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MYCOBACTERIOLOGY AND AEROBIC ACTINOMYCETES

QuantiFERON-TB Performs Better in Children, Including Infants, than in Adults with Active Tuberculosis: a Multicenter Study

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ABSTRACT Immunological tests, including the QuantiFERON-TB Gold In-Tube (QFT-IT) assay, represent an important aid for diagnosing active tuberculosis (TB) and latent TB infections in children, but concerns about their use in children <5 years of age persist. This is a multicenter retrospective study comparing a population of 226 children to 521 adults with pulmonary or extrapulmonary TB. The aim was to evaluate the QFT-IT performance, analyzing both qualitative and quantitative results, according to age, birthplace, and disease localization. Compared to culture, QFT-IT sensitivity was 93.9%, 100%, and 94.4% in children ≤2, 2 to 5, and 5 to 16 years of age, respectively, and was significantly higher than that in adults (81.0%) ($P < 0.0001$). The rate of indeterminate test results for children (2.2%) was significantly lower than that for adults (5.2%) ($P < 0.0001$). In children, QFT-IT sensitivity was not affected by disease localization or birthplace (Italy born versus foreign born). Interferon gamma (IFN-γ) values in response to TB antigen and mitogen were significantly higher in children than in adults (TB antigen, median of 10 versus 1.66 IU IFN-γ/ml; mitogen, median of 10 versus 6.70 IU IFN-γ/ml; $P < 0.0001$). In summary, this study supports the use of QFT-IT as a complementary test for the diagnosis of pediatric TB even under 2 years of age. Our observations could be applicable to the new version of the test, QuantiFERON-TB Gold Plus, which has recently been shown to have similar sensitivity in active TB, although data in children are still lacking.

KEYWORDS interferon gamma values, QuantiFERON-TB Gold In-Tube, active disease, adults, age, children, tuberculosis

According to the latest World Health Organization (WHO) report, pediatric tuberculosis (TB) accounted for 1 million new cases in 2017, 10% of the total cases estimated worldwide (1). In 2016, 158 TB cases were ascribed to children in Italy (3.9% of all notified cases), with a notification rate in line with those of other countries in the European region (2). However, these data might be an underestimate, as pediatric TB diagnosis is hampered by several factors, including nonspecific clinical presentation, difficulties isolating *Mycobacterium tuberculosis* complex, less cavitation and fewer bacilli, and more frequent extrapulmonary localizations, compared to adults (3, 4).

Current guidelines regarding the management of pediatric TB recommend using clinical, radiological, microbiological, and immunological approaches to improve diag-

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nostic outcomes (5, 6). In order to increase sensitivity, microbiological diagnosis must be performed using a multisampling approach, consisting of serial (or consecutive) collection of biological samples from the respiratory tract, such as gastric aspirates, nasopharyngeal aspirates, or sputum samples (for older children or adolescents), or samples from extrapulmonary sites, such as lymph node aspirates, biopsy specimens, cerebrospinal fluid samples, or urine samples, depending on TB localization (7, 8).

The Xpert MTB/RIF system (Cepheid, Sunnyvale, CA, USA), a fully automated real-time PCR system that simultaneously detects the *M. tuberculosis* genome and mutations conferring rifampin resistance, has recently been endorsed by the WHO as the most sensitive rapid test for TB diagnosis with paucibacillary respiratory samples (9). The Xpert MTB/RIF system performed well with samples from pediatric patients, with a sensitivity of 86.9% and a specificity of 99.7% (10, 11).

The tuberculin skin test (TST), an indirect immunological test, has several limitations, such as low sensitivity and cross-reactivity with nontuberculous mycobacteria and BCG vaccine (12, 13). Interferon gamma (IFN-γ) release assays (IGRAs), such as the QuantiFERON-TB Gold In-Tube (QFT-IT) assay, are more specific than the TST, correlate better with TB exposure, and represent a more sensitive method of detecting active TB, at least in settings in which the prevalence of the disease is low (14–17). Although IGRAs have become useful additional tests for the diagnosis of active TB in adults, some uncertainties persist about their use in children, particularly those $<$5 years of age (18). Increasing amounts of data analyzing IGRA performance in this vulnerable population suggest that IGRAs are more accurate than the TST as a supporting tool for the diagnosis of TB in children, due to better specificity and the presence of a control for immunoreactivity (19–22).

The aim of this study was to analyze the performance of the QFT-IT assay for children in three different age groups, in comparison with adults with active TB. We analyzed both qualitative and quantitative IFN-γ responses to TB antigen and mitogen according to age, disease localization, and birthplace.

MATERIALS AND METHODS

Study design and population. This was a retrospective multicenter study performed in four Italian hospitals (Sant'Orsola-Malpighi University Hospital in Bologna, University Hospital in Modena, Regina Margherita University Children's Hospital in Turin, and University Hospital in Padua). QFT-IT results for 747 consecutive patients (226 children and 521 adults) with TB disease over 9 years were analyzed. The inclusion criteria included QFT-IT testing performed no more than 15 days before or after the start of TB treatment. QFT-IT testing was a standard of care in these centers. Clinical and demographic information, including birthplace, were collected from medical files. The study was approved by the ethics committee of the lead institution of this study, Sant'Orsola-Malpighi University Hospital (protocol no. 275/2017/OssN).

Diagnosis of TB disease. The diagnosis of TB disease was made by the physicians according to WHO criteria (23). TB cases included bacteriologically confirmed cases, based on culture and/or Xpert MTB/RIF positivity, and clinically confirmed cases, based on clinical, pathological, and radiological findings consistent with the disease and no improvement after a full course of antibiotics, followed by clinical improvement with anti-TB treatment. All patients with a diagnosis of active TB were monitored until recovery.

QFT-IT assay. The QFT-IT assay (Qiagen, Hilden, Germany) was performed and interpreted according to the manufacturer's instructions (24). Venous blood was collected in three distinct tubes, i.e., one containing *M. tuberculosis*-specific antigens (ESAT-6, CFP10, and TB7.7), one containing a nonspecific mitogen (phytohemagglutinin) as the positive control, and an empty tube (nil) as the negative control. The tubes were incubated at 37°C for 18 h and, after centrifugation, the IFN-γ release was measured with an enzyme-linked immunosorbent assay (ELISA) and converted to international units (IU) per milliliter using a standard curve elaborated by QFT-IT Analysis software. Tubes that were not analyzed immediately were stored at 4°C. Positive results were defined as nil-corrected antigen values of $\geq$0.35 IU IFN-γ/ml. If the nil-corrected mitogen value was $<$0.50 IU/ml and/or if the nil value was $>$8.0 IU/ml, then the test was considered indeterminate.

Statistical analysis. The quantitative analysis of QFT-IT results was carried out with background (nil)-corrected IFN-γ responses to TB antigen and mitogen. Since the QFT-IT test cannot accurately determine IFN-γ values of $>$10 IU/ml, a value of 10 IU/ml was conventionally attributed to plateau values in all analyses, as already adopted in the literature (25).

Percentages were used to describe categorical variables, while medians were calculated for continuous variables. Comparisons of QFT-IT results according to age group, birthplace, and disease localization were performed with χ^2 tests, the Mann-Whitney test was used to compare medians, and Fisher's exact test was used to compare sensitivities. Correlations between age and IFN-γ levels in response to TB

TABLE 1 QFT-IT qualitative results and sensitivity in adults and children with bacteriologically confirmed TB

Patient group	No. of patients	No. (%)			Sensitivity (95% CI) (%)
		Positive result	Negative result	Indeterminate result	
Adults	521	400 (76.8)	94 (18.0)	27 (5.2)	81.0 (77.2–84.3)
PTB	324	254 (78.4)	53 (16.4)	17 (5.2)	82.7 (77.9–86.7)
EPTB	197	146 (74.1)	41 (20.8)	10 (5.1)	78.1 (71.3–83.6)
Children	127	120 (94.5)	3 (2.4)	4 (3.1)	97.6 (92.5–99.4)
≤2 yr	33	31 (93.9)	2 (6.1)	0 (0)	93.9 (78.4–98.9)
2.1–5 yr	27	26 (96.3)	0 (0)	1 (3.7)	100 (84.0–100)
5.1–16 yr	67	63 (94.0)	1 (1.5)	3 (4.5)	94.4 (90.5–99.9)
PTB	98	96 (98.0)	2 (2.0)	0	98.0 (92.1–99.6)
EPTB	29	24 (82.8)	3 (10.3)	2 (6.9)	88.9 (69.7–97.1)

antigen and mitogen were expressed by Pearson's coefficient. Statistical analysis was performed using GraphPad Prism v8.0.1 (GraphPad, San Diego, CA, USA). Statistical significance was set at *P* values of <0.05.

RESULTS

Qualitative QFT-IT results for adults and children with active TB. A total of 747 patients with TB disease were enrolled in this study, including 521 adults (>16 years of age) and 226 children (≤16 years of age). All 521 adult cases (100%) and many pediatric TB cases (127 cases [56.2%]) were bacteriologically confirmed; for 99 children (43.8% of pediatric cases), the diagnosis was based on clinical and radiological findings.

QFT-IT results were positive for 215 (95.2%) of 226 children with TB, compared to 76.8% of adults ($P < 0.0001$). The proportion of indeterminate test results was significantly smaller for children (2.2%) than for adults (5.2%; $P < 0.0001$), and such results were always due to an inadequate response to the positive control (mitogen).

A total of 214 patients (28.6%) with TB were born in Italy, and 533 (71.4%) were foreign born. Overall, foreign-born patients had a higher rate of QFT-IT positivity (85.7%) than did Italian-born patients (73.8%; $P = 0.0005$). However, the proportions of children with positive QFT-IT results were similar for Italian-born children (96.2%) and foreign-born children (94.8%). Italian children were QFT-IT positive more frequently than were Italian adults (96.2% versus 66.5%; $P = 0.0001$); similar results were obtained for foreign-born patients (94.8% versus 81.4%; $P < 0.0001$).

To further investigate the role of age in QFT-IT performance, the pediatric population was divided into three subgroups, i.e., ≤2 years of age ($n = 56$), 2.1 to 5 years of age ($n = 54$), and 5.1 to 16 years of age ($n = 116$). There were no significant differences in positive QFT-IT results (92.9%, 96.3%, and 95.7%, respectively) or indeterminate QFT-IT results (1.8%, 1.85%, and 2.6%, respectively) among the three age groups.

Considering only cases of bacteriologically confirmed TB and excluding indeterminate QFT-IT results, the sensitivity of QFT-IT testing was 97.6% in children, compared to 81.0% in adults ($P < 0.0001$) (Table 1). No significant differences in QFT-IT sensitivity were observed for children in the different age groups, i.e., 93.9%, 100%, and 94.4% for children ≤2, 2 to 5, and 5 to 16 years of age, respectively. Regarding age and disease localization, QFT-IT sensitivity was significantly higher in children than in adults for pulmonary TB (PTB) (98.0% versus 82.7%; $P = 0.0003$) but not for extrapulmonary TB (EPTB) (88.9% versus 78.1%; $P = 0.2960$) (Table 1).

Quantitative QFT-IT responses to TB antigen and mitogen for adults and children. Quantitative IFN-γ values in response to TB antigen were available for 218 children (96.5%) and 521 adults (100%), and IFN-γ values in response to mitogen were available for 134 children (58.8%) and 520 adults (99.8%). IFN-γ values in response to TB antigen and mitogen are shown in Fig. 1A and B, respectively. The median TB antigen response value for children was 10 IU IFN-γ/ml, significantly higher than that for adults (1.66 IU IFN-γ/ml; $P < 0.0001$). IFN-γ values in response to mitogen were also

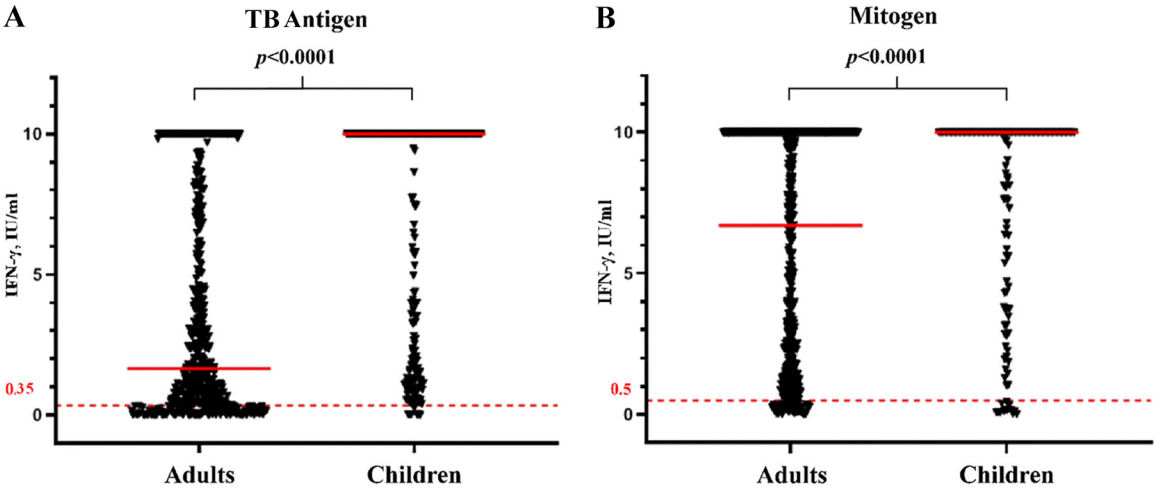

FIG 1 Quantitative QFT-IT results for TB patients according to age. Individual nil-corrected IFN-γ levels in response to TB antigen (A) and mitogen (B) in adults and children are reported. Median values are indicated as red lines. The cutoff values for positive and indeterminate QFT-IT results are represented by dotted red lines at 0.35 and 0.50 IU/ml, respectively. Significant *P* values are shown.

statistically higher for children than for adults (median of 10 versus 6.70 IU IFN-γ/ml; $P < 0.0001$). Furthermore, no significant differences ($P = 0.9386$) in IFN-γ production in response to TB antigen were observed among children ≤2 years of age (median of 10 IU IFN-γ/ml), 2.1 to 5 years of age (median of 10 IU IFN-γ/ml), and 5.1 to 16 years (median of 9.49 IU IFN-γ/ml). Similarly, no significant differences ($P = 0.5027$) in IFN-γ production in response to mitogen were observed among children ≤2 years of age (median of 10 IU IFN-γ/ml), 2.1 to 5 years of age (median of 9.7 IU IFN-γ/ml), and 5.1 to 16 years of age (median of 10 IU IFN-γ/ml).

When analyzed by birthplace, Italian-born children showed significantly greater IFN-γ production in response to TB antigen than did Italian adults (median of 10 versus 0.98 IU IFN-γ/ml), and the same was observed for foreign-born children (median of 9.41 versus 2.06 IU IFN-γ/ml; $P < 0.0001$) (Fig. 2A). With respect to the localization of

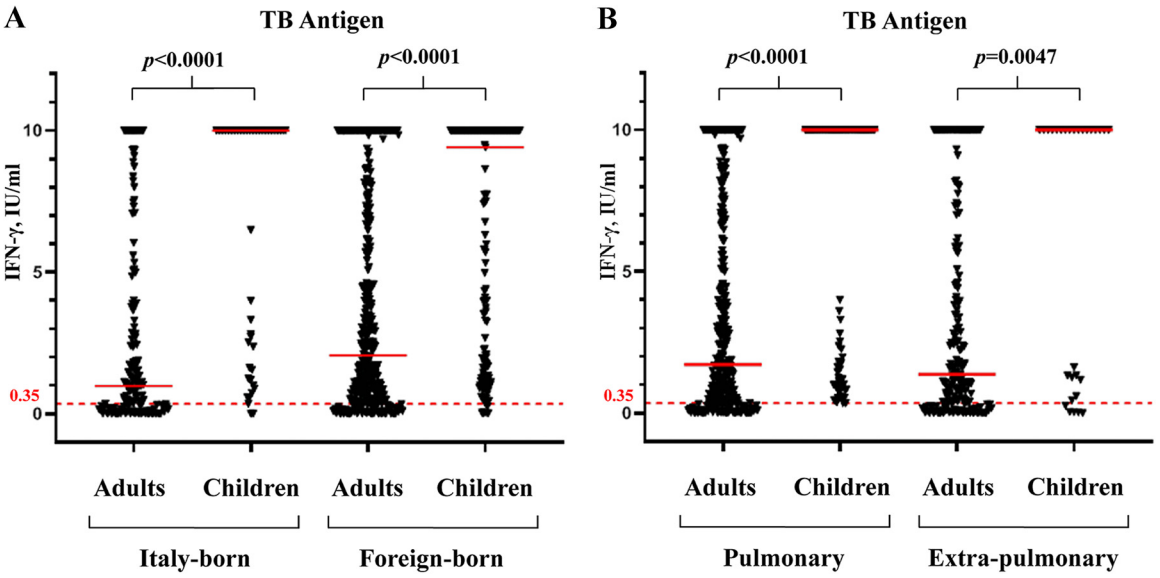

FIG 2 Quantitative QFT-IT results for adults and children according to birthplace and TB localization. Individual nil-corrected IFN-γ levels in response to TB antigen in adults and children according to birthplace (A) and TB localization (B) are reported. Median values are indicated as red lines. The cutoff value for positive QFT-IT results is represented by dotted red lines at 0.35 IU/ml. Significant *P* values are shown.

bacteriologically confirmed TB, children always had significantly greater responses to TB antigen than adults for both PTB (median of 10 versus 1.71 IU IFN-γ/ml; $P < 0.0001$) and EPTB (median of 10 versus 1.37 IU IFN-γ/ml; $P = 0.0047$) (Fig. 2B).

Linear regression analysis showed that there were significantly lower IFN-γ responses to both TB antigen (Pearson's correlation coefficient $r = -0.3111$ [95% confidence interval [CI], −0.3749 to −0.2444]; $P < 0.0001$) and mitogen (Pearson's $r = -0.2086$ [95% CI, −0.2807 to −0.1340]; $P < 0.0001$) with increasing age. In contrast, when the pediatric population was analyzed, age did not influence IFN-γ responses to either TB antigen (Pearson's $r = -0.0602$ [95% CI, −0.1915 to 0.07330]; $P = 0.3767$) or mitogen (Pearson's $r = 0.0746$ [95% CI, −0.09621 to 0.2411]; $P = 0.3917$) (see Fig. S1 in the supplemental material).

DISCUSSION

In this multicenter study, we evaluated qualitative and quantitative QFT-IT results for 226 children and 521 adults with TB disease. We showed that the overall QFT-IT sensitivity in children with bacteriologically confirmed TB was significantly higher than that in adults (97.6% versus 81.0%), regardless of disease localization. Our results are in agreement with the systematic review and meta-analysis conducted by Laurenti et al., in which the QFT-IT performance in immunocompetent children with TB was 89.6% (95% CI, 79.7 to 95.7%) (26).

The rate of positive QFT-IT results for native adults with TB (66.5%) was lower than that for foreign-born patients with TB (81.4%), and this could be explained by the older age of Italian-born TB patients, compared to foreign-born patients. In contrast, Italian-born children with TB showed a rate of positive QFT-IT results similar to that for foreign-born children (96.2% and 94.8% respectively), indicating that TB burden did not influence QFT-IT results in children.

In our study, the test performed well for children with TB regardless of age; the high QFT-IT sensitivity (93.9%) in children ≤2 years of age is in agreement with data obtained in a previous study by the Italian Society of Pediatric Infectious Diseases with 105 children with TB in the first 2 years of age (27). In the quantitative analysis, IFN-γ values in response to TB antigen showed a significantly greater response in children than in adults with TB (median of 10 versus 1.66 IU IFN-γ/ml), regardless of the children's country of origin or disease localization. Furthermore, regression analysis demonstrated a significant reduction in TB antigen-stimulated IFN-γ responses with increasing age, whereas specific responses to TB antigen in children were age independent.

Previous reports described widely variable rates of indeterminate QFT-IT results in children; some authors showed a clear association with young age (28–30), and others suggested that host immune status, comorbidities, and preanalytical processing of blood specimens might influence indeterminate QFT-IT results (31). In our previous study, we reported an overall low rate of indeterminate results (3.9%) in a large cohort of children tested with the QFT-IT assay for different reasons (32); indeterminate results were mostly distributed among children with acute infections, such as pneumonia, whereas they did not occur among children with active TB. In this study, the rate of indeterminate test results for children was even lower (2.2%) and was significantly lower than that for adults (5.2%). A recent study by Kay and colleagues reported an overall rate of indeterminate IGRA results of 3.7% (12 of 322 children) and found that 4 of 5 children <2 years of age with indeterminate IGRA results had central nervous system (CNS) disease, suggesting that CNS disease may be a stronger driver of indeterminate test results than age (33). In our population, only 1 of 4 children with CNS disease had an indeterminate QFT-IT result.

Furthermore, the median IFN-γ value in response to mitogen was statistically higher in children than in adults (10 versus 6.7 IU IFN-γ/ml; $P \leq 0.0001$) and the regression analysis of IFN-γ responses to mitogen suggested that the immune system of children with TB is not impaired in its ability to mount an immune response. We reported a similar trend in a previous study with a large cohort of children with different diagno-

ses, in addition to TB, who were screened with the QFT-IT assay (34). In contrast, the regression analysis of results as a function of age in adults with TB demonstrated that there were lower IFN-γ responses to mitogen with increasing age, confirming that responses to mitogen are significantly affected by age (35–37). We could not evaluate the presence of immunosuppressive conditions other than TB disease, in both populations. However, lower IFN-γ responses in older patients were previously described by Tebruegge et al. (37), demonstrating significant decreases in mitogen-induced IFN-γ responses due to immunosenescence, independent of immunosuppressive conditions. The better performance of QFT-IT in childhood could be due to the greater proportion of lymphocytes in the blood, particularly in children $<$2 years of age, where they account for approximately 60% of leukocytes (in comparison to 30% in adults) (38).

Our study has some limitations. First, due to the retrospective design of this study, we analyzed the performance of the QuantiFERON-TB Gold In-Tube assay and not the latest version, the QuantiFERON-TB Gold Plus (QFT-Plus) assay, which was recently introduced into the market (39). However, we can speculate that our observations would be applicable to QFT-Plus, which has been shown to agree substantially with QFT-IT results and has similar sensitivity in active TB (40, 41). Additionally, quantitative IFN-γ values in response to mitogen were not available for all of the pediatric patients. Follow-up findings for patients with negative or indeterminate QFT-IT results were not recorded, and thus possible conversions were not captured. Lastly, the specificity of QFT-IT was not assessed in this study. However, we previously evaluated QFT-IT specificity, comparing children with active TB to those with symptoms and/or signs of TB but with a final diagnosis of TB exclusion, and we obtained a specificity of 99.3% (32).

In conclusion, this is the largest Italian study of QFT-IT performance in children with TB disease, analyzing both qualitative and quantitative results, compared to adult TB patients. We found higher sensitivity and fewer indeterminate results for QFT-IT in children with TB than in adults, regardless of age (even $<$2 years), patient birthplace, or disease localization. IFN-γ values in response to TB antigen and mitogen were significantly higher in children than in adults with TB, suggesting the use of IGRAs as complementary tests for the diagnosis of pediatric TB even in infants.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.01048-19.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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