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The Magnitude of Interferon Gamma Release Assay Responses in Children With Household Tuberculosis Contact is Associated With Tuberculosis Exposure and Disease Status

Lena Ronge, MD[*], **Rosa Sloot, PhD**[*], **Karen Du Preez, MD, PhD**[*], **Alexander W. Kay, MD**[†], **H. Lester Kirchner, PhD**[‡], **Harleen M. S. Grewal, MD, PhD**[§,¶], **Anna M. Mandalakas, MD, PhD**[†,^], **Anneke C. Hesseling, MD, PhD**[*,^]

[*]Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Stellenbosch University, Cape Town, South Africa

[†]The Global Tuberculosis Program, Texas Children's Hospital, Department of Pediatrics, Baylor College of Medicine, Houston, Texas, USA

[‡]Department of Population Health Sciences, Geisinger Clinic, Danville, Pennsylvania, USA

[§]Department of Clinical Science, BIDS group, Faculty of Medicine, University of Bergen, Bergen, Norway.

[¶]Department of Microbiology, Haukeland University Hospital, Bergen, Norway.

Abstract

Background—The clinical utility of the magnitude of interferon gamma (IFNγ) in response to mycobacterial antigens is unknown. We assessed the association between quantitative IFNγ response and degree of *M. tuberculosis* exposure, infection and tuberculosis (TB) disease status in children.

Methods—We completed cross-sectional analysis of children (≤15 years) exposed to an adult with bacteriologically confirmed TB, 2007–2012 in Cape Town, South Africa. IFNγ values were reported as concentrations and spot forming units (SFU) for the QuantiFERON-TB Gold In-Tube (QFT-GIT), and T-SPOT.*TB*, respectively. Random effects linear regression was used to investigate the relation between the *M. tuberculosis* contact score, clinical phenotype (TB diseased, infected, uninfected) and IFNγ response as outcome, adjusted for relevant covariates.

Results—We analyzed data from 669 children (median age 63 months; IQR=33–108). A one-unit increase in *M. tuberculosis* contact score was associated with an increase of IFNγ 0.60 IU/ml (95%CI=0.44–0.76), and IFNγ SFU 2 counts (95%CI=1–3). IFNγ response was significantly lower among children with *M. tuberculosis* infection compared to children with TB disease (β = −1.42, 95%CI=−2.80- −0.03) for the QFT-GIT, but not for the T-SPOT.*TB*. This association was strongest among children 2–5 years (β =−2.35, 95%CI=−4.28- −0.42) and absent if <2 years.

Correspondence: Lena Ronge, Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 241, Cape Town 8000, South Africa, Tel: +27 79 3190588 or +49 171 7451519; lena@rongeonline.de.

[^]Authors contributed equally

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Discussion—The magnitude of IFNγ response correlated with the degree of recent *M. tuberculosis* exposure, measured by QFT-GIT and T-SPOT.*TB*, and was correlated with clinically relevant TB phenotypes, using the QFT-GIT. IFNγ values are not only useful in estimating the risk of *M. tuberculosis* infection but may also support the diagnosis of TB disease in children.

Keywords

IFNγ; tuberculosis; diagnosis; children; magnitude

INTRODUCTION

The World Health Organization (WHO) estimated that there were 10.0 million new cases of tuberculosis (TB) worldwide in 2018, of which 1.1 million cases were in children <15 years of age [1]. Young children are at increased risk of rapid progression to active TB after recent *M. tuberculosis* infection, and have a high risk of developing severe forms of disease [2]. Timely provision of TB preventive therapy in children prevents progression to disease while early diagnosis of disease averts additional TB-related morbidity and mortality [3].

The diagnosis of TB in children is hampered by the paucibacillary nature of the disease in most young children, and practical challenges in sample collection [4]. Existing diagnostic tests typically detect *M. tuberculosis* in less than 30% of children treated for TB [5]. Measures of mycobacterial burden might distinguish the continuum of clinical stages of TB, from exposure to *M. tuberculosis,* infection, to subclinical and incipient disease, and also manifestations of overt disease [6]. It has been shown that the magnitude of interferon gamma (IFNγ) detected by commercial interferon gamma release assays (IGRAs) in response to *M. tuberculosis* specific antigens, correlates to some extent with TB disease status in children and adults [7–10]. It is, however, unclear if the level of IFNγ produced by the host represents the extent of mycobacterial burden in the host, or whether it simply reflects heterogeneity in the human immune response to *M. tuberculosis*, which is influenced by age and several other factors [11].

In this study, we assessed whether the magnitude of IFNγ production, measured by two commercial IGRAs, QuantiFERON-TB Gold In-Tube (QFT-GIT) and T-SPOT.*TB*, was associated with the degree of recent *M. tuberculosis* exposure, and if it varied between children with TB disease, *M. tuberculosis* infection, and children that were disease and infection free.

MATERIALS AND METHODS

Study design and setting

Cross-sectional analysis was completed using data from a community-based cohort study conducted between December 2007 and June 2012 in Cape Town, Western Cape Province, South Africa. The reported incidence of TB in Cape Town in 2012 was 741 per 100,000 [12]. The estimated annual risk of *M. tuberculosis* infection in the three study communities was 3.2%−5.8% [13–15]. The population prevalence of human immunodeficiency virus (HIV) among adults was estimated at 19.2% in 2010 in the province [16]. Vertical HIV

transmission was <5% during the study period [17]. According to national guidelines, bacillus Calmette-Guérin (BCG) vaccination was routinely administered to all newborns at birth, with >90% coverage in the province in 2012 [12]. TB preventive therapy (TPT) – 6 months of daily isoniazid (10–15 mg/kg per day) was routinely recommended in children <5 years of age and in HIV-infected children with documented TB exposure to an infectious (bacteriologically confirmed) adult with pulmonary TB.

Study Population

The study population has been previously described [18–20]. This sub-analysis included children recruited from households with consecutively routinely diagnosed adult (≥18 years) drug-susceptible TB source cases, within three months of the source case starting TB treatment. HIV-infected and uninfected child household contacts were eligible if 3 months to 15 years of age, and if written informed consent was obtained from the parent or legal guardian. Children were excluded if they weighed <5 kg, had laboratory-documented anemia (Hb <9 mg/dl), were on anti-tuberculosis treatment for TB disease, or had a documented tuberculin skin test (TST) administered in the preceding 12 weeks. Enrolment was deferred in children who had received live measles or polio vaccine within the past 6 weeks, or had severe acute illness.

Data collection

In the larger cohort study, child household contacts were followed at 3, 6 and 15 months after enrolment. We report on baseline data here only. Details of data collection have been reported elsewhere [18,20]. Data on BCG vaccination (scar and vaccination records), previous TB treatment and current TB preventive therapy (history and TB clinic treatment card) and stunting (defined as moderate for height-for-age z-scores (HAZ) ≤−2-≥3 and severe for HAZ <−3) was collected using structured case report forms. Detailed information on *M. tuberculosis* exposure was captured, and described under “TB contact score”, below. Chest radiography (CXR) was independently interpreted by two physicians blinded to patient personal and clinical status using a standard pediatric TB radiologic classification tool. In all children, regardless of TST and IGRA results, at least 2 respiratory samples for gastric aspirates (< 5-year-olds) and sputum collections in older children were completed for TB microbiological testing (Mycobacteria Growth Indicator Tube, Becton, Dickinson, and company, Sparks, MD, USA). Phlebotomy was completed (6 ml-8 ml) depending on age, to complete the T-SPOT.*TB* (Oxford Immunotec, Abingdon, UK) and Quanti-FERON®-TB Gold In-Tube (Cellestis, Carnegie, VIC, Australia, since 2011: Qiagen) according to the manufacturers’ guidelines [21,22]. TST was placed after phlebotomy for IGRAs had been completed, using 2 tuberculin units of purified protein derivative RT23 (Statens Serum Institute, Copenhagen, Denmark). TST was read at 48–72 hours and considered positive if ≥10 mm in HIV uninfected and ≥5 mm in HIV infected children. All children with unknown or negative HIV status underwent HIV testing using a HIV-1/2 rapid test (Abbott Determine™ HIV-1/2 rapid test, Abbott Diagnostic Division Hoofddorp, The Netherlands). A positive or intermediate HIV test was followed by a confirmatory laboratory-based test. Polymerase chain reaction and HIV enzyme-linked immunosorbent assays were used for children under the age of 18 months and for children ≥18 months, respectively. Children

with positive maternal HIV status and negative HIV test results were classified as HIV exposed-uninfected.

TB contact score—*M. tuberculosis* exposure among child household contacts was quantified using the contact score, a linear composite measure of *M. tuberculosis* exposure, ranging from 0 (absence of documented household exposure in the past 3 months) to 10 (highest degree of household exposure) [20]. Since some categories had <5 children in the relevant category, we grouped children for this analysis into five categories: group 1 (contact score 0–2), group 2 (contact score 3–4), group 3 (contact score 5–6), group 4 (contact score 7–8), group 5 (contact score 9–10) for ease of use. Outcomes were also presented using the original 10 category contact score, to prevent loss of efficiency due to grouping.

TB clinical phenotype—TB disease was defined based on a standard protocol case definition relevant to contact investigation studies, where disease status is ascertained in the context of active surveillance [19]. Prevalent TB was defined as TB disease diagnosed within 60 days of enrolment. A 60-day window period was used to allow for pending mycobacterial culture results and CXR results (e.g., CXR may have been repeated and a child clinically re-assessed). Children who had a positive TST at baseline and had no TB disease detected within 60 days of enrolment, were considered to have 'evidence of *M. tuberculosis* infection (TB infection)'. Children who had a negative TST at baseline and were free of disease in the first 60 days after enrolment were classified as having 'no evidence of *M. tuberculosis* infection or TB disease'.

The primary outcomes of interest were quantitative IGRA responses. QFT-GIT values were reported continuously (IFNγ concentration in IU/ml). QFT-GIT cannot accurately measure absolute IFNγ values greater than 10 IU/ml, therefore such values were treated as 10 IU/ml. T-SPOT.*TB* values were reported as the number of spot forming units (SFU). Both IFNγ responses were calculated as TB antigen responses minus the assays' negative control responses (Nil). Children with indeterminate, borderline, invalid (according to manufacturers' guidelines [21,22]) and children with missing IGRA results were excluded from this analysis (Table 1). In most children >5 years, the QFT-GIT and not the T-SPOT.*TB,* was preferentially completed, due to blood volume and funding constrains. The clinical research team was blinded to IGRA test results.

Statistical methods

Random effects linear regression analysis was used to assess the relation between the *M. tuberculosis* contact score [as a categorical (5-group) and a continuous (10-group) variable], TB clinical phenotype (diseased, infected, and uninfected), and QFT-GIT and T-SPOT.*TB* responses (IFNγ and SFU, respectively) as outcomes of interest. The random effects approach specified household as the clustering variable to account for lack of independency (given multiple potential child contacts per household). Multivariable models were adjusted for relevant covariates, including gender, age, HIV status, HIV exposure, BCG vaccination status, previous TB treatment history, current TB preventive therapy, stunting and study community. TB clinical phenotype models were additionally adjusted for contact score and

stratified by age group (<2, 2-<5 years and ≥5 years). Effect estimates were reported as crude and adjusted regression coefficients with 95% confidence intervals.

We assessed the level of agreement between the TST and IGRA test results using Cohen's kappa coefficient [23]. Agreement was assessed for different TST cut-offs and for study communities, to verify whether stratified analysis was required. STATA version 14.2 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP) was used for analysis.

Ethical considerations

The research ethics committees of Stellenbosch University, Baylor College of Medicine, Case Western Reserve University, and local health authorities approved the study. Confidentiality of data was maintained at all times; only de-identified data were used.

RESULTS

Study population

The overall cohort study enrolled 1,343 children [18]. Of these, 669 (50%) children had documented household *M. tuberculosis* exposure, 96% to pulmonary TB, and were eligible for inclusion in analysis (Table 1). There were slightly more females (53%), and 47% were younger than 5 years of age. Almost all children were HIV-negative (99%). Most children (86%) had either a BCG scar noted, or a record of a BCG vaccination; 5% reported previous TB treatment. At enrolment, 28/318 (9%) of eligible children (<5 years of age or HIV-infected) were currently on TB preventive therapy. About one third of child contacts were stunted, either moderately (24%) or severely (10%). A valid QFT-GIT test result was available for 640/669 (96%) of children, of which 124 children had IFNγ level > 10 IU/ml. 403/669 (60%) children had a valid T-SPOT.*TB* test result available. 658/669 (98%) had a valid TST result. We included 32/669 (5%) children with evidence of TB disease; of those, 8 had confirmed TB, 24 had unconfirmed disease. Of those remaining, 276/669 children (41%) had evidence of *M. tuberculosis* infection (positive TST), 350/699 (52%) children had neither evidence of *M. tuberculosis* infection nor evidence of TB disease, and 11 children (2%) did not have TST results available and were not categorized.

TB contact score and quantitative IGRA results

Higher median IFNγ levels were found in children with increasing level of *M. tuberculosis* exposure, varying from 0.03 (IQR=0–1.24) among children in contact group 1, to 6.93 (IQR=0.28–10) among children in group 5 (Table 2). Adjusted analysis showed that the difference in IFNγ level between children in contact group 1 and 5 was 4.83 IU/ml (95%CI=3.27–6.40), with an increase in IFNγ level of 0.60 IU/ml (95%CI=0.44–0.76) given a one-unit increase in contact score (Table 2).

Likewise, higher IFNγ SFU counts were found with increasing *M. tuberculosis* exposure. The median IFNγ SFU count in children ranged from 1 (IQR=0–9) in contact group 1, to 16 (IQR=1–41) in group 5 (Table 3). In adjusted analysis, the difference in IFNγ SFU count

between children in contact group 1 and 5 was 16 counts (95%CI=7–25), with an increase in IFNγ SFU of 2 counts (95%CI=1–3), given a one-unit increase in contact score (Table 3).

Agreement between TST and binary IGRA results

There was substantial agreement between having a positive QFT-GIT and TST (kappa 0.78, p<0.001), and between positive T-SPOT.*TB* and TST status (kappa 0.76, p<0.001) (see Table, Supplemental Digital Content 1 and 2). Using a TST cut off of 5 mm for all children did not significantly alter the agreement between IGRA and TST. The agreement between the two IGRA tests was also high (kappa 0.82, p<0.001) (see Table, Supplemental Digital Content 3).

TB clinical phenotype and quantitative IGRA results

The median IFNγ level in children with TB disease (10, IQR=2.98–10.00) was twice as high compared with children with *M. tuberculosis* infection (5.76, IQR=1.81–10) (p=0.087) (Table 4, Supplemental Digital Content 4 (figure)). In the adjusted model, IFNγ response was significantly lower among child contacts with *M. tuberculosis* infection compared with children with disease (β=−1.42, 95%CI=−2.80- −0.03) (p=0.045). This association was stronger among children between 2 and 5 years of age (β=−2.35, 95%CI=−4.28- −0.42) (p=0.018) and in children 5 years of age or older (β=−2.24, 95%CI=−4.34- −0.14) (p=0.036), and was absent among children younger than 2 years of age (β=−0.15, 95%CI= −-2.95–3.26) (0.923) (Table 4).

Children with TB disease had higher, however statistically not significant, median IFNγ SFU counts (40, IQR=16–41) than children with *M. tuberculosis* infection (32, IQR=13–40) (p=0.633) and, than children without evidence of *M. tuberculosis* infection or disease (0, IQR=0–2) (p<0.001) (Table 5, Figure 1a-c). In adjusted analysis, there was no significant difference in IFNγ SFU response between children with *M. tuberculosis* infection and those with disease across all age groups (Table 5).

DISCUSSION

Currently, binary results of IGRAs cannot distinguish between *M. tuberculosis* infection and TB disease status. However, the magnitude of IFNγ response may reflect mycobacterial burden, and can thereby assist in the clinical diagnosis of disease [24,25]. Our study findings suggest that there is a relationship between *M. tuberculosis* burden (as a proxy for TB infection and disease status) and the magnitude of IFNγ production, in children.

The magnitude of IFNγ response was associated with the degree of *M. tuberculosis* exposure among children for both the QFT-GIT and T-SPOT.*TB*. Well-quantified TB exposure has been shown to be a reliable surrogate of *M. tuberculosis* infection in children [20,26,27]. Our findings suggest that in settings where IGRAs are used routinely as tests of infection, quantitative analysis of IGRAs could be applied to target TB preventive therapy, if data on the exposure risk is incomplete or unavailable.

We found that, among children ≥2 years of age, QFT-GIT IFNγ levels were significantly higher in children with TB disease compared with children with *M. tuberculosis* infection.

Our findings from a high TB burden setting with high levels of community transmission, are consistent with a study by Lombardi et al., which found higher levels of QFT-GIT IFNγ in children <5 years with TB disease than those with *M. tuberculosis* infection, in a low TB burden setting [28]. Lattore et al., using the QFT-GIT and the T-SPOT.*TB,* observed no significant difference of IFNy responses between children with TB disease and those with *M. tuberculosis* infection [29]. In the latter study, children were older (median age >9 years) and children with TB disease were recruited from a different source than those with *M. tuberculosis* infection, hampering comparability with our results, which were obtained using a single household contact investigation approach in all children.

Overlapping confidence intervals of IFNγ levels in the three clinical phenotype groups in our study precluded us from establishing clinically relevant diagnostic cut-offs to distinguish *M. tuberculosis* infection from disease. This could be partly explained by the lower observed IFNγ levels in active disease [30] and the inverse correlation between IFNγ production and TB disease severity noted by others [31]. Furthermore, the association between IFNγ values and TB clinical phenotype was only significant after adjustment for relevant epidemiological and clinical variables.

Children below 2 years of age have the highest risk of progressing to TB and the clinical diagnosis is the most challenging in this group. Diagnostic tests aimed at this group are critically needed. Among children <2 years, the magnitude of IFNγ responses were not associated with TB clinical phenotype. This is consistent with the finding that children <2 years of age with confirmed TB are less likely to have a positive IGRA test than older children [32]. A possible explanation for this finding is the limited proinflammatory cytokine response to mycobacteria observed in younger children [33], which may have affected the magnitude of IFNγ responses. We had low indeterminate values for both IGRAs, which are reassuring. We are not aware of any data providing an immunological explanation for the higher IFNγ response observed in children 2-<5 years, compared with children ≥ 5 years. A possible explanation could be that older children may have been more likely to have had remote infection with *M.tuberculosis*. Our finding emphasizes the importance of considering age in characterizing *M. tuberculosis*-specific responses to distinguish between TB disease and infection.

The association found between TB clinical phenotype and QFT-GIT IFNγ response was absent for IFNγ SFU values produced by T-SPOT.*TB*. However, the sample for T-SPOT.*TB* was smaller than for QFT-GIT. The QFT-GIT has been shown to be a more reproducible assay with less variability than the T-SPOT.*TB* [34]. This could have resulted in more precise estimates with narrower confidence intervals for QFT-GIT IFNγ responses, compared with IFNγ SFU values for the T-SPOT.TB. The recently FDA-approved QuantiFERON-TB Gold Plus (QFT-Plus) has been proposed to improve the detection of TB infection through stimulation of CD8+ T cells, also in children. Data from adults have shown that the CD8+ response was higher in those with TB disease than in those with *M. tuberculosis* infection [35,36]. Evidence from pediatric studies remains sparse [37,38]. The clinical utility of binary and quantitative IFNγ values from the QFT-Plus requires evaluation in children.

This study had several limitations. First, TST positivity was used as a surrogate for *M. tuberculosis* infection. Although the TST remains the only test of infection routinely recommended in high-TB burden settings, IGRAs correlate better with recent *M. tuberculosis* exposure and have a higher accuracy in diagnosing *M. tuberculosis* infection in children [39]. We expect this have only minimally influenced our outcomes, especially since the agreement between TST and IGRA was high. Second, our data did not support evaluation of the association between other clinical markers of bacterial burden and IFNγ response among children with TB disease. Third, most children in our study were HIV negative. Immunosuppression by HIV infection can result in a diminished antigen response, resulting in a low negative predictive value of the IGRA in HIV positive individuals [40]. Therefore, our findings might not be generalizable to HIV-infected children. Lastly, we did not consider that some children classified as having no evidence of *M. tuberculosis* infection and no TB disease, might have developed TB after our observation period of 60 days. The IFNγ responses of these children at baseline might be different from those who never developed disease. Ultimately, it would be critical to predict which children are at highest risk for TB progression after exposure, to ensure that children most at risk receive TPT. For example, Andrews et al. observed an increased risk of incident TB among young children with QFT conversion at very high IFNγ values [10].

In conclusion, the magnitude of IFNγ response was correlated with the degree of *M. tuberculosis* exposure, measured by QFT-GIT and T-SPOT.*TB,* in children with recent household exposure. Among children ≥2 years of age, QFT IFNγ levels were significantly higher in those with TB disease compared with children with *M. tuberculosis* infection. Our study includes a well-characterized cohort recruited using a household contact investigation strategy, with a large sample size, and meticulously captured clinical and epidemiological data. These data add to the limited evidence of potential clinical use of IGRA in children. Our data indicate that IFNγ values are not only useful in estimating the risk of *M. tuberculosis* infection, but may also support the diagnosis of disease in young children. Future longitudinal studies should explore the use of clinically relevant cut-off IFNγ values in clearly defined TB clinical groups across the spectrum of pediatric TB, from *M. tuberculosis* exposure, infection, prevalent and incident TB disease, to validate these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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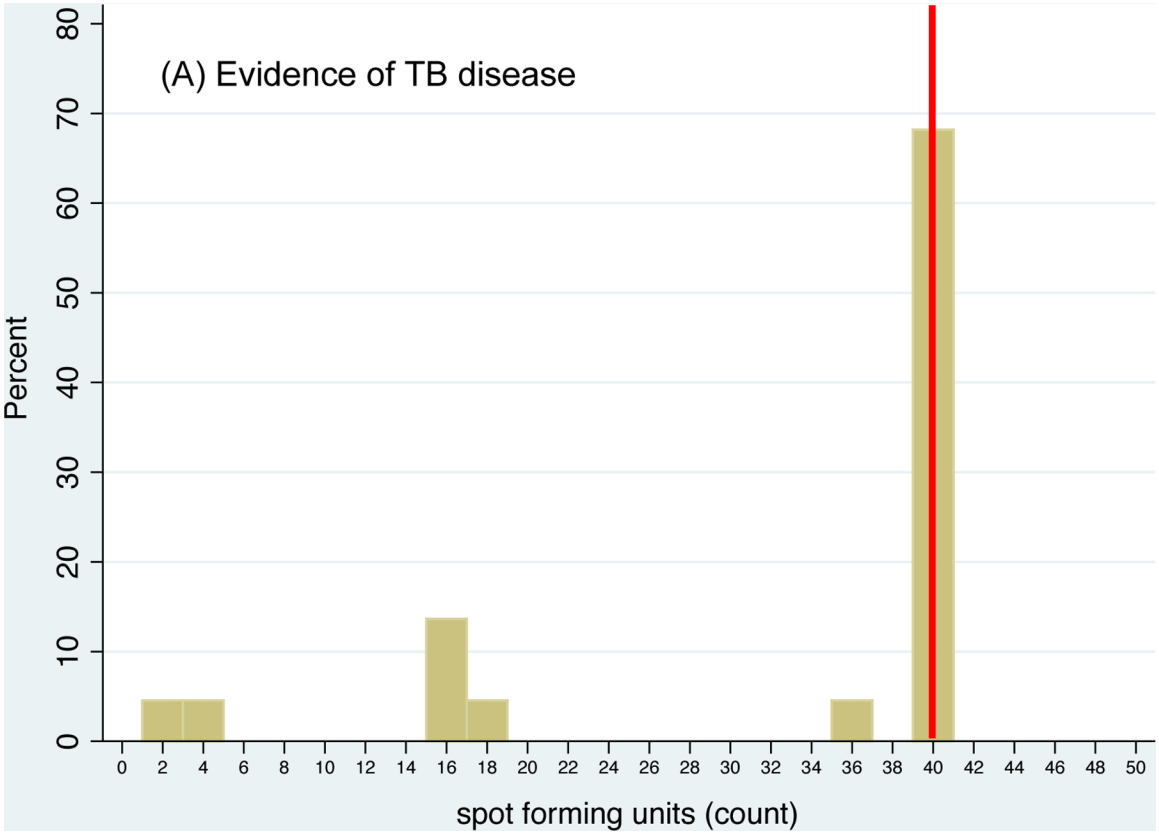

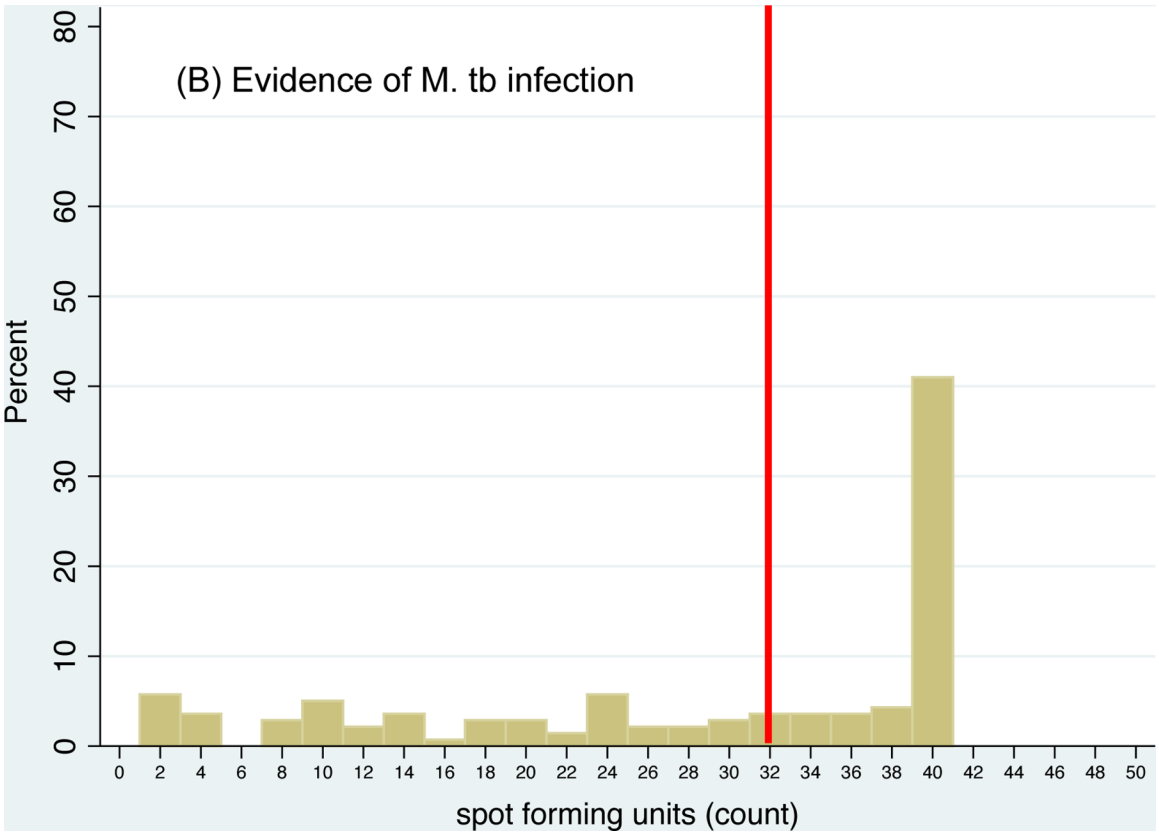

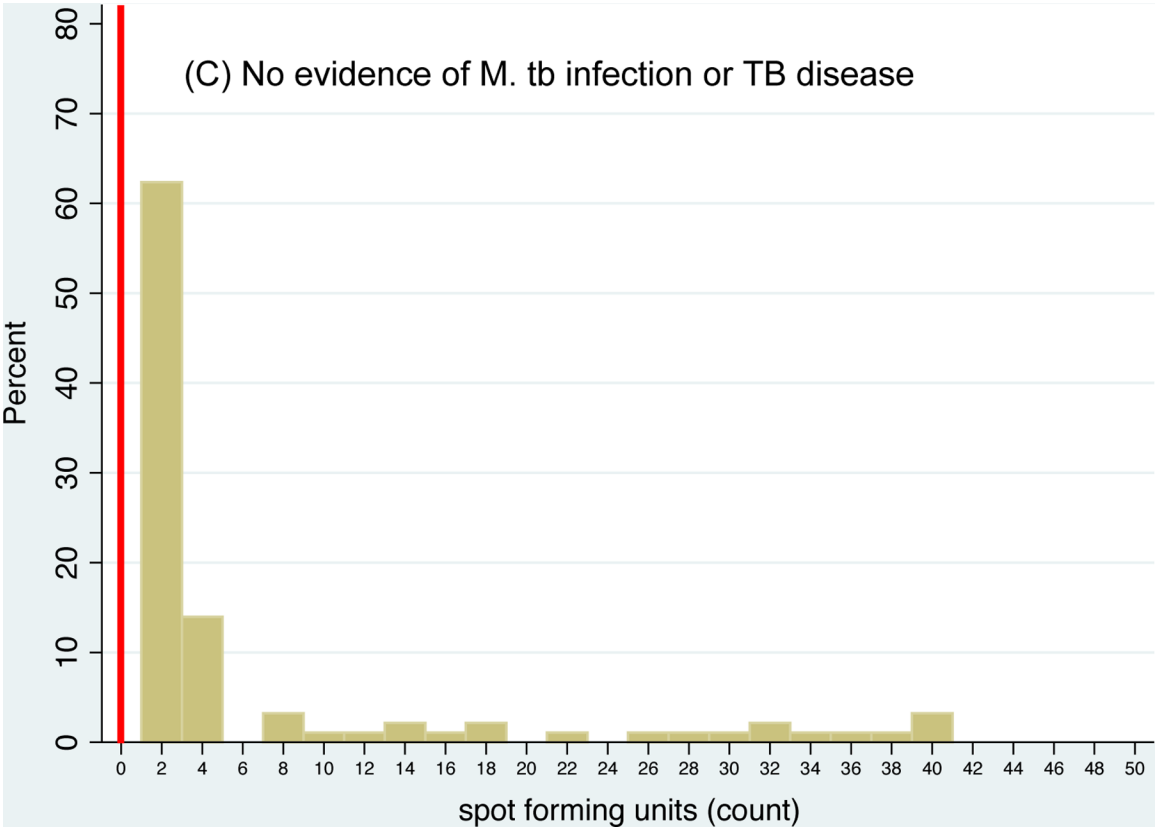

Figure 1.

A-C. Distribution of IFNγ SFU measured by T-SPOT.*TB* among children with household tuberculosis exposure with (A) Evidence of TB disease, (B) Evidence of *M. tb* infection, (C) No evidence of *M. tb* infection or TB disease

—— = median IFNγ SFU

Abbreviations: *M. tb*=*M. tuberculosis*

Table 1.

Baseline characteristics of children with household tuberculosis exposure (n=669)

	Total study population n (%) / median [IQR]
Total	669
Gender	
Male	311 (46)
Female	355 (53)
Unknown	3 (1)
Age (months)[1]	63 [33–108]
Age <2 years	112 (17)
Age 2-<5 years	202 (30)
Age ≥5 years	352 (53)
TB contact score[2]	5 [4–7]
TB clinical phenotype	
Evidence of TB disease	32 (5)
Evidence of *M. tb* infection	276 (41)
No evidence of *M. tb* infection or TB disease	350 (52)
Unknown	11 (2)
HIV infected	
Negative	664 (99)
Positive	5 (1)
Unknown	0 (0)
HIV-exposed, uninfected[3]	
No	505 (76)
Yes	42 (6)
Unknown	122 (18)
BCG vaccination status[4]	
No	91 (14)
Yes	578 (86)
Unknown	0 (0)
Previous TB treatment	
No	633 (95)
Yes	36 (5)
Unknown	0 (0)
Current TB preventive therapy	
No	641 (96)
Yes	28 (4)
Unknown	0 (0)

	Total study population n (%) / median [IQR]
Stunting	
No Stunting	437 (65)
Moderate Stunting	162 (24)
Severe Stunting	64 (10)
Unknown	6 (1)
IFNγ level (IU/ml)[5,6]	0.4 [0.0–6.7]
QFT-GIT[7]	
Negative	309 (46)
Positive	331 (50)
Indeterminate	8 (1)
Missing	21 (3)
IFNγ Spot forming units (count)[5]	2 [0–31]
T-SPOT.*TB*[7]	
Negative	234 (35)
Positive	169 (25)
Borderline	9 (1)
Missing[8]	257 (38)
Tuberculin skin test induration (mm)[5]	7 [0–15]
Mantoux Tuberculin Skin Test result[9]	
Negative	356 (53)
Positive	302 (45)
Unknown	11 (2)
Study community	
Ravensmead	250 (37)
Uitsig	293 (44)
Khayelitsha	126 (19)

Abbreviations: IQR=interquartile range, *M. tb=M. tuberculosis*

[1] data missing for N=3

[2] 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 Aug;16(8):1033–9.

[3] Positive maternal HIV status; child participant HIV-uninfected

[4] BCG scar noted, or record of vaccination available

[5] missing, indeterminate, borderline and invalid test values were excluded

[6] values > 10 IU/ml were treated as 10 IU/ml (N=124)

[7] according to manufacturer's definition

[8] 210/257 (82%) of missing values were among children aged ≥5 years, due to funding constraints

[9] positive if ≥10 mm in HIV-uninfected and ≥5 mm in HIV-infected children

Table 2.

Univariate and multivariate linear regression analysis estimating the effect of tuberculosis exposure on the IFNγ response measured by QFT-GIT in IU/ml among children with household tuberculosis exposure

	Total number (n)[#]	Median IFNγ (IQR)	Unadjusted regression coefficient β (95%CI)	p-value	Adjusted regression coefficient β (95%CI)[*]	p-value
TB contact score (1–10)[+]	640	0.44 (0.01–6.74)	0.55 (0.39–0.71)	<0.001	0.60 (0.44–0.76)	<0.001
TB contact group						
1	22	0.03 (0–1.24)	0		0	
2	156	0.06 (0–1.72)	0.65 (−0.52–1.83)	0.227	0.72 (−0.49–1.93)	0.242
3	266	0.54 (0.01–6.8)	2.04 (0.85–3.22)	0.001	2.07 (0.84–3.30)	0.001
4	144	2.02 (0.02–9.47)	2.67 (1.38–3.97)	<0.001	2.77 (1.41–4.12)	<0.001
5	52	6.93 (0.28–10)	4.30 (2.72–5.88)	<0.001	4.83 (3.27–6.40)	<0.001

Abbreviations: CI=confidence interval, IQR=interquartile range

[#]Contacts with indeterminate or missing QFT-GIT test results were excluded from the analysis

[*]Multivariable model is adjusted for: gender, age, HIV status, HIV-exposed (uninfected), BCG vaccination, previous TB treatment, current IPT, stunting, study community

[+]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 Aug;16(8):1033–9.

Table 3.

Univariate and multivariate linear regression analysis estimating the effect of tuberculosis exposure on the IFNγ response measured by T-SPOT.*TB* in SFU among children with household tuberculosis exposure

	Total number (n)[#]	Median IFNγ SFU (IQR)	Unadjusted regression coefficient β (95%CI)	p-value	Adjusted regression coefficient β (95%CI)[*]	p-value
TB contact score (1–10)[+]	403	2 (0–31)	2 (1–3)	<0.001	2 (1–3)	<0.001
TB contact group						
1	14	1 (0–9)	0		0	
2	105	1 (0–11)	2 (−5–9)	0.635	2 (−5–9)	0.609
3	170	2 (0–36)	8 (1–15)	0.029	8 (1–16)	0.031
4	79	10 (0–32)	9 (2–16)	0.017	10 (2–18)	0.020
5	35	16 (1–41)	14 (5–23)	0.002	16 (7–25)	<0.001

Abbreviations: CI=confidence interval, IQR=interquartile range

[#]Contacts with invalid, borderline or missing T-SPOT.*TB* test results were excluded from the analysis

[*]Multivariable model is adjusted for: gender, age, HIV status, HIV exposed (uninfected), BCG vaccination, previous TB treatment, current IPT, stunting, study community

[+]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 Aug;16(8):1033–9.

Table 4.

Univariate and multivariate linear regression analysis estimating the effect of tuberculosis clinical phenotype on the IFNγ response measured by QFT-GIT in IU/ml among children with household tuberculosis exposure, stratified by age

TB clinical phenotype	No. of patients*	Median IFNγ in IU/ml (IQR)	Unadjusted regression coefficient β (95%CI)	p-value	Adjusted regression coefficient β (95%CI)#	p-value
Total population						
Evidence of TB disease	30	10 (2.98–10.00)	0		0	
Evidence of *M. tb* infection	271	5.76 (1.81–10.00)	−1.32 (−2.81–0.16)	0.080	−1.42 (−2.80− −0.03)	0.045
No evidence of *M. tb* infection or TB disease	330	0.02 (0.00–0.09)	−6.46 (−7.90− −5.01)	<0.001	−6.37 (−7.73− −5.02)	<0.001
Age <2 years						
Evidence of TB disease	11	6.55 (0.61–10.00)	0		0	
Evidence of *M. tb* infection	20	6.87 (1.11–9.87)	−0.08 (−3.27–3.10)	0.958	0.15 (−2.95–3.26)	0.923
No evidence of *M. tb* infection or TB disease	72	0.01 (0.00–0.03)	−5.06 (−7.80− −2.32)	<0.001	−4.29 (−6.98− −1.61)	0.002
Age 2−<5 years						
Evidence of TB disease	12	10.00 (8.51–10.00)	0		0	
Evidence of *M. tb* infection	61	9.69 (2.18–10.00)	−1.53 (−3.81–0.74)	0.185	−2.35 (−4.28− −0.42)	0.018
No evidence of *M. tb* infection or TB disease	117	0.01 (0.00–0.06)	−7.84 (−9.95− −5.73)	<0.001	−8.33 (−10.21− −6.45)	<0.001
Age ≥ 5 years						
Evidence of TB disease	7	10 (4.72–10.00)	0		0	
Evidence of *M. tb* infection	190	5.01 (1.81–10.00)	−2.12 (−4.84–0.61)	0.128	−2.24 (−4.34− −0.14)	0.036
No evidence of *M. tb* infection or TB disease	141	0.03 (0.00–0.27)	−6.66 (−9.38− −3.95)	< 0.001	−6.74 (−8.83− −4.65)	< 0.001

Abbreviations: CI=confidence interval, IQR=interquartile range, *M. tb=M. tuberculosis*

*Contacts with indeterminate or missing QFT-GIT test results and contacts with invalid or missing TST results were excluded from the analysis

#Multivariable model is adjusted for: gender, age, contact score, HIV status, HIV-exposed (uninfected), BCG vaccination, previous TB treatment, current IPT, stunting, study community

Table 5.

Univariate and multivariate linear regression analysis estimating the effect of tuberculosis clinical phenotype on the IFNγ response measured by T-SPOT.*TB* in SFU among children with household tuberculosis exposure, stratified by age

TB clinical phenotype	No. of patients*	Median IFNγ SFU (IQR)	Unadjusted regression coefficient β (95%CI)	p-value	Adjusted regression coefficient β (95%CI)#	p-value
Total population						
Evidence of TB disease	25	40 (16–41)	0		0	
Evidence of *M. tb* infection	147	32 (13–40)	−2 (−9–5)	0.633	−1 (−7–5)	0.758
No evidence of *M. tb* infection or TB disease	228	0 (0–2)	−26 (−33– −19)	<0.001	−24 (−31– −18)	<0.001
Age <2 years						
Evidence of TB disease	10	28 (3–41)	0		0	
Evidence of *M. tb* infection	18	33 (10–40)	1 (−14–16)	0.897	−1 (−15–13)	0.879
No evidence of *M. tb* infection or TB disease	66	0 (0–1)	−22 (−34– −10)	0.001	−22 (−34– −10)	0.001
Age 2–<5 years						
Evidence of TB disease	13	40 (39–41)	0		0	
Evidence of *M. tb* infection	51	37 (22–41)	−2 (−11–7)	0.699	−4 (−12–3)	0.260
No evidence of *M. tb* infection or TB disease	105	0 (0–1)	−31 (−39– −22)	<0.001	−31 (−39– −24)	<0.001
Age ≥5 years						
Evidence of TB disease	2	26 (17–35)	0		0	
Evidence of *M. tb* infection	78	26 (12–40)	−1 (−15–12)	0.850	1 (−13–14)	0.940
No evidence of *M. tb* infection or TB disease	57	1 (0–3)	−20 (−33– −7)	0.003	−19 (−33– −5)	0.009

Abbreviations: CI=confidence interval, IQR=interquartile range, *M. tb=M. tuberculosis*

*Contacts with invalid or missing T-SPOT.*TB* results and contacts with invalid or missing TST results were excluded from the analysis

#Multivariable model is adjusted for: gender, age, contact score, HIV status, HIV-exposed (uninfected), BCG vaccination, previous TB treatment, current IPT, stunting, study community