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Malnutrition and Helminth Infection Affect Performance of an Interferon γ–Release Assay

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Malnutrition and Helminth Infection Affect Performance of an Interferon γ–Release Assay

WHAT'S KNOWN ON THIS SUBJECT: The use of interferon γ–release assays, including the QuantiFERON-TB Gold In-Tube assay (QFT-IT), is hampered by insufficient data regarding performance among high-risk pediatric subpopulations, including children with immunomodulating conditions such as malnutrition and intestinal helminth infection.

WHAT THIS STUDY ADDS: Interferon γ responses were blunted in the setting of malnutrition and helminth infection, leading to more indeterminate QFT-IT results. QFT-IT results should be interpreted with caution when performed on children affected by such conditions.

abstract

OBJECTIVE: We sought to compare the tuberculin skin test (TST) to the QuantiFERON-TB Gold In-Tube assay (QFT-IT) and assess the effects of malnourishment and intestinal helminth infection on QFT-IT results.

METHODS: In this population-based cross-sectional study from Dhaka, Bangladesh, we screened children for latent tuberculosis infection with the QFT-IT and TST. We assess the agreement between the TST and QFT-IT, risk factors associated with indeterminate QFT-IT results, and magnitude of interferon γ (IFN-γ) production.

RESULTS: Three hundred and two children (aged 11–15.3 years) were enrolled, including 93 (30.8%) who were malnourished. Of 251 participants who provided stool samples, 117 (46.6%) were infected with *Ascaris lumbricoides* and/or *Trichuris trichiura*. TST results were positive (≥10 mm) for 101 (33.4%) children and negative for 201 (66.6%) children. QFT-IT results were positive for 107 (35.4%) children, negative for 121 (40.1%) children, and indeterminate for 74 (24.5%) children. Agreement between the tests was moderate ($\kappa = 0.55$ [95% confidence interval: 0.44–0.65]; $P < .0001$) when excluding indeterminate results. Children with indeterminate QFT-IT results were separately compared with children with positive and negative QFT-IT results; malnutrition ($P = .0006$ and .0003), and helminth infection ($P = .05$ and .02), and the statistical interaction between these 2 terms ($P = .03$ and .004) were associated with indeterminate results. Higher levels of IFN-γ in response to tuberculosis antigens were associated with positive TST results ($P < .0001$); lower levels were associated with malnutrition ($P = .02$).

CONCLUSIONS: Malnutrition and helminth infections were associated with indeterminate QFT-IT results. Therefore, the presence of such conditions may limit the interpretability of QFT-IT results in children. *Pediatrics* 2010;126:e1522–e1529

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KEY WORDS
latent tuberculosis infection, interferon γ–release assay, pediatrics, malnutrition, helminth infection

ABBREVIATIONS
TB—tuberculosis
LTBI—latent tuberculosis infection
TST—tuberculin skin test
BCG—bacille Calmette-Guérin
IFN—interferon
IGRA—interferon γ–release assay
QFT-IT—QuantiFERON-TB Gold In-Tube
IQR—interquartile range
Th—T helper

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Tuberculosis (TB) is a leading global cause of morbidity and mortality in children. Timely diagnosis and treatment of TB infection and disease are especially important for pediatric populations. Once infected with *Mycobacterium tuberculosis*, immunocompetent adults have a 5% to 10% lifetime risk of progressing to have TB disease. Children have an increased risk: within 2 years of infection, nearly half of infants and up to 15% of older children will develop TB disease.[1] Those who do not develop TB disease in childhood will serve as reservoirs during adulthood.

Identification and treatment of latent TB infection (LTBI) is a proposed key strategy for global TB control.[2] However, the lack of a gold-standard test to detect LTBI makes accurate diagnosis difficult. The tuberculin skin test (TST), the most widely used method of diagnosing LTBI, is fraught with limitations including poor specificity because of cross-reactivity with nontuberculous mycobacteria and the bacille Calmette-Guérin (BCG) vaccine and poor sensitivity among people who may be anergic as a result of malnutrition, HIV/AIDS, or immunosuppressive states.[3] The BCG vaccine's limited performance is magnified in populations who are at the highest risk (young children and immunocompromised hosts) of progressing from infection to TB disease.[4]

Interferon γ (IFN-γ)–release assays (IGRAs), such as the QuantiFERON-TB Gold In-Tube assay (QFT-IT), have been developed as possible replacements for the TST. The QFT-IT has the potential to be more specific than the TST by using antigens encoded within region-of-difference 1 (early secreted antigenic target, 6 kDa [ESAT-6] and culture filtrate protein, 10 kDa [CFP-10]) and region-of-difference 11 (TB7.7) of the *M tuberculosis* genome, antigens that are not included in the BCG vaccine and most nontuberculous mycobacteria, respectively.[5] However, IGRAs quantify cell-mediated immune responses; therefore, the sensitivity may be limited in immunocompromised populations. When using TB disease as an indicator for LTBI, IGRAs have been successful at diagnosing LTBI in adults[3]; a meta-analysis by Pai et al[6] revealed a pooled sensitivity of 70% and specificity of 99% from studies that used the QFT-IT. Although the replacement of the TST by IGRAs may not be imminent in resource-limited settings, IGRAs are increasingly being recommended in resource-rich countries for the diagnosis of LTBI in adults. It should be noted that recommendations for the use of IGRAs in children have been limited by a lack of evidence among high-risk pediatric subpopulations.

Recently, more pediatric IGRA studies have emerged[7]; however, validation studies among children with immune dysfunction have been few and focused on children immunosuppressed from treatment for rheumatologic conditions, malignancies, and organ transplantation.[8,9] Malnutrition and helminth infection are other immunomodulating states that may lead to anergic TST responses,[10,11] but their effect on IGRA performance has not been well characterized. One study of hospitalized children from rural India suspected to have TB disease showed no association between malnutrition and QFT-IT result.[12] To our knowledge, no studies have assessed the effect of helminth infection on IGRA performance.

Because many nations are adopting guidelines to use IGRAs, additional investigation of the performance of these tests among a variety of pediatric hosts is imperative. We sought to compare the QFT-IT with the TST in detecting LTBI among a cohort of children with a high burden of malnutrition and helminth infection from a TB-endemic area and assess whether malnutrition, helminth infections, BCG-vaccine status, or previous TB contact influenced QFT-IT results.

METHODS

Setting and Study Population

This was a cross-sectional study performed on children from Mirpur, Bangladesh. Mirpur is a densely populated urban area within Dhaka, where overcrowding and extreme poverty contribute to poor sanitary conditions and a high burden of malnutrition. The average household in this region of Mirpur contains 6 people, and more than 95% of the families live on less than $1 US per day.[13]

The study population included children previously enrolled in the Field Studies of Human Immunity to Amebiasis Study, an ongoing longitudinal cohort study established in 1998 that targets preschool-aged children.[14] All children in the cohort were invited to participate in this community-based TB-screening study. Children were excluded if they had a known history of TB disease. Any child with a positive TST result was referred to the government TB clinic for additional evaluation; if found to have TB disease, treatment was given according to national guidelines.[15]

Procedures

Trained field workers used a uniform questionnaire to assess current symptoms of TB, history of TB, TB contacts, or BCG vaccination, and socioeconomic status. BCG-vaccination history was confirmed by the presence of a BCG scar observed by field workers. Anthropometric measurements were obtained at enrollment, including height and weight to calculate BMI. Malnutrition was defined by a gender-specific BMI-for-age *z* score of less than −2 SDs, and severe malnutrition was defined by a gender-specific BMI-

for-age *z* score of less than or equal to −3 SDs.[16] Because of the low nationwide HIV prevalence (estimated as <0.1%), information on HIV status was not collected as a part of this study.[17]

The QFT-ITs (Cellestis, Carnegie, Australia) were performed and interpreted according to the package insert[18] by experienced laboratory technicians who were blinded to all clinical information. Briefly, blood was collected into 3 heparin-containing tubes, including a positive control (mitogen, phytohemagglutinin), a negative control (heparin), and a TB-antigen tube (which contained antigens ESAT-6, CFP-10, and TB-7.7). Tubes were shaken for 10 to 15 seconds after blood collection and again in the laboratory before incubation. Within 16 hours of collection, and generally within 3 hours, the tubes were incubated at 37°C for 16 to 24 hours. After centrifugation, plasma was harvested and stored at −80°C until enzyme-linked immunosorbent assay tests were run. Quality-control tests were performed according to the manufacturer's recommendations. Results were calculated by subtracting the IFN-γ level of the negative control tube from the IFN-γ level of the respective TB-antigen and mitogen tubes. A test result was positive if the net IFN-γ response to the TB antigens was ≥0.35 IU/mL, regardless of the mitogen response; a test result was negative if the net IFN-γ value was <0.35 IU/mL and there was sufficient mitogen response (>0.50 IU/mL); and a test result was indeterminate if there was excessive IFN-γ production from the negative control tube (>8.0 IU/mL) or an insufficient net mitogen response (<0.50 IU/mL) plus insufficient net TB-antigen response (<0.35 IU/mL).

The TST was placed by a trained tester using 2 tuberculin units of purified protein derivative RT23 by the Mantoux method.[15] Results were measured in millimeters of induration 72 hours after placement. A TST result was defined as positive if the induration was ≥10 mm.[15] TSTs were performed after phlebotomy for the QFT-IT.

A stool sample was requested from all participants at the initial visit. Fresh specimens were transported to the laboratory at the International Centre for Diarrhoeal Disease Research, Bangladesh for ova and parasite evaluation by microscopy. The Kato-Katz method was used for quantifying *Ascaris lumbricoides* and *Trichuris trichiura* infection by counting eggs per gram of stool. A fresh specimen was defined as one that was collected, transported to the laboratory, and examined within 6 hours of collection. Infection was defined as having any positive egg count in stool. Because of low regional prevalence of other geohelminths, including hookworm and *Strongyloides stercoralis* (both estimated to be <1%), these helminths were not assessed (M.A., unpublished data, 2010).

Statistical Analyses

Agreement between the TST and QFT-IT was assessed by using κ statistics. Multinomial logistic regression analysis was performed to assess risk factors associated with an indeterminate QFT-IT result; the effects of age, gender, previous TB contact, BCG vaccination, any helminth infection, nutritional status, and TST results were examined. Statistical interactions between these terms, if significant, were included in the analysis. The magnitude of IFN-γ production was regressed on the above-listed risk factors. The magnitude of helminth burden was regressed on TST induration and IFN-γ production. Statistical significance was defined by a *P* value of ≤.05. Analyses were performed by using SPSS 17.0 (SPSS Inc, Chicago, IL) and SAS 9.1 (SAS Institute, Inc, Cary, NC).

Ethics

Ethical approval to conduct this study was obtained from the International Centre for Diarrhoeal Disease Research, Bangladesh and the University of Virginia. Informed consent was obtained from the children's caregiver, and eligible children assented.

RESULTS

Between April and June 2009, 314 children were enrolled: 302 children were included in the initial analysis, and because stool data were unavailable for 51 children, the final regression analysis included 251 children (Fig 1). Characteristics of the study participants are listed in Table 1. The mean age was 13.1 years (SD: 1.25 years). Previous BCG vaccination was confirmed in 239 children (79.1%); 30 children (9.9%) reported a current or past TB contact. Of those with a positive TST result who were evaluated for TB disease, 1 child was found to have culture-confirmed TB. Ninety-three children (30.8%) were malnourished, including 26 children (8.6%) who were severely malnourished. Of 251 participants who provided a stool sample, 117 (46.6%) were infected with either *A lumbricoides* and/or *T trichiura*. *Ascaris* infection was found in 88 children (35.1%; median number of eggs per g of stool: 8016 [interquartile range (IQR): 3240–14 736]), among whom 29 (33%) had a light level of infection, 51 (58%) had moderate infection, and 8 (9%) had heavy infection, as defined by the World Health Organization.[19] *Trichuris* infection was found in 74 children (29.5%; median number of eggs per g of stool = 270 [IQR: 186–890]), among whom 56 (75.7%) had light infection, 14 (18.9%) had moderate infection, and 4 (5.4%) had heavy infection. Both helminths were found in 45 children (17.9%).

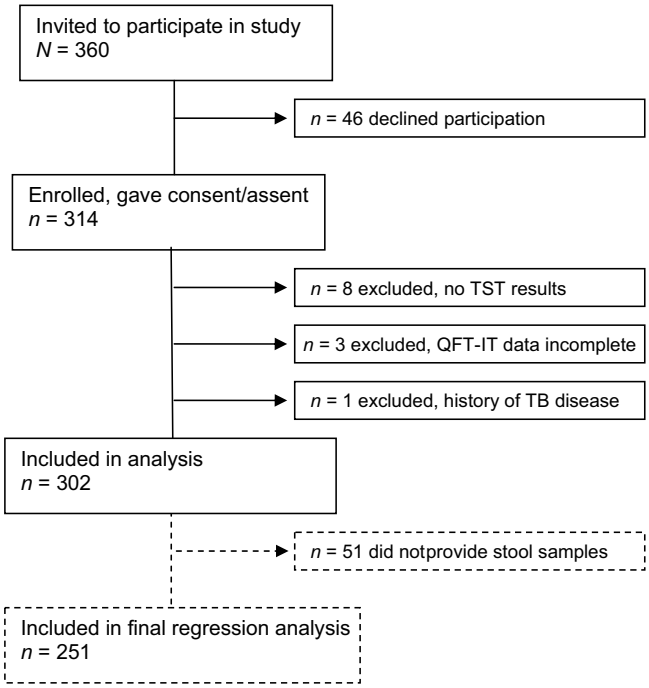

FIGURE 1
Flow diagram of enrollment.

TABLE 1 Baseline Demographic and Clinical Information of Study Participants

	All Results ($N = 302$)
Age, mean (SD), y	13.10 (1.25)
Gender, *n* (%)	
Male	140 (46.4)
Female	162 (53.6)
BCG-vaccination status, *n* (%)	
Positive	239 (79.1)
Negative	59 (19.5)
Unknown	4 (1.4)
Positive TB-contact history	30 (9.9)
BMI	
Mean (SD)	16.1 (2.2)
Malnourished, *n* (%)[a]	93 (30.8)
Severely malnourished, *n* (%)[a]	26 (8.6)
Helminth infection ($n = 251$), *n* (%)	
Ascaris	88 (35.1)
Trichuris	74 (29.5)
Ascaris and/or *Trichuris*	117 (46.6)
Both *Ascaris* and *Trichuris*	45 (17.9)
TST result, mean (SD), mm	5.9 (6.2)

[a] Malnourished was defined as a BMI *z* score of less than −2 SDs for age and gender; severely malnourished was defined as a BMI-*z* score of less than or equal to −3 SD for age and gender.

TST results were positive for 101 children (33.4%) and negative for 201 (66.6%) (Table 2). QFT-IT results were positive for 107 (35.4%) children, negative for 121 (40.1%) children, and indeterminate for 74 (24.5%) children. Figure 2 displays the absolute QFT-IT values based on the TST results. The agreement between the tests can only be compared between the concordant groups; thus, after excluding the indeterminate results, the agreement was moderate ($\kappa = 0.55$ [95% confidence interval: 0.44−0.65]; $P < .0001$). Discordant results were found in 51 patients (16.9%).

Of those with indeterminate results, 3 subjects' results were attributed to excessive background IFN-γ production, whereas 71 subjects' results were a result of an insufficient IFN-γ response to mitogen. Baseline characteristics for those with indeterminate results are listed in Table 3.

For 251 subjects with complete records of outcome and risk factors, multinomial logistic regression analysis was used to compare QFT-IT results with indeterminate QFT-IT results as the reference group (Table 4). When children with indeterminate QFT-IT results were compared with those with positive results, malnutrition ($P = .0006$), any helminth infection ($P = .05$), the statistical interaction between these 2 terms ($P = .03$), negative TST result ($P < .0001$), and older age ($P = .04$) were associated with indeterminate results. When those with indeterminate results were compared with those with negative results, only malnutrition ($P = .0003$), helminth infection ($P = .02$), and the statistical interaction between these terms ($P = .004$) were associated with indeterminate QFT-IT results. Among both comparison groups, children with 1 risk factor, either malnutrition or helminth infection, were at increased risk of having an indeterminate QFT-IT result. The presence of both risk factors also increased the risk of having an indeterminate result but not to the extent of malnutrition alone. Gender, previous TB contact, and BCG-vaccination status showed no association with indeterminate QFT-IT results.

After adjusting for BCG-vaccination status, helminth infection, TB-contact history, age, and gender, linear regression revealed that the log-transformed magnitude of IFN-γ responses to TB antigens was associated with TST size and malnu-

TABLE 2 Comparison of QFT-IT and TST Results ($N = 302$)

TST Result	QFT-IT Result			Total
	Positive	Negative	Indeterminate	
Positive, *n* (%)	72	16	13	101 (33.4)
Negative, *n* (%)	35	105	61	201 (66.6)
Total, *n* (%)	107 (35.4)	121 (40.1)	74 (24.5)	302 (100.0)

The agreement between the 2 tests was calculated by comparing the positive/positive and negative/negative results. The agreement was moderate ($\kappa = 0.55$ [95% confidence interval: 0.44−0.65]; $P < .0001$) after excluding indeterminate results.

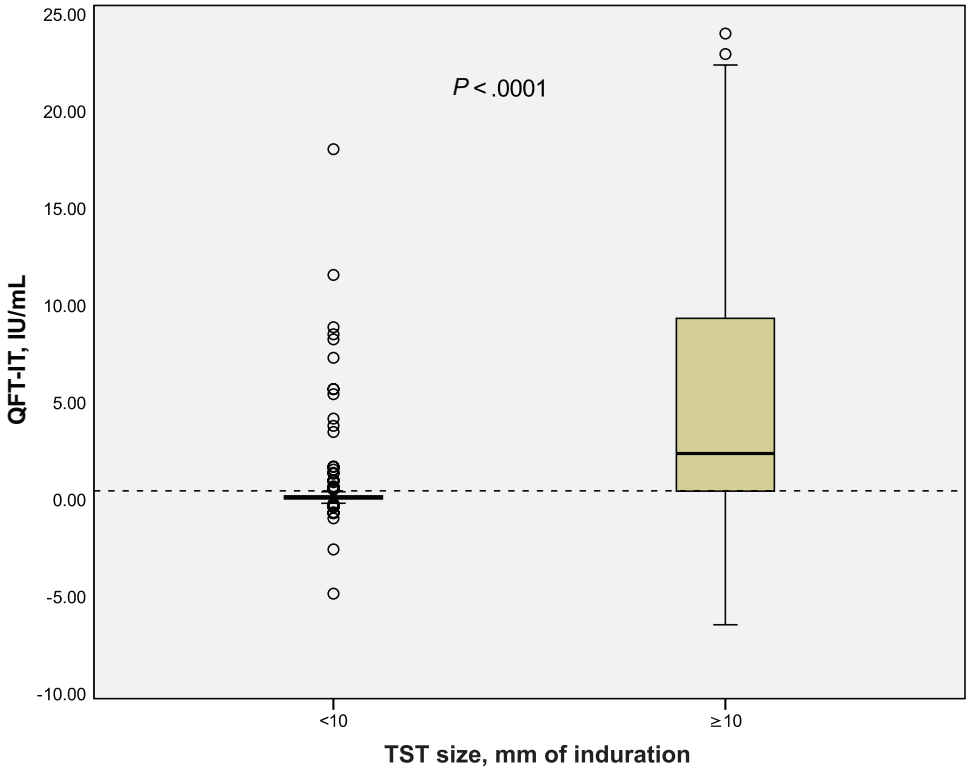

FIGURE 2

Absolute QFT-IT values based on TST results. Boxes represent the median value and the IQR; whiskers represent a distance up to 1.5 times the IQR; and the dotted line represents the cutoff value for a positive QFT-IT result: 0.35 IU/mL (measured as the difference between the IFN-γ response to TB antigens and the IFN-γ production in the negative control tube).

TABLE 3 Demographic and Clinical Characteristics of Study Participants With Indeterminate QFT-IT Results ($N = 74$)

	Excessive Background IFN-γ ($N = 3$)	Low IFN-γ Response to Mitogen ($N = 71$)
Age (IQR), y	13.3 (13.2–14.9)	12.5 (11.0–15.3)
Female gender	2	37
Median (IQR) TST size, mm	8 (6–9)	0 (0–7)
Malnourished, *n*	1	29
Any helminth infection, *n*	0[a]	29[b]
Both helminth infections, *n*	0[a]	8[b]
History of BCG vaccination, *n*	1[c]	62

[a] Stool data were available for 1 of the 3 children.
[b] Stool data were available for 59 of the 71 children.
[c] BCG-vaccine data were available for 1 of the 3 children.

trition. Larger TST induration (≥10 mm) and IFN-γ production to TB antigens were positively correlated ($P < .0001$); those with a TST induration of ≥10 mm had 6.2-fold greater IFN-γ production compared with those with a TST induration of <10 mm. Those who were malnourished were more likely to have lower IFN-γ responses to TB antigens ($P = .02$); the presence of malnutrition corresponded to 36% lower production of IFN-γ.

Regression analysis of the burden of helminth infection (in number of eggs per g of stool) revealed no significant association with size of TST induration or magnitude of IFN-γ production after phytohemagglutinin or TB-antigen stimulation (data not shown).

DISCUSSION

This community-based study in which we compared TSTs with QFT-ITs for detection of LTBI among 302 children from Bangladesh revealed a high proportion of indeterminate QFT-IT results. Risk factors associated with an indeterminate QFT-IT result in this cohort included malnourishment and the presence of infection with *A lumbricoides* and/or *T trichiura*.

From previous studies of IGRAs in children, widely discrepant proportions of indeterminate results, ranging from 0% to 35%, have been reported.[9,12] Numbers varied on the basis of the population under study[20] and the type of IGRA used.[8,21,22] Pediatric studies with higher proportions of indeterminate results have generally included more immunocompromised patients.[8,9] However, only 1 study[9] has been able to docu-

TABLE 4 Multinomial Regression Analysis According to Risk Factors for Indeterminate QFT Response ($N = 251$)

	Indeterminate QFT vs Positive QFT Results ($N = 181$), P	Indeterminate QFT vs Negative QFT Results ($N = 195$), P
Age	.04	NS
Female gender	NS	NS
TST result < 10 mm	<.0001	NS
Malnutrition	.0006	.0003
Any helminth infection	.05	.02
Malnutrition (helminth infection)	.03	.004
Previous household TB contact	NS	NS
History of BCG vaccination	NS	NS

NS indicates not significant.

ment a significant association between impaired immunity and indeterminate IGRA results, which parallels the performance of IGRAs in immunocompromised adults.[23–26]

IGRA performance depends on intact cellular immune responses; however, malnutrition and infection with helminths are known to alter such responses.[11,27,28] Cell-mediated immunity, specifically T-helper 1 (Th1)-type immunity, is thought to be essential for TB control.[29] The effects of malnutrition and helminth infection on cell-mediated immunity that are relevant to TB include reduced IFN-γ production and altered balance between Th1-type and Th2-type responses, respectively.[30]

In the severely malnourished host, the absolute number and function of T lymphocytes are compromised,[28] which corresponds to reduced IFN-γ production in human and experimental animal studies.[31–33] This phenomenon is problematic when applied to tests that rely on IFN-γ production. As demonstrated in our cohort, malnourished children are at greater risk of having an indeterminate QFT-IT result. A vast majority of these indeterminate results were caused by low IFN-γ production from T cells stimulated with phytohemagglutinin, the mitogen used as a positive control. This tendency to lower IFN-γ production may represent the effects of malnutrition.

Infection with *A lumbricoides* and/or *T trichiura* also was associated with indeterminate QFT-IT results in our cohort. Helminth infections are known to elicit a Th2 response from the host. In the setting of coinfection with helminths and *M tuberculosis*, the balance of the immune response may be skewed toward a Th2 response rather than a Th1 response,[34] which may lead to low IFN-γ production in response to mitogen and TB antigens and, ultimately, an indeterminate QFT-IT result.

Results of studies of humans infected with helminths and *M tuberculosis* suggest that coinfection alters the number and function of T-cell subsets.[11,34] Compared with healthy control subjects or patients with TB alone, coinfected patients were more likely to have fewer $CD3^+$, $CD4^+$, $CD8^+$, and natural killer T-cell subsets.[34] Other studies have examined the effect of *A lumbricoides* infection on immunologic responses and outcomes among a cohort of patients with leprosy, another mycobacterial disease that depends on intact Th1 responses for control.[35] In comparison to patients with leprosy alone, those who were coinfected with intestinal helminths were more likely to have severe forms of leprosy and lower levels of IFN-γ. A mechanism that potentially explains helminth infection–related alterations in T-cell responses can be found from a study[11] that compared the immune activation status and cellular responses to purified protein derivative among people with and without chronic helminth infections from TB-endemic areas. Peripheral blood mononuclear cells from people with chronic helminth infection were found to have a greater expression of cytotoxic T-lymphocyte antigen 4 (CTLA-4), a negative regulator of T-cell responses that leads to downregulation of Th1-type cytokines. These peripheral blood mononuclear cells also proliferated poorly after stimulation with purified protein derivative, which was attributed to the presence of CTLA-4. However, when correlating the magnitude of helminth burden to the size of TST induration or magnitude of IFN-γ production, we found no associations. The degree of helminth infection in our cohort was mild to moderate, which may have contributed to this lack of association. We cannot rule out the possibility that high worm burdens may have a greater influence on TST size or IFN-γ production than mild-to-moderate infections.

A negative TST result also was associated with indeterminate QFT-IT results when compared with those with positive QFT-IT results, which is consistent with our findings that the magnitude of IFN-γ production to TB antigens is directly related to TST size. Although it is plausible that many of these children with negative TST results were actually anergic, that remains unconfirmed because of the lack of a gold standard for anergy and LTBI testing. Older age was statistically associated with indeterminate QFT-IT results. However, we doubt the biological significance of this finding, because the range of ages from this cohort was narrow (11.0–15.3 years). In addition, other studies have found associations between children of younger ages (generally <5 years) and indeterminate IGRA results.[9,21,36]

Indeterminate results can occur because of technical and host factors. Technical factors include improper

phlebotomy leading to insufficient blood collection and/or hemolysis, insufficient agitation of blood in the antigen-coated tubes, and improper processing.[37] It is unlikely that the indeterminate results from this cohort were caused by technical error; all laboratory procedures were verified by an independent party before commencing the study, the tubes were shaken immediately after collecting blood and again in the laboratory before processing, and all tests met quality-control standards. We were unable to obtain additional blood samples from children with indeterminate results to repeat the QFT-IT. Commonly described host factors include a relative deficiency or dysfunction of T lymphocytes, which leads to insufficient IFN-γ production. Among all 74 children with indeterminate results in this cohort, malnutrition and/or helminth infection could be found in 52 (70.3%). Of these 2 risk factors, malnutrition seemed to be more strongly associated with indeterminate QFT-IT results. Conversely, there were children in this cohort who were malnourished or infected with helminths but were still able to have definitive QFT-IT results, which suggests that other unmeasured risk factors also may contribute to the risk of having an indeterminate QFT-IT result.

Our study is not without limitations. The community-based screening design may have influenced the predictive value of the results. Participants have been followed prospectively for approximately 1 year, and none have developed TB disease; therefore, we have no gold standard to compare the true performance of the QFT-IT or TST. Because we focused on *A lumbricoides* and *T trichiura*, the most prevalent geohelminths in Dhaka, we are unable to generalize these results to all helminths that may coexist in many areas.

CONCLUSIONS

Many national guidelines, including those from the United States, Canada, the United Kingdom, Netherlands, France, Switzerland, and Japan, recommend caution when using IGRAs in children.[38] Despite suggestions to use different cutoff values for immunocompromised children as a means to improve performance, further studies that use TB disease as a surrogate for LTBI in children with immune dysfunction are needed to validate the best threshold. Until then, interpretation of these tests requires special consideration when performed on children who are malnourished or infected with helminths.

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