Evaluation of the QuantiFERON-Tuberculosis Gold Plus Assay in Children with Tuberculosis Disease or Following Household Exposure to Tuberculosis

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Abstract. Interferon-gamma release assays are increasingly used in children to establish evidence of tuberculosis (TB) infection and to assist in the diagnosis of TB disease. The QuantiFERON-TB Gold In-Tube assay is being phased out in favor of a next-generation test, the QuantiFERON-TB Gold Plus (QFT-Plus) assay. The QFT-Plus assay is designed with two antigen tubes to differentially stimulate CD4⁺ and CD8⁺ T cells. The performance of this assay has been documented extensively in adults but has not yet been evaluated in children. Here, we compare the performance of the two assays in a cohort of 46 children exposed to TB and 12 children diagnosed with TB disease in Eswatini. The tests demonstrated excellent concordance in both TB disease (100% agreement, Cohen's kappa = 1) and TB infection (96% agreement, Cohen's kappa = 0.91). Most of the children with household exposure tested negative for TB infection by both tests, indicating the ongoing need for new tests for TB infection that can be easily implemented in TB high-burden settings at minimal cost.

INTRODUCTION

The global burden of tuberculosis (TB) infection in children is estimated at 50 million.¹ To achieve the global community's End TB targets, there is an urgent need to scale up treatment of TB infection.² Current rates of isoniazid preventive therapy uptake among children living with HIV (CLHIV) are unknown, but likely inadequate, based on estimates of < 50% of adults receiving preventive therapy.³ Furthermore, treatment of infection in high-risk people, children < 5 years of age and CLHIV, following household exposure to TB, is poorly implemented.³ The most cost-effective means of contact management among children < 5 years of age is treatment of all high-risk asymptomatic household TB contacts.⁴ Nevertheless, < 15% of eligible children receive preventive treatment.³

In low-burden, highly resourced settings, interferon-gamma release assays (IGRA) are essential tools in the navigation of the TB contact management cascade.^{5,6} In high-burden resource-constrained settings, IGRAs are not considered feasible, cost-effective, or sensitive enough among high-risk contacts (children < 5 years of age and CLHIV). In Eswatini, all high-risk household contacts are presumed infected and empirically offered treatment for TB infection. With this in mind, it is important to evaluate the performance of new tools to measure TB infection, such as the QuantiFERON-TB Gold Plus (QFT-Plus) assay, to evaluate changes in performance that could impact implementation strategies.

The QFT-Plus was recently introduced to replace the QuantiFERON-TB Gold In-Tube (QFT-GIT). The QFT-Plus incorporates two antigen tubes, with peptide pools of different amino acid lengths, to differentially stimulate CD4⁺ and CD8⁺ T cells. It was designed to increase the sensitivity for detecting the immune response to TB in subpopulations such as children, people living with HIV (PLHIV), and recently infected individuals. Thus far it has demonstrated similar performance characteristics to the QFT-GIT, among adults,^{7–11} with limited

data in subpopulations and some suggestion of benefit in PLHIV, recently infected individuals, and those with severe TB disease.^{8,12–15}

Here, we evaluate the performance of the QFT-Plus compared with that of the QFT-GIT in a critical subpopulation of children with household exposure to TB disease and in those treated for TB disease.

METHODS

We conducted a comparative analysis of the QFT-Plus and QFT-GIT (QIAGEN, Germantown, MD) in children enrolled through a clinical cohort between February 2016 and July 2017. Children and adolescents aged 0–19 years were prospectively enrolled from two TB clinics in Mbabane, Eswatini, following household exposure to TB disease, with an exposure score of 5 or greater,¹⁶ or when initiating treatment for TB disease. The catchment area included children living in urban and peri-urban households in the area surrounding Mbabane. Information on age, gender, immune status, and intensity of exposure were collected.¹⁶ The exposure score includes details on living and sleeping arrangements with the index case, in addition to measures of index case infectiousness.

Whole blood samples were collected in lithium heparin tubes, and the QFT-Plus and QFT-GIT assays were performed and interpreted according to the manufacturer's protocol.¹⁷ If performed at the same time, single negative control and mitogen tubes were used for both assays. All indeterminate results were repeated with the same specimen, and if a valid result was obtained, it was accepted.

The clinical cohort was described using descriptive statistics. Test agreement was assessed using Cohen's kappa statistic. Chi-square tests were used to evaluate the performance of each assay in subpopulations of children with TB household exposure and TB disease. As there remains no reference standard for TB infection, TB exposure scores have been developed in an effort to improve the accuracy of household exposure as a surrogate measure of TB infection. Estimates of sensitivity and specificity for exposure scores were calculated compared with a definition of TB infection

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TABLE 1

Cohort characteristics of children with household TB exposure and children diagnosed with TB disease

	TB exposed	TB disease	P-value (Chi ²)
	N = 46	N = 12	
Age (years)			0.16
0–4	21 (46)	5 (42)	–
5–9	17 (37)	2 (16)	–
10–19	8 (17)	5 (42)	–
Gender			0.29
Female	27 (59)	5 (42)	–
HIV			< 0.0001
+	1 (2)	5 (42)	–
–	35 (76)	7 (58)	–
Unknown	10 (22)	0	–
Exposure score			–
5–6	14 (30)	–	–
7–8	23 (40)	–	–
9–10	9 (20)	–	–
Result: QFT-Gold			0.005
Indeterminate	2 (4)	4 (33)	–
Positive	15 (33)	5 (42)	–
Negative	29 (63)	3 (25)	–
Result: QFT-Plus			0.001
Indeterminate	1 (2)	4 (33)	–
Positive	15 (33)	5 (42)	–
Negative	30 (65)	3 (25)	–

QFT = QuantiFERON-TB; TB = tuberculosis.

defined by the result of the QFT-Plus test (Stata 14.0, College Station, TX, and Prism 7, San Diego, CA). Of note, the QFT tests included in this analysis were performed at a single time point and did not consider subsequent conversion.

The Baylor-Swaziland Children's Foundation, Baylor College of Medicine Institutional Review Board, and the Swaziland Scientific and Ethics Council approved this study. All participants provided written informed consent in compliance with the guidelines for the Protection of Human Subjects and the Declaration of Helsinki.

RESULTS

Participant characteristics are described in Table 1. The age distribution was similar in both groups, but children with TB disease (5/12, 42%) were more likely than exposed

children (1/46, 2%) to be HIV positive. The median CD4 count in CLHIV with TB disease was 79 cells/mm³ (95% confidence interval [CI]: 13–233 cells/mm³). Children with TB disease were more likely to have an indeterminate test result with both assays (4/12, 33% versus 2/46, 4% for QFT-Gold and 4/12, 33% versus 1/46, 2% for QFT-Plus). Among children with TB disease, 3/5 CLHIV had indeterminate results compared with 1/7 without HIV.

In 44/46 children with household TB exposure, agreement between the QFT-GIT and QFT-Plus was 96% (Cohen's kappa of 0.91, 95% CI: 0.78–1.00). Test agreement was 100% in children with TB disease. There was one participant with TB exposure who had discordant results in T1 and T2 tubes (T2 was negative) in the QFT-Plus assay among all participants.

Subgroup analysis did not reveal significant differences in test performance (Tables 2 and 3). In exposed children, all indeterminate results occurred in children aged 0–4 years with both tests. A higher percentage of children with confirmed TB disease had a positive QFT-GIT or Plus (4/5, 80%) when compared with children with clinically diagnosed TB (1/7, 14%). Among children with household exposure, only 33% had positive QFT-GIT and QFT-Plus results; this was lower among children < 5 years of age or those living with HIV (18%).

This cohort consisted of children with significant TB exposure, defined as an exposure score of at least 5 out of 10 points.¹⁶ A score of 7 or more increased the likelihood of a positive QFT-Plus (odds ratio: 4.1, 95% CI: 0.78–21.5), but the QFT-Plus failed to identify two children with a score of less than 7 (sensitivity of a contact score of 7 or higher versus QFT-Plus = 86%, specificity = 38%). Both QFT-Plus and GIT may have underestimated TB infection in this cohort because 78% (36/46) of children were tested within 12 weeks of index case treatment initiation.

DISCUSSION

Achievement of END TB targets is dependent on the treatment of TB infection, yet few eligible individuals complete treatment for TB infection. Children and CLHIV are at risk for developing TB disease following exposure. Household exposure is often used as a surrogate measure for TB infection in high-burden settings because IGRAs are not feasible to

TABLE 2

Comparison of QFT-Gold In-Tube and Plus performance among subgroups of children exposed to tuberculosis (N = 46)

	QFT-Gold result			Chi ² (P)	QFT-Plus result			Chi ² (P-value)
	Ind	Negative	Positive		Ind	Negative	Positive	
	N (%)	N (%)	N (%)		N (%)	N (%)	N (%)	
Age (years)								
0–4	2 (100)	16 (55)	3 (20)	0.11	1 (100)	16 (53)	4 (27)	0.37
5–9	0	9 (31)	8 (53)		0	10 (33)	7 (46)	
10–19	0	4 (14)	4 (27)		0	4 (14)	4 (27)	
Gender				0.30				0.39
Female	2 (100)	18 (62)	7 (47)		1 (100)	19 (63)	7 (47)	
Male	0	11 (38)	8 (53)		0	11 (37)	8 (57)	
HIV				0.8				0.9
+	0	1 (3)	0		0	1 (3)	0	
–	2 (100)	22 (76)	11 (73)		1 (100)	22 (73)	12 (80)	
Unknown	0	6 (21)	4 (27)		0	7 (23)	3 (20)	
Contact score				0.01				0.23
5–6	2 (100)	11 (38)	1 (7)		1 (100)	11 (37)	2 (13)	
7–8	0	11 (38)	12 (80)		0	12 (40)	11 (74)	
9–10	0	7 (24)	2 (13)		0	7 (23)	2 (13)	

QFT = QuantiFERON-TB.

TABLE 3
 QuantiFERON-TB Gold In-Tube and Plus performance among subgroups of children with tuberculosis disease ($N = 12$)

	QFT-Gold result			Chi ² (P-value)	QFT-Plus result			Chi ² (P-value)
	Ind N (%)	Negative N (%)	Positive N (%)		Ind N (%)	Negative N (%)	Positive N (%)	
Age (years)								
0–4	1 (25)	2 (67)	2 (40)	0.24	1 (25)	2 (67)	2 (40)	0.24
5–9	2 (50)	0	0	–	2 (50)	0	0	–
10–19	1 (25)	1 (33)	3 (60)	–	1 (25)	1 (33)	3 (60)	–
Gender				0.06				0.06
Female	1 (25)	3 (100)	1 (20)		1 (25)	3 (100)	1 (20)	
Male	3 (75)	0	4 (80)		3 (75)	0	4 (80)	
HIV				0.2				0.2
+	3 (75)	1 (33)	1 (20)		3 (75)	1 (33)	1 (20)	
–	1 (25)	2 (67)	4 (80)		1 (25)	2 (67)	4 (80)	
Unknown	0	0	0		0	0	0	
Disease type								
Confirmed	1 (25)	0	4 (80)	0.06	1 (25)	0	4 (80)	0.06
Clinical	3 (75)	3 (100)	1 (20)	–	3 (75)	3 (100)	1 (20)	–

QFT = QuantiFERON-TB.

implement or sufficiently sensitive in high-risk populations. Hence, tests with better performance in these subpopulations are needed. Here we explored the performance of the new QFT-Plus assay in children with TB exposure and disease in a high-burden TB setting. QuantiFERON-TB Gold In-Tube and QFT-Plus performed comparably in children with TB exposure and those treated for TB disease. We found no evidence of differential performance between the two assays in this cohort. This is consistent with prior studies in adults that have largely demonstrated similar performance characteristics. Although the QFT-Plus has shown some hints of superior sensitivity among patients with recent TB infection, who are HIV infected or with severe TB disease,^{8,12,15} the findings have been subtle and we are most likely underpowered to identify such differences. Although the use of QFT-Plus requires an additional 1–1.5 mL of blood, this was not an obstacle in our study setting, which routinely provides care for a high number of CLHIV and offers in-house phlebotomy.

It is worth noting the low rates of positive QFT results among this population of household contacts and children treated for TB disease. In high-burden TB settings, treatment of high-risk household contacts without testing is the most cost-effective approach.⁴ This approach reduces barriers to preventive therapy initiation and eliminates the risk of a false-negative test in contacts at high risk for developing TB disease. The rate of TB infection following exposure may be underestimated in this cohort because of a failure to capture subsequent QFT conversion. However, similar to a recent systematic review of household contacts performed by the World Health Organization, a minority of children < 5 years of age had immunologic evidence of TB infection,¹⁸ suggesting there is still a need for new tools to assess TB infection among this population.

Among all children with TB disease, only 42% tested positive, compared with 80% among children with confirmed disease, likely attributable to the imperfect sensitivity of the QFT test and the challenges of accurately diagnosing TB disease, particularly among CLHIV. Children with severe TB disease are also at risk for an anergic response; however, one would expect this to more heavily impact children with confirmed disease. Similar to that in low-burden TB settings, tests of TB infection in high-burden settings could support the

clinical diagnosis of TB in critical populations of children, if they are cost-effective to implement.

Overall, although limited by the small sample size and the lack of a true gold standard measurement of TB infection, this study demonstrates that QFT-GIT and QFT-Plus perform comparably in children with TB exposure and TB disease. Further studies with larger sample sizes are needed to better understand the performance of the QFT-Plus in children. These data also underscore the need for improved diagnostics for TB infection that can be implemented effectively in high-burden settings and potentially enhance the critical delivery of preventive treatment.

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