Diagnostic Accuracy of QuantiFERON-TB Gold Plus Assays in Children and Adolescents with Tuberculosis Disease

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In 2016, a new interferon-gamma release assay, QuantiFERON-TB Gold Plus, was introduced. We conducted a cross-sectional multicenter study, involving 158 children and adolescents with tuberculosis disease. The overall sensitivity of the assay was 82.9% (IQR 77.0%-88.8%), indicating that in children this test does not have higher sensitivity than previous generation interferon-gamma release assays. *(J Pediatr 2020;223:212-5).*

In 2017, an estimated 1 million children developed tuberculosis (TB) globally.[1] Microbiologic confirmation of TB in children is challenging because of its paucibacillary nature and the difficulties in obtaining sputum samples. Therefore, the diagnosis is often based on epidemiologic risk factors, suggestive clinical and radiologic findings, and positive immunodiagnostic tests (ie, tuberculin skin test [TST] and/or interferon-gamma release assay [IGRA]).

IGRAs are functional immunoassays that rely on the detection of interferon-gamma produced by T lymphocytes following stimulation with TB-specific peptides.[2] Their sensitivity is reduced in immunocompromised individuals and young children, who are at greater risk of progression from latent TB infection (LTBI) to TB disease, and severe and disseminated forms of disease.[2] In resource-rich settings, IGRAs are widely used for LTBI screening and as adjunctive tools in the diagnostic work-up of suspected TB disease.[3] Previous data show that in Europe QuantiFERON-TB assays (Cellestis/Qiagen, Carnegie, Australia) are used more widely than T-SPOT.*TB* assays (Oxford Immunotec, Oxford, United Kingdom) in routine clinical practice.[4]

In 2016, the QuantiFERON-TB Gold in-Tube (QFT-GIT) assay was phased out and the QuantiFERON-TB Gold Plus (QFT-Plus) assay was launched (Cellestis/Qiagen). Compared with the QFT-GIT, the QFT-Plus includes the original antigen tube (TB1, coated with ESAT-6 and CFP-10, but lacking TB7.7), and an additional antigen tube, TB2, that contains short peptides from CFP-10 designed to detect interferon-gamma responses generated by both CD4+ and CD8+ T lymphocytes. Data about the performance of QFT-Plus assays in children and adolescents remain limited.[5,6] This study aimed to determine the sensitivity of QFT-Plus assays in children and adolescents with TB disease in a low TB-burden setting.

BCG	Bacillus Calmette-Guérin
IGRA	Interferon-gamma release assay
LTBI	Latent TB infection
QFT-GIT	QuantiFERON-TB Gold in-Tube
QFT-Plus	QuantiFERON-TB Gold Plus
pTBred	Spanish Pediatric TB Research Network
TST	Tuberculin skin test
TB	Tuberculosis

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Supported by "Subvencions per a la Intensificació de Facultatius Especialistes" (Departament de Salut de la Generalitat de Catalunya, Programa PERIS 2016-2020) (SLT008/18/00193 [to A.N-J.]). M.T. has received QuantiFERON assays at reduced pricing or free of charge for TB diagnostics projects from the manufacturer (Cellestis/Qiagen) in the past, and has received support for conference attendance from Cepheid. The manufacturers had no influence on the study design, data collection, analysis or interpretation, writing of the manuscript or decision to submit the data for publication. The other authors declare no conflicts of interest.

https://doi.org/10.1016/j.jpeds.2020.02.025

Methods

We performed a cross-sectional study within the Spanish Pediatric TB Research Network (pTBred),[7] which includes 83 participating centers. Patients <18 years of age diagnosed with TB are eligible for inclusion in the pTBred database. Data are collected using Research Electronic Data Capture electronic data capture tools, hosted at Instituto de Investigación Sanitaria Gregorio Marañón. Approval for pTBred was obtained from the Hospital Carlos III Madrid Ethics Committee (ref.P13/12). Informed consent was obtained from parents/guardians at inclusion. By June 2019, 741 patients with TB disease had been included in the pTBred database.

For this study, patients included from September 2016 to June 2019 in whom QFT-Plus tests had been performed at initial diagnosis were eligible. All QFT-Plus assays were performed in fully accredited diagnostic laboratories at each participating institution, and their results interpreted according to manufacturer's instructions (www.quantiferon.com/wp-content/uploads/2017/04/English_QFTPlus_ELISA_R04_022016.pdf). In brief, QFT-Plus results were classified as positive (ie, TB1-nil and/or TB2-nil ≥0.35 IU/mL), negative or indeterminate; where available, quantitative background-corrected antigen responses were also collected.

In pTBred, the diagnosis of TB disease is based on epidemiologic, clinical, radiologic, and microbiologic findings according to consensus criteria published elsewhere.[8] Cases are classified as microbiologically confirmed (ie, by culture or molecular tests) or probable TB, and disease severity is categorized as per established criteria.[9] TSTs were performed by intradermal injection of 2 tuberculin-units of purified protein derivative (PPD RT23; Statens Serum Institut, Copenhagen, Denmark), with results read after 48-72 hours. The cut-offs for a positive TST result were based on national guidelines: ≥5 mm induration in children assessed for clinically or radiologically suspected TB and children with TB contact; ≥10 mm in children undergoing new-entrant screening. Other variables collected were age, sex, bacillus Calmette-Guérin (BCG) vaccination history, comorbidities, hemoglobin levels, lymphocyte counts, C-reactive protein, and erythrocyte sedimentation rate at presentation.

Data are reported as proportions with 95% CIs or medians with IQR. Sensitivity was calculated based on the proportion of positive QFT-Plus results; indeterminate results were considered negative for this analysis. Total percentage agreement and Cohen kappa coefficient (κ) were used to quantify concordance between TST and QFT-Plus results; indeterminate QFT-Plus results were excluded from this analysis. Statistical analyses were performed using SPSS v 24 (IBM, Armond, New York), with statistical significance defined as a P value of <.05.

Results

During the study period, 168 children diagnosed with TB disease were included in the pTBred database; 158 had QFT-Plus results: 131 were positive (82.9%), 25 negative (15.8%), and 2 indeterminate (1.3%, both because of insufficient mitogen responses) (**Table I**). The median age was 5.3 (IQR 2.4-11.6) years. Most patients had been born in Spain (73.4%) and were BCG-unvaccinated (73.4%). Nine (5.7%) had significant comorbidities: Down syndrome (n = 3), malnutrition (n = 2) and acute lymphoblastic leukemia, Crohn's disease, STAT-1 deficiency, and auto-immune thrombocytopenia (n = 1, each). The main reasons for assessment were clinically or radiologically suspected TB (53.8%) and contact tracing (40.5%). Seventy-eight (49.4%) children were tested for HIV-infection; all had negative results. Most patients (75.3%) had isolated intra-thoracic disease (**Table II**). Almost one-half (46.8%) were microbiologically confirmed.

The overall sensitivity of QFT-Plus was 82.9% (77.0%-88.8%). The assay sensitivity was ≥75% in all subgroups analyzed (**Table I**), except for the subgroup of patients with negative TST results (44.0% [23.1%-65.9%]). QFT-Plus sensitivity was not significantly affected by sex, BCG vaccination, reason for TB screening, TB disease location and severity, or microbiologic confirmation (78.4% in confirmed and 86.9% in unconfirmed cases; P = .079). However, the analyses showed that the assay had lower sensitivity in children <5 years of age than in older children (78.7% vs 86.7%), although this was not statistically significant. No significant differences were observed in hemoglobin, lymphocyte count, or inflammatory marker levels between patients with positive and those with negative QFT-Plus results (data not shown).

TSTs were performed in 146 patients, and the result was positive in 121 (82.9% [76.7%-89.1%]). In this subgroup, 11 (7.5%) patients had a TST+/QFT- and 11 (7.5%) a TST-/QFT+ discordant result constellation. Concordance between QFT-Plus and TST was moderate (84.3%, $\kappa = 0.454$), and BCG vaccination status did not have a significant impact on concordance (BCG nonvaccinated patients: 82.4%, κ = 0.448 vs BCG-vaccinated patients: 91.3%, $\kappa = 0.465$; $P = .478$). In the subgroup of BCG-vaccinated cases (n = 27), 22 (81.5%) had intrathoracic disease and 5 (18.5%) extrathoracic disease, and 16 (59.3%) of those cases were microbiologically-confirmed.

Concordance between qualitative TB1 and TB2 results was very good (96.9%, $\kappa = 0.894$). Quantitative results were available for 105 of 131 patients with positive QFT-Plus results. In 101 cases, both TB1-nil and TB2-nil were positive, and only 1 of the 2 tubes produced a positive result in 4 patients (TB1-nil positive vs TB2-nil positive, n = 2 each). Median TB1-nil and TB2-nil concentrations were similar (4.60 [2.42-7.79] and 4.90 [2.39-8.13] IU/mL respectively; $P = .451$) and correlated strongly (r = 0.923; $P < .001$). There were no significant associations between the magnitude of background-corrected antigen responses and age, sex, BCG vaccination status, reason for assessment, TST result, disease site or severity, microbiological confirmation status, or levels of hemoglobin, lymphocyte count, C-reactive protein, and erythrocyte sedimentation rate (data not shown).

Table I. QFT-Plus assay results in correlation to clinical characteristics, TST results, and microbiological results in the 158 children with TB disease included in the study population

Characteristics	Total number* n = 158	Positive QFT-Plus n = 131	Negative QFT-Plus n = 25	Indeterminate QFT-Plus n = 2	Sensitivity, % (95% CI) 82.9 (77.0-88.8)	*P* value†
Age						.389
<5 y	75 (47.0)	59 (78.7)	15 (20.0)	1 (1.3)	78.7 (69.2-88.2)	
≥5 y	83 (53.0)	72 (86.7)	10 (12.0)	1 (1.3)	86.7 (79.3-94.2)	
Sex						.769
Female	80 (50.6)	68 (85.0)	11 (13.7)	1 (1.3)	85.0 (77.0-93.0)	
Male	78 (49.4)	63 (80.8)	14 (17.9)	1 (1.3)	80.7 (71.8-89.7)	
BCG vaccination status						.220
Nonvaccinated	116 (73.4)	93 (80.1)	22 (19.0)	1 (0.9)	80.2 (72.8-87.5)	
Vaccinated	27 (17.1)	23 (85.2)	3 (11.1)	1 (3.7)	85.2 (70.9-99.5)	
Unknown	15 (9.5)	15 (100)	0 (0)	0 (0)	100 (79.6-100)	
Reason for TB assessment						.093
Contact tracing	64 (40.5)	48 (75.0)	15 (23.4)	1 (1.6)	75.0 (64.1-85.9)	
Clinically/radiologically suspected TB	85 (53.8)	75 (88.2)	10 (11.8)	0 (0)	88.2 (81.2-95.2)	
New-entrant screening	9 (5.7)	8 (88.9)	0 (0)	1 (11.1)	88.9 (63.3-100)	
Tuberculin skin test						<.001
Negative	25 (15.8)	11 (44.0)	13 (52.0)	1 (4.0)	44.0 (23.1-64.9)	
Positive	121 (76.6)	109 (90.1)	11 (9.1)	1 (0.8)	90.1 (84.7-95.5)	
Not done	12 (7.6)	11 (91.7)	1 (8.3)	0 (0)	91.7 (73.3-100)	
Disease site[9]						.185
Only intrathoracic disease	119 (75.3)	95 (79.8)	22 (18.5)	2 (1.7)	79.8 (72.5-87.2)	
Extra- +/− intrathoracic disease	39 (24.7)	36 (92.3)	3 (7.7)	0 (0)	92.3 (83.6-100)	
Disease severity[9]						.231
Nonsevere	102 (64.5)	81 (79.4)	19 (18.6)	2 (2.0)	79.4 (70.6-86.1)	
Severe	51 (32.3)	45 (88.2)	6 (11.8)	0 (0)	88.2 (76.6-94.5)	
Unknown	5 (3.2)	5 (100)	0 (0)	0 (0)	100 (56.6-100)	
Microbiological confirmation						.079
No	84 (53.2)	73 (86.9)	9 (10.7)	2 (2.4)	86.9 (79.5-94.3)	
Yes	74 (46.8)	58 (78.4)	16 (21.6)	0 (0)	78.4 (68.8-88.0)	

All data are expressed as numbers and percentages, except where stated otherwise.
*Percentage in this column refers to proportion of patients in each subgroup.
†*P* values based on χ^2.tests comparing data from 2 or more subgroups.

Discussion

This large study on the performance of QFT-Plus assays in children and adolescents with TB disease showed sensitivity of QFT-Plus assay of 82.9%, highlighting that approximately 1 in 5 children with TB have a false-negative test result when this assay is used as an adjunctive test. Importantly, this shows that in the context of TB disease, the new generation assay does not perform better than previous generation QFT assays, which had a pooled sensitivity of 83% in a well-designed meta-analysis that included data from 31 pediatric studies.[2] Our results are in accordance with head-to-

Table II. Comparison of QFT-Plus assay performance according to disease site, severity of disease (classified according to criteria proposed by Wiseman et al[9]) and microbiological confirmation status in the 158 children with TB disease included in the study population

Site of disease		n (%)*	Severe disease†	Microbiological confirmation†	Positive QFT-Plus result†
Intra-thoracic	Ghon focus	17 (14.3)	6 (35.3)	3 (17.6)	13 (76.5)
	Ghon complex	77 (64.7)	18 (23.4)	37 (48.1)	58 (75.3)
	Adult type disease	4 (3.4)	3 (75.0)	2 (50.0)	4 (100)
	Pleural disease	5 (4.2)	0 (0.0)	2 (40.0)	4 (80.0)
	Pleural and parenchymal disease	10 (8.4)	2 (20.0)	6 (60.0)	10 (100)
	Cardiac disease	1 (0.8)	1 (100)	1 (100)	1 (100)
	Not specified	5 (4.2)	Not specified	2 (40)	5 (100)
	Total	119/158 (75.3)	30 (25.2)	52 (43.7)	95 (79.8)
Extrathoracic	Peripheral lymphadenitis	20 (51.3)	2 (10.0)	12 (60.0)	18 (90.0)
	Abdominal disease	7 (17.9)	7 (100)	3 (42.9)	6 (85.7)
	Bone and joint disease	4 (10.3)	4 (100)	1 (25.0)	4 (100)
	Brain disease	7 (17.9)	7 (100)	4 (57.1)	7 (100)
	Bone & joint and brain disease	1 (2.6)	1 (100)	1 (100)	1 (100)
	Total	39/158 (24.7)	21 (53.9)	21 (53.9)	36 (92.3)

All data are expressed as numbers and percentages.
*Percentages in this column refer to subgroups according to intrathoracic and extrathoracic disease.
†Percentages in these columns refer to subgroups according to site of disease.

head studies in adults, which also have not observed improved sensitivity.[10,11] Our data also show that the second, newly added TB2 tube only identifies a very small proportion of additional cases that do not show positive responses in the TB1 tube (2 out of 158 patients; 1.3%).

Two studies investigated the performance of QFT-Plus assays in children.[5,6] One retrospective study from Vietnam, which only included 33 patients with confirmed TB disease, reported the sensitivity of the assay was only 54% in this subgroup.[5] In a study from Eswatini, which compared QFT-Plus and QFT-GIT assays and only included 12 children with TB disease, the sensitivity of both assays was only 42%, although, importantly, several patients had HIV-infection with low CD4+ lymphocyte counts.[6]

In our cohort, the concordance between TST and QFT-Plus results was only moderate, and the sensitivity of the QFT-Plus was lowest in patients with a negative TST result. Importantly, the sensitivities of the QFT-Plus assay and TST were identical, and equal proportions had TST+/QFT- or TST-/QFT+ result discordance, combined observed in 14% of the study population. This observation lends further weight to the common pediatric practice of performing both a TST and an IGRA in parallel in patients with suspected TB disease.[3]

We did not identify any definitive factors impacting significantly on the performance of QFT-Plus assays. Unexpectedly, assay sensitivity was higher in patients with unconfirmed disease (vs those with microbiologically confirmed disease) and also in cases with extra-thoracic disease (vs those with intrathoracic disease). Our data also indicate that the assays may have lower sensitivity in children <5 years of age than in older children (78.7% vs 86.7%). However, none of the comparisons between subgroups were statistically significant, potentially because of the comparatively small size of those subgroups. Future studies should specifically investigate whether age impacts on the performance of QFT-Plus assays, as some previous studies, including our own, have shown that QFT-GIT performance is impaired in young children.[12]

Our study has some limitations. In Spain, malnutrition and HIV-infection are rare and our results, therefore, may not be applicable to high TB-burden settings where these conditions are common. Furthermore, microbiologic confirmation was only obtained in approximately one-half of the cases, reflecting the difficulties in achieving confirmation in children. Because only patients with TB disease were included, we were unable to determine the specificity of the QFT-Plus assay in uninfected children or performance in patients with LTBI. Finally, we did not include other IGRAs in the study, precluding direct comparisons with those assays.

Our results suggest QFT-Plus assays do not perform better than the previous generation QFT-GIT assays as an adjunctive test for the diagnosis of TB in children and adolescents in a low TB-burden setting. Considering that approximately 1 in 5 patients had a false-negative QFT-Plus result, the assay cannot be used as a rule-out test. Further studies investigating the impact of age on assay performance in greater detail are warranted. ■

Submitted for publication Dec 16, 2019; last revision received Jan 27, 2020; accepted Feb 12, 2020.

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Data Statement

Data sharing statement available at www.jpeds.com.

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