB bacteriologically confirmed by induced sputum among children at Bugando Medical Centre, Tanzania. *Int J Tuberc Lung Dis.* 2016;20(2):228-234.

21. Zar HJ, Hanslo D, Apolles P, Swingler G, Hussey G. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study. *Lancet.* 2005;365(9454):130-134.

22. Salim N, Schindler T, Abdul U, et al. Enterobiasis and strongyloidiasis and associated co-infections and morbidity markers in infants, preschool- and school-aged children from rural coastal Tanzania: a cross-sectional study. *BMC Infect Dis.* 2014;14:644.

23. Cuevas LE, Browning R, Bossuyt P, et al. Evaluation of tuberculosis diagnostics in children: 2. Methodological issues for conducting and reporting research evaluations of tuberculosis diagnostics for intrathoracic tuberculosis in children. Consensus from an expert panel. *J Infect Dis.* 2012;205 Suppl 2:S209-215.

24. Steiner A, Hella J, Gruninger S, et al. Managing research and surveillance projects in real-time with a novel open-source eManagement tool designed for under-resourced countries. *J Am Med Inform Assoc.* 2016;23(5):916-923.

25. Graham SM, Ahmed T, Amanullah F, et al. Evaluation of tuberculosis diagnostics in children: 1. Proposed clinical case definitions for classification of intrathoracic tuberculosis disease. Consensus from an expert panel. *J Infect Dis.* 2012;205 Suppl 2:S199-208.

26. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity *World Health Organization.* 2011.

27. WHO. The WHO Child Growth Standards *World Health Organization.* 2006.

28. Chabala C, Chongwe G, Jumbe-Marsden E, Somwe SW. Missed opportunities for screening child contacts of smear-positive tuberculosis in Zambia, a high-prevalence setting. *Int J Tuberc Lung Dis.* 2017;21(1):53-59.

29. Seddon JA, Shingadia D. Epidemiology and disease burden of tuberculosis in children: a global perspective. *Infect Drug Resist.* 2014;7:153-165.

30. Wood R, Johnstone-Robertson S, Uys P, et al. Tuberculosis transmission to young children in a South African community: modeling household and community infection risks. *Clin Infect Dis.* 2010;51(4):401-408.

31. Marquez C, Chamie G, Achan J, et al. Tuberculosis Infection in Early Childhood and the Association with HIV-exposure in HIV-uninfected Children in Rural Uganda. *Pediatr Infect Dis J.* 2016;35(5):524-529.

32. Bianchi L, Galli L, Moriondo M, et al. Interferon-gamma release assay improves the diagnosis of tuberculosis in children. *Pediatr Infect Dis J.* 2009;28(6):510-514.

33. Masoumi Asl HMM, Alborzi AM, Pourabbas BP, Kalani MM. QuantiFERON-TB Gold and Tuberculin Skin Test for the Diagnosis of Latent Tuberculosis Infection in Children. *Iran J Med Sci.* 2015;40(5):411-417.

34. World Health Organization. Roadmap for childhood tuberculosis: towards zero deaths. *WHO.* 2013.

35. Singh AR, Kharate A, Bhat P, et al. Isoniazid Preventive Therapy among Children Living with Tuberculosis Patients: Is It Working? A Mixed-Method Study from Bhopal, India. *J Trop Pediatr.* 2017.

36. World Health Organization. WHO guidance for national tuberculosis programmes on the management of tubeculosis in children. *WHO.* 2014;Second edition.

37. Perez-Porcuna TM, Ascaso C, Malheiro A, et al. *Mycobacterium tuberculosis* infection in young children: analyzing the performance of the diagnostic tests. *PLoS One.* 2014;9(5):e97992.

38. Thomas TA, Mondal D, Noor Z, et al. Malnutrition and helminth infection affect performance of an interferon gamma-release assay. *Pediatrics.* 2010;126(6):e1522-1529.

39. Powell DA. Interferon gamma release assays in the evaluation of children with possible *Mycobacterium tuberculosis* infection: a view to caution. *Pediatr Infect Dis J.* 2009;28(8):676-677.

40. Haustein T, Ridout DA, Hartley JC, et al. The likelihood of an indeterminate test result from a whole-blood interferon-gamma release assay for the diagnosis of *Mycobacterium tuberculosis* infection in children correlates with age and immune status. *Pediatr Infect Dis J.* 2009;28(8):669-673.

41. Diese M, Shrestha L, Pradhan B, et al. Bottlenecks and opportunities for delivering integrated pediatric HIV services in Nepal. *Curr Opin HIV AIDS.* 2016;11 Suppl 1:S21-29.

42. Kancheya N, Luhanga D, Harris JB, et al. Integrating active tuberculosis case finding in antenatal services in Zambia. *Int J Tuberc Lung Dis.* 2014;18(12):1466-1472.

43. Marais BJ. Improving access to tuberculosis preventive therapy and treatment for children. *Int J Infect Dis.* 2017;56:122-125.

Figure legends

Figure 1: Trends in the distribution of semiquantitative quantiferon (QFT) results (IFN-γ values) among 226 children 6-59 months of age at recruitment and at the 3-month follow-up visit in Temeke district, Dar es Salaam, Tanzania between October 2015 and September 2016

Supplementary Digital Content legend

Supplementary Digital Content 1. (figure) Selection of study participants and blood samples.

Supplementary Digital Content 2. (text) Standard Operating Procedure: Control child invitation

Supplementary Digital Content 3. (table) TB screening tool

Supplemental Digital Content 4. (table) Baseline demographic, socioeconomic and clinical characteristics of 301 children under the age of 5 years, enrolled between October 2015 and September 2016, and their parents/caregivers in Temeke district, Dar es Salaam, Tanzania.

Supplemental Digital Content 5. (figure) Screening algorithm and diagnosis of TB among enrolled children

Supplemental Digital Content 6. (table) Determinants of quantiferon (QFT) conversion and reversion at three-month follow-up visit among under-fives in Dar es Salaam, Tanzania

Table 1. Prevalence of latent tuberculosis infection (LTBI) based on composite quantiferon (QFT) results among children aged below 5-years in Temeke, Dar es Salaam, Tanzania enrolled between October 2015 and September 2016.

Characteristics	TB exposed (n=186)			TB unexposed (n=115)		
	QFT result			QFT result		
	Positive	Negative	Indetermin	Positive	Negative	Indetermin
Total n (%)	38 (20)	144 (78)	4 (2)	23 (20)	87 (76)	5 (4)
Age (months), median (IQR)	33 (22-43)	26 (17-	29 (13-47)	26 (17-43)	24 (12-37)	20 (18-34)
Age groups (months)						
6-23	12 (32)	59 (41)	2 (50)	10 (43)	43 (491)	3 (60)
24-59	26 (68)	85 (59)	2 (50)	13 (57)	44 (51)	2 (40)
Female	26 (68)	77 (53)	1 (25)	12 (52)	38 (44)	2 (40)
BCG scar diameter (mm)						
No scar	9 (24)	29 (20)	2 (50)	1 (4)	9 (10)	0
1-4	16 (42)	71 (49)	2 (50)	14 (61)	46 (53)	4 (80)
>4	13 (34)	44 (31)	0	8(35)	32 (37)	1 (20)
HIV positive	0	2 (1.4)	0	1 (4)	1 (1.2)	0
>3people in the sleeping	10 (26)	31 (22)	2 (50)	5 (22)	20 (23)	1 (20)
Mother index [1]	15 (39)	39 (27)	0			
Index other primary	9 (24)	33 (23)	3 (75)	-	-	-
Sleep same bed as index [1]	28 (74)	87 (60)	3 (75)	-	-	-
Multiple indexes in h/hold [1]	5 (13)	13 (9)	0	-	-	-
Likely infected with TB [1]	25 (66)	82 (57)	3 (75)	-	-	-
Coprevalent TB cases [2]	1 (3)	2 (1.4)	0	1 (4)	1 (1.2)	0
Incident TB cases [3]	2 (5)	1 (0.7)	0	0	0	0
IPT during follow-up	29 (76)	101 (70)	3 (75)	1 (4)	0	0
Any helminth infection	10 (26)	32 (22)	2 (50)	8 (35)	17 (20)	1 (20)

LTBI defined as positive QuantiFERON-TB-Gold at either baseline or 3-month follow-up (composite QFT result). Children who developed TB during follow-up are included in this analysis.

IPT, isoniazid prophylactic therapy; QFT, quantiferon; LTBI, latent tuberculosis infection; TB, tuberculosis

[1] Applicable to TB-exposed group only based on TB exposure score based on Mandalakas et. al. [11]

[2] Diagnosed within 3 months of enrollment

[3] Diagnosed after 3 months post enrollment

Table 2. Risk factors for LTBI among children aged below 5 year in Temeke, Dar es Salaam, Tanzania.

Characteristics	All	TB exposed (n=182)		TB unexposed (n=110)	
n (%)	292	OR (95% CI)	p value	OR (95% CI)	p value
Age groups (months)			0.29		0.61
6-23	124 (42)	1		1	
24-59	168 (58)	1.50 (0.70-3.22)		1.27 (0.51-3.21)	
Sex			0.10		0.47
Male	139 (48)	1		1	
Female	153 (52)	1.89 (0.88-4.02)		1.41 (0.56-3.52)	
BCG scar diameter (mm)			0.63		0.39
No scar	48 (16)	1		1	
1-10	244 (84)	0.81 (0.34-1.90)		2.54 (0.30-21.13)	
HAZ			0.36		0.49
Normal	231 (79)	1		1	
Stunted	61 (21)	1.48 (0.64-3.40)		1.45 (0.50-4.24)	
TB clinical sign and symptoms			0.22		0.11
None	143 (49)	1		1	
At least one	149 (51)	1.58 (0.76-3.26)		0.45 (0.17-1.18)	
Hemoglobin level (g/dl)			0.17		0.48
Not anemic ≥11.5	99 (34)	1		1	
Anemic <11.5	189 (65)	0.60 (0.29-1.24)		0.71 (0.27-1.84)	
Lymphocyte count[2]			0.33		0.24
Normal	226 (77)	1		1	
Abnormal	66 (23)	0.60 (0.22-1.67)		1.79 (0.68-4.69)	
Any helminth infection			0.59		0.13
Negative	225 (77)	1		1	
Positive	67 (23)	1.25 (0.55-2.84)		2.20 (0.80-6.02)	
Mother index case[1]			0.14		-
No	238 (82)	1		-	
Yes	54 (18)	1.76 (0.83-3.71)		-	
Sleep same bed as index case[1]			0.13		-
No	177 (61)	1		-	
Yes	115 (39)	1.83 (0.83-4.06)		-	
Household income per month			0.36		0.19
<100	105 (36)	1		1	
≥100	187 (64)	1.41 (0.67-2.98)		0.53 (0.20-1.38)	

Household size			0.003		0.25
>5 people	121 (40)	1		1	
1-5 person	173 (60)	3.57 (1.54-7.69)		2.86 (0.64-5.6)	

Nine children with indeterminate QFT results were excluded in this analysis. LTBI was defined as a positive QuantiFERON-TB-Gold (QFT) test at either baseline or 3-month follow-up.

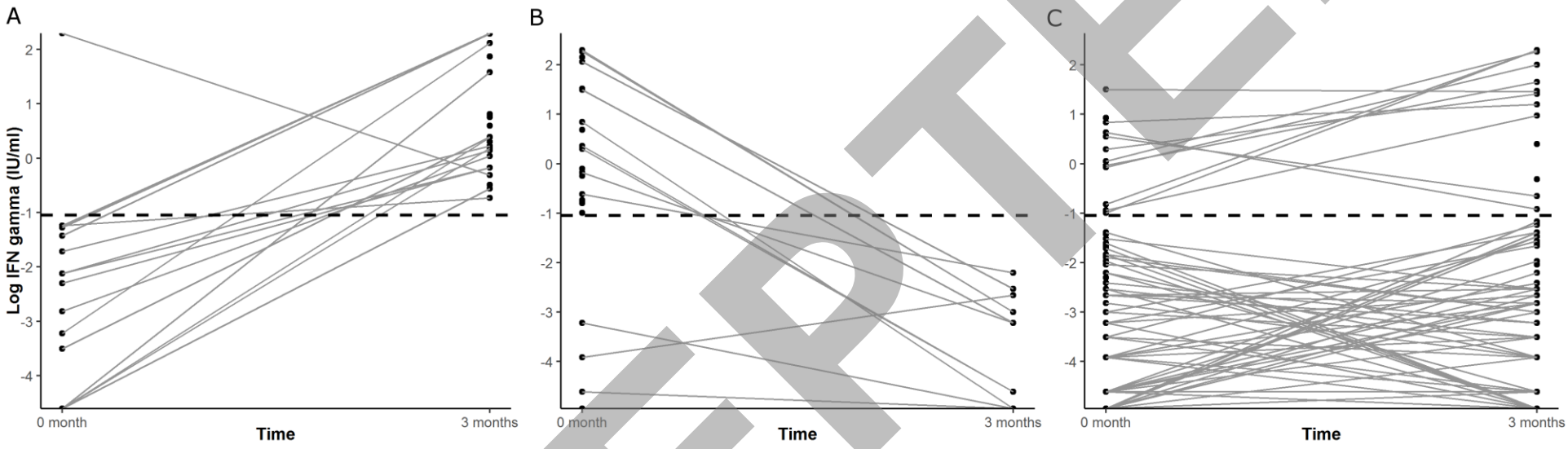
H/hold, household; HIV, human immunodeficiency virus; USD, United States dollars (1 USD=2,190 Tanzanian shillings)

Estimates derived from bivariate mixed logistic regression models.

1 Applicable to TB-exposed group only based on TB exposure score based on Mandalakas et al. [11]

2 Lymphocytes, normal 2.1-9.5, abnormal <2.1 or >9.5 $10^3/\mu l$

Figure 1



Dashed lines represent cut off -1.05