A Comparison of Interferon-γ and IP-10 for the Diagnosis of Tuberculosis

WHAT'S KNOWN ON THIS SUBJECT: IP-10 is a novel immunologic marker for tuberculosis (TB) infection. It has been suggested that IP-10 may perform better in children compared with the QuantiFERON test, but only a few studies have investigated IP-10 for diagnosing active TB in children.

WHAT THIS STUDY ADDS: This study is the first to investigate IP-10 and QuantiFERON for diagnosing TB in children by using consensus classifications. Both IP-10 and QuantiFERON exhibited poor performance in children from a high-burden setting, and performance was especially compromised in young children.

abstract

OBJECTIVE: Interferon-γ and IP-10 release assays are diagnostic tests for tuberculosis infection. We have compared the accuracy of IP-10 and QuantiFERON-TB Gold In-tube [QFT-IT] in Tanzanian children suspected of having active tuberculosis (TB).

METHODS: Hospitalized Tanzanian children with symptoms of TB were tested with the QFT-IT and IP-10 tests and retrospectively classified into diagnostic groups. Adults with confirmed TB were assessed in parallel.

RESULTS: A total of 203 children were included. The median age was 3.0 years (interquartile range: 1.2–7.0), 38% were HIV infected, 36% were aged <2 years, and 58% had a low weight-for-age. IP-10 and QFT-IT test performance was comparable but sensitivity was low: 33% (1 of 3) in children with confirmed TB and 29% (8 of 28) in children with probable TB. Rates of indeterminate responders were high: 29% (59 of 203) for IP-10 and 26% (53 of 203) for QFT-IT. Age <2 years was associated with indeterminate test outcome for both IP-10 (adjusted odds ratio [aOR]: 2.2; $P = .02$) and QFT-IT (aOR: 2.4; $P = .01$). TB exposure was associated with positive IP-10 test outcome (aOR: 3.6; $P = .01$) but not with positive QFT-IT outcome (aOR 1.4; $P = .52$). In 102 adults, test sensitivity was 80% for both tests ($P = .248$).

CONCLUSIONS: Although IP-10 and QFT-IT performed well in Tanzanian adults, the tests exhibited an equally poor performance in diagnosing active TB in children. Test performance was especially compromised in young children. Neither test can be recommended for use in hospitalized children in high-burden settings. *Pediatrics* 2014;134: e1568–e1575

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KEY WORDS

children, diagnosis, interferon-γ release assay, IP-10, tuberculosis

ABBREVIATIONS

aOR—adjusted odds ratio
CI—confidence interval
CXR—chest radiograph
IFN-γ—interferon-γ
IGRA—interferon-γ release assay
PHA—phytohemagglutinin
PTB—pulmonary tuberculosis
QFT-IT—QuantiFERON-TB Gold In-tube
TB—tuberculosis

Dr Holm performed the IP-10 laboratory measurements, performed data analysis, drafted the initial manuscript, and revised the final manuscript; Dr Rose conceptualized and designed the study, coordinated and supervised data collection at the field site, created the study database and initial data analyses, and critically reviewed and revised the manuscript; Dr Kimaro performed quality assurance of the data collection tools, supervised data collection, and reviewed the final manuscript; Drs Bygbjerg and Mfinanga oversaw implementation of the study and reviewed the final manuscript; Dr Ravn conceptualized and designed the study, co-invented the IP-10 enzyme-linked immunosorbent assay used for IP-10 measurements, supervised data collection, and critically reviewed and revised the manuscript; and Dr Ruhwald co-invented the IP-10 enzyme-linked immunosorbent assay used for IP-10 measurements, supervised IP-10 laboratory analysis and data analysis, and critically reviewed and revised the manuscript. All authors approved the final manuscript as submitted.

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Tuberculosis (TB) is a major contributor to childhood morbidity and mortality, especially in high endemic countries. The World Health Organizations estimates that at least 500 000 children become ill and 70 000 die of TB disease each year.[1] Children aged <2 years are at greatest risk of disease progression, and up to 40% of infected children develop TB disease.[2]

TB diagnosis is difficult in children because of the diverse clinical presentation of the disease and the poor performance of diagnostic tests. Positive culture result or nucleic acid amplification test confirmation of TB disease is rare in high-burden countries, mainly due to the paucibacillary nature of *Mycobacterium tuberculosis*, difficulties in obtaining specimens, and limited access to diagnostic facilities.[3,4]

In 2009, interferon-γ (IFN-γ) release assays (IGRAs), such as the QuantiFERON-TB Gold in-tube (QFT-IT) (Qiagen, Düsseldorf, Germany), were approved in the United States as additional diagnostic tools in children with clinical suspicion of active TB.[5] However, IGRA sensitivity is compromised in the young and immunosuppressed,[6–8] and there is little evidence of the test's accuracy in children with active TB from high-burden settings.[9]

IP-10 release assays are emerging as alternatives to the IGRAs.[10,11] IP-10 is a 7.2-kilodalton chemokine released primarily by the antigen-presenting cells upon T-cell stimulation, and IP-10 release assays measure IP-10 responses in whole blood upon stimulation with the *M tuberculosis*–specific antigens (ESAT-6, CFP-10, and TB7.7). We and others have shown that IP-10 and IGRAs have comparable accuracy in adults infected with *M tuberculosis*.[10,12–14] Although only a few studies have assessed the accuracy of IP-10 as a marker for childhood TB, all current evidence suggests that IP-10 performs on par with QFT-IT in children or may be even less affected by age and HIV infection.[10,15–22]

The goal of the present study was to examine the accuracy of the IP-10 release assay in a large group of Tanzanian children with symptoms of active TB. We compared the accuracy of IP-10 versus IFN-γ in the QFT-IT and identified factors associated with positive and indeterminate test outcomes.

METHODS

Study Population

This trial was a substudy of a prospective study conducted at Muheza District Hospital, Tanzania, in 2008–2010.[23] The aim of the prospective study was to evaluate QFT-IT performance for diagnosing active TB in children. Children aged <15 years with signs and symptoms of active TB were included consecutively from the pediatric ward and outpatient departments. Adults with active TB were included as a measure of comparison and for validation of the laboratory set-up.

In adults, active TB was defined as positive results on sputum microscopy by using Ziehl-Neelsen staining confirmed by either positive *M tuberculosis* culture or fluorescence microscopy. Underweight was defined as BMI <18.5 for adults and weight-for-age *z* score less than or equal to −2 for children. Pulmonary tuberculosis (PTB) exposure was defined as a verbal report of close contact to a case of either confirmed or suspected PTB. Follow-up was conducted 2 and 6 months after inclusion, and children who did not return for scheduled follow-up were actively tracked in the villages within 7 to 12 months after inclusion. We have included in the present study previously published data on QFT-IT results[18] to compare IP-10 and QFT-IT performances.

Case Definitions

Children were classified in accordance with recently published consensus case definitions for intrathoracic TB.[24] Children were included in the analysis if they fulfilled ≥1 of the following signs or symptoms of TB: cough ≥14 days, fever ≥7 days, or weight-for-age *z* score less than or equal to −2 with no response to nutritional rehabilitation. Included children were divided into 5 diagnostic classification groups (Fig 1) on the basis of the following criteria: (1) microbiologic confirmation, defined as ≥1 culture of sputum/gastric wash specimen positive for *M tuberculosis*; (2) chest radiographs (CXRs) categorized as either "suggestive of TB" or "not suggestive of TB" on the basis of readings by 3 blinded experts by using standardized recording forms (children with missing CXRs were categorized as "CXR not suggestive of TB"); (3) confirmed PTB exposure within 24 months; (4) good clinical response to anti-TB treatment, defined as resolved symptoms after receiving a full course of anti-TB therapy; and (5) confirmation of an alternative diagnosis.

Because the aim of the present study was to evaluate accuracy of immunologic tests, the sixth consensus criterion[24] (ie, immunologic evidence of *M tuberculosis* infection) was excluded from the algorithm (Fig 1).

IFN-γ and IP-10 Measurements

Venous blood from the study participants was collected into the QFT-IT tubes at inclusion and incubated at 37°C for 16 to 24 hours. Nil, TB antigen, and phytohemagglutinin (PHA) tubes were centrifuged immediately after incubation, and the supernatants were stored at −70°C until IFN-γ was measured by using the QFT-IT enzyme-linked immunosorbent assay at the NIMR–Mbeya Medical Research Program Laboratory, Tanzania. Results were reported as positive, negative, or indeterminate according to manufacturer's instructions (Qiagen). Aliquots of plasma from the QFT-IT tubes were transported on dry ice to University Hospital Hvidovre (Hvidovre, Denmark) and stored at −80°C. Plasma

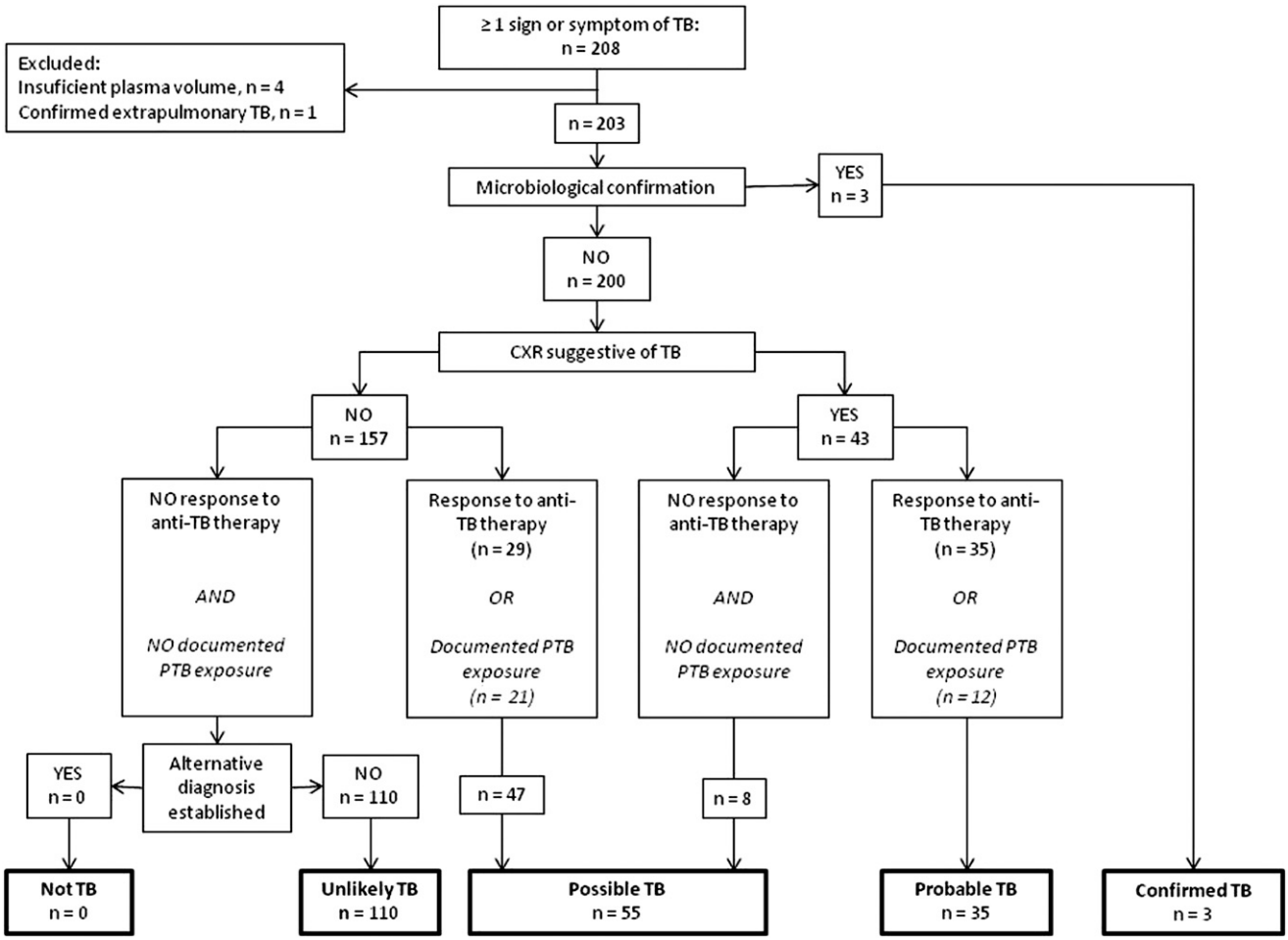

FIGURE 1
Flowchart of children's diagnostic classification using criteria modified from Graham et al.[24]

samples were then thawed and IP-10 measured in duplicates by using an enzyme-linked immunosorbent assay and analyzed according to a predefined algorithm.[25]

Statistical Analysis

Nonparametric tests (Mann-Whitney *U* test and Kruskal-Wallis test) were used to compare continuous variables. Fisher's exact test was used to compare result rates between groups. Test agreement was assessed by using κ statistics and McNemar's test for marginal homogeneity. Trends were assessed by using the Cochran-Armitage test and Spearman's rank. Logistic regression analysis was used for risk factor assessment for positive and indeterminate outcomes; odds ratios were adjusted (aOR) for gender, age, HIV infection, and low weight-for-age findings. In addition, PTB exposure was included in the analysis for positive outcome. Level of significance was set to $\leq$.05, and all tests were 2-sided. Statistical analysis was performed by using SAS Enterprise Guide version 4.3 (SAS Institute, Inc, Cary, NC) and GraphPad Prism version 5 (GraphPad Software, Inc, La Jolla, CA).

Ethical Considerations

The study protocol was approved by the Tanzanian Medical Research Coordinating Committee (NIMR/HQ/R.8a/Vol IX/584) and evaluated by the Danish Central Ethical Committee, with no objections. Written informed consent was obtained from the immediate caretaker before inclusion. Results were reported according to Standards for Reporting of Diagnostic Accuracy guidelines.[26]

RESULTS

Population Characteristics

Adults

In total, 107 adults were eligible for the study; 5 were excluded due to insufficient sample material, and 102 adults were thus available for data analysis. The adults were predominantly male subjects with a median age of 39 years (interquartile range: 28.0–52.0), 27% were HIV-positive, and 62% were underweight (BMI $<$18.5) (Table 1).

Children

A total of 211 children were included in the prospective study by Rose et al.[23] Of these, 8 children were excluded from further analysis; 3 children did not meet ≥1 of the symptoms suggestive of TB, 4 children had insufficient plasma recovery, and 1 was excluded because the child had TB peritonitis and was therefore unsuitable for intrathoracic TB classification. Nine children were included without having CXR data. Alternative diagnoses could not be verified for any of the children, rendering no children in the not TB category. Thus, 203 children were classified according to consensus guidelines as having the following: confirmed TB (n = 3), probable TB (n = 35), possible TB (n = 55), unlikely TB (n = 110), and not TB (n = 0) (Fig 1, Table 1).

The children's median age was 3.0 years (interquartile range: 1.2–7.0). Sixty percent (121 of 203) of the children were male, 36% (73 of 203) were aged <2 years, 38% (76 of 201) were HIV infected, and 58% (105 of 180) had low weight-for-age findings. Thirty-five percent (71 of 201) of the children had been exposed to TB within 2 years, and 34 of these had close contact with a confirmed PTB case. The overall 6-month mortality for children was 17% (36 of 203); one-third (11 of 36) of these deaths occurred during hospital admission. Children had comparable baseline characteristics across TB classification groups, except for a relatively higher proportion of male subjects and a lower proportion of HIV-infected children in the unlikely TB group.

IP-10 and IFN-γ Levels

We compared IP-10 and IFN-γ levels in adults and children and found that children had lower IP-10 nil levels, whereas PHA-induced IP-10 responses were comparable to adult responses (Table 2). For IFN-γ, children had both lower nil and lower PHA-induced levels compared with adults. For the children, we found no correlation between age (months) and PHA-induced IP-10 (r^2 = 0.001 [confidence interval (CI): –0.11 to 0.17], P = .659) or IFN-γ responses (r^2 = 0.006 [CI: –0.06 to 0.22], P = .253) (data not shown).

There were no significant differences in TB-antigen–induced IP-10 or IFN-γ responses across children's classification groups. However, PHA-induced IP-10 responses were lower in the unlikely TB group compared with the 3 other groups.

IP-10 and QFT-IT Accuracy in Adults

In adults, rates of positive responders were 72% (73 of 102) for the IP-10 test and 75% (77 of 102) for the QFT-IT (P = .248) (Table 3). Indeterminate rates were also comparable: 11% (11 of 102) indeterminate responders for the IP-10 test and 6% (6 of 102) for the QFT-IT (P = .096). Agreement between the 2 tests was 83% (κ = 0.62, P < .0001) (data not shown). Sensitivity (positivity rate after excluding indeterminate results) was 80% (73 of 91) for IP-10 and 80% (77 of 96) for QFT-IT. When using a combination of the 2 tests, defined as at least 1 positive result in either test, sensitivity reached 84% (81 of 97).

IP-10 and QFT-IT Accuracy in Children

We found low rates of positive responders in all classification groups. Even after excluding indeterminate results, the proportion of positive test responders was only 33% (1 of 3) in the confirmed TB group and 29% (8 of 28) of the probable TB group for both IP-10 and QFT-IT (Table 3).

For IP-10, there was a stepwise increase of positive responders across the classification groups, with the rate of positive responders being lowest in the unlikely TB group and steadily increasing toward the confirmed TB group (test for trend, P = .019). There was no such trend for positive QFT-IT responders (test for trend, P = .327).

TABLE 1 Characteristics of the Study Population

Characteristic	Confirmed	Probable TB	Possible TB	Unlikely TB	Adults
No. of children	3	35	55	110	102
Median age					
Years	10	3.5	3.5	2.5	39.0
IQR in each diagnostic group	10.0–14.0	2.2–6.0	1.6–7.0	0.9–6.7	28.0–52.0
Age, y[a]					
<2	—	7 (20)	17 (31)	49 (45)	—
2–4.9	—	15 (43)	16 (29)	21 (19)	—
5–9.9	—	10 (28)	14 (25)	24 (22)	—
>10	3 (100)	3 (9)	8 (15)	16 (15)	—
Male gender[a]	2 (67)	27 (77)	35 (64)	57 (52)	76 (75)
Positive HIV status[a]	2 (67)	18 (51)	29 (53)	27 (25)	28 (27)
Low weight-for-age (z score less than or equal to –2 SD)[a]	2 (67)	17 (52)	30 (64)	56 (58)	—
TB exposure[a]					
Confirmed	1 (33)	12 (34)	21 (38)	0 (0)	—
Suspect/confirmed	1 (33)	18 (51)	31 (56)	20 (19)	
BMI <18.5[b]	—	—	—	—	63 (62)
Follow-up status[a]					
Healthy	2 (67)	29 (83)	43 (78)	75 (68)	—
Still ill	—	3 (9)	3 (6)	12 (11)	—
Dead	—	3 (9)	9 (16)	23 (21)	—

Missing data: 2 children had no HIV status data, 23 children had no z score data, 3 children had no exposure data, and 1 child with confirmed TB had no follow-up. Group comparability: In the unlikely TB group, there was a higher proportion of male subjects compared with the probable TB group (P = .01). In the unlikely TB group, there was a lower proportion of HIV-positive subjects compared with the probable TB group (P = .006) and the possible TB group (P = .001). IQR, interquartile range.

[a] Values given as n (% of diagnostic group).

[b] Values given as n (% total).

TABLE 2 Biomarker Levels in Adults and in Children's Diagnostic Groups

Group	*N*	IP-10			QFT-IT		
		Nil	TB Antigen	PHA	Nil	TB Antigen	PHA
Adults	102	1.34* (0.86–1.96)	6.48 (1.31–10.60)	3.00*** (1.13–5.67)	0.37** (0.22–0.60)	2.36 (0.4–5.95)	2.29**** (0.77–7.93)
All children	203	0.88* (0.46–1.20)	0.30 (0.02–0.86)	3.03*** (0.89–6.55)	0.15** (0.09–0.35)	0.01 (0.00–0.11)	1.43**** (0.38–5.85)
Children							
Confirmed TB	3	0.85 (0.15–1.67)	0.37 (0.00–6.05)	4.72[a] (2.64–8.13)	0.06 (0.04–0.56)	0.01 (0.00–2.13)	5.54[b] (2.04–10.00)
Probable TB	35	0.93 (0.48–2.52)	0.43 (0.04–1.85)	4.88[a] (2.32–7.85)	0.15 (0.12–0.26)	0.03 (0.00–0.40)	2.11[b] (0.46–5.76)
Possible TB	55	0.92 (0.53–1.64)	0.31 (0.05–0.90)	4.21[a] (1.95–8.06)	0.14 (0.09–0.38)	0.01 (0.00–0.10)	1.72[b] (0.69–7.46)
Unlikely TB	110	0.80 (0.41–1.10)	0.21 (0.00–0.71)	2.17[a] (0.70–4.27)	0.16 (0.08–0.38)	0.01 (0.00–0.08)	1.29[b] (0.32–5.43)

IP-10: median nanograms per milliliter (interquartile range [IQR]). IFN-γ: median international units per milliliter (IQR). Nil levels have been subtracted from TB antigen (TB Ag) and PHA responses.

Comparison of children versus adults: Nil: *IP-10, $P = .0007$; **IFN-g, $P < .0001$; TB Ag, ***IP-10, $P = .777$; ****IFN-g, $P = .042$.

Comparison of responses across diagnostic groups (Kruskal-Wallis test): TB Ag: [a] IP-10, $P = .0004$; [b] IFN-g, $P = .2110$.

Comparison of PHA IP-10 responses: unlikely TB versus confirmed TB: $P = .117$, unlikely TB versus probable TB: $P = .003$, unlikely TB versus possible TB: $P = .001$.

However, proportions of positive IP-10 and QFT-IT results were comparable within all classification groups except in the possible TB group, in which we found more positive IP-10 test results compared with QFT-IT ($P = .025$).

Overall agreement between IP-10 and QFT-IT test results in children was 69% (141 of 203; $\kappa = 0.47$, $P < .0001$).

Indeterminate Results

In children, the overall indeterminate rate was 29% (59 of 203) for IP-10 and 26% (53 of 203) for QFT-IT ($P = .355$). IP-10 and QFT-IT indeterminate rates were comparable within classification groups (Table 3). Across groups, there was a higher proportion of both IP-10 and QFT-IT indeterminate responders in the unlikely TB group.

All indeterminate results were due to low PHA-induced responses, except 1 indeterminate QFT-IT test in the unlikely TB group due to a high nil value.

Predictors of Positive Test Results

Multiple logistic regression analysis was used to assess potential factors associated with positive outcome. The following factors were assessed: PTB exposure, low weight-for-age values, HIV infection, age <2 years, and gender. Indeterminate responders were excluded from analysis. PTB exposure was associated with positive IP-10 test results (aOR: 3.6 [CI: 1.3 to 9.9], $P = .011$), but we found no association between positive QFT-IT results and PTB-exposure (aOR: 1.4 [CI: 0.5 to 3.4], $P = .515$).

Risk Factors for Indeterminate Test Results

Possible risk factors included in the logistic regression analysis for indeterminate results were HIV infection, low weight-for-age findings, age <2 years, and gender. Of these, only young age was associated with indeterminate results for both IP-10 (aOR: 2.2 [CI: 1.12 to 4.34], $P = .023$) and QFT-IT (aOR: 2.4 [CI: 1.21 to 4.92], $P = .012$).

DISCUSSION

In the present study, we compared the accuracy of IP-10 versus QFT-IT for diagnosing active TB in a large group of severely ill Tanzanian children with signs and symptoms of TB. Our primary finding was poor performance by both the IP-10 and QFT-IT in children, with low rates of

TABLE 3 Rate of Positive, Negative, and Indeterminate IP-10 and QFT Responders

Group	IP-10				QFT-IT				Combined
	Negative	Positive	Indeterminate	Positive Excluding Indeterminate Results	Negative	Positive	Indeterminate	Positive Indeterminate Results	Positive Excluding Double Indeterminate Results
Children									
Confirmed TB	67 (2/3)	33 (1/3)	0 (0/3)	33 (1/3)	67 (2/3)	33 (1/3)	0 (0/3)	33 (1/3)	33 (1/3)
Probable TB	57 (20/35)	23 (8/35)	20 (7/35)	29 (8/28)	57 (20/35)	23 (8/35)	20 (7/35)	29 (8/28)	35 (11/31)
Possible TB	65 (36/55)	16* (9/55)	18** (10/55)	20 (9/45)	76 (42/55)	7* (4/55)	16** (9/55)	9 (4/46)	18 (9/49)
Unlikely TB	55 (61/110)	6 (7/110)	38** (42/110)	10 (7/67)	55 (60/110)	12 (13/110)	35*** (37/110)	18 (13/73)	20 (17/85)
Adults									
Confirmed TB	18 (18/102)	72 (73/102)	11 (11/102)	80 (73/91)	19 (19/102)	75 (77/102)	6 (6/102)	80 (77/96)	84 (81/97)

Combined positive results: positive IP-10 and/or positive QFT-IT result after exclusion of double indeterminate results. Data are % of group (n/group n).

* IP-10 versus QFT-IT positivity rate in the possible TB group, $P = .025$.

** IP-10 indeterminate rate in possible TB versus unlikely TB, $P = .012$.

*** QFT-IT indeterminate rate in possible TB versus unlikely TB, $P = .026$.

positive test responders in children classified with confirmed and probable TB and overall high rates of indeterminate test responders. Second, we found an association between PTB exposure and positive IP-10 test outcome, whereas no such association was observed for QFT-IT. Third, we found that young age was associated with indeterminate test results for both tests.

IP-10 and QFT-IT Accuracy

This assessment is the first analysis of IP-10's accuracy head to head with QFT-IT in hospitalized children from a high endemic setting. Both IP-10 and QFT-IT performed poorly, with low rates of positive responders and high rates of indeterminate results.

Numerous studies report poor IGRA performance in children with confirmed or presumed TB. Recent meta-analyses show that sensitivity of QFT-IT for diagnosing active TB in children varies between 50% and 100%,[27,28] whereas the sensitivity of IP-10 ranges between 43% and 91%.[10] Hence, we did expect some reduction in test accuracy. However, we were surprised to find positivity rates in high-risk children to be <35% and overall indeterminate rates >25% in our population.

These findings may be partly explained by the composition of the study population. First, diagnostic sensitivity in children is generally lower in TB high-burden countries, such as Tanzania, compared with low-burden countries.[28] To our knowledge, only 3 previous studies regarding IP-10 and QFT-IT for diagnosing active TB in children were conducted in high-burden countries; 1 study reported a sensitivity of 81.1% for IP-10, and for QFT-IT, sensitivity reports varied between 53% and 63%.[17,29,30] Second, our population had a high prevalence of HIV infection, malnutrition, and young children, all factors that have previously been associated with poor IGRA performance.[6,7,11,31–33] Third, because mortality rates were high among the tested children, it is likely that immunosuppression caused by severe and chronic illness may have affected test outcomes.[23] Because confirmation of TB diagnosis in children is difficult, poor test accuracy could also be due to misclassification and overdiagnosis, falsely lowering test sensitivity.[4,34]

Technical Set-up

IGRAs are complicated tests posing an array of potential challenges when implemented in low-resource settings. Therefore, a parallel inclusion of adults with confirmed TB was performed to validate the preanalytical workflow, laboratory analysis, and dried ice sample transportation to Copenhagen. In this group of 102 adults, both the QFT-IT and IP-10 test performed on par with previous studies,[10] thus refuting the possibility of a technical cause for the low sensitivity found among the children.

Positive Test Outcome

In this study, we found that IP-10, but not QFT-IT, was positively associated with PTB exposure. We also found a trend of increasing rates of positive IP-10 test responders across classification groups, whereas this was not the case for QFT-IT. This trend may suggest that the IP-10 test is better correlated to the risk factors and symptoms of TB used in the children's diagnostic classification. This finding is in line with other recent evaluations demonstrating a significantly better association between risk of infection and IP-10 positivity compared with the QFT-IT.[19,35,36] However, the trend analysis reflects a small number of discordant cases in the unlikely and possible TB groups, whereas IP-10 and QFT-IT exhibited equal performance in the groups of higher disease certainty. Thus, we cannot draw any final conclusions pointing to a potential benefit of the IP-10 test.

Indeterminate Test Outcome

Both IP-10 and QFT-IT had high rates of indeterminate responders in children (29% vs 26%; $P = .355$). Most evidence, including our data, indicate that the performance of QFT-IT is compromised in young children.[6,7,31] It has been suggested that IP-10 may be superior to IFN-γ for diagnosing TB in this age group.[15,16] In our study, we find associations between indeterminate results and young age (<2 years) for both QFT-IT and IP-10 and hence no indication that 1 test is superior to the other. We and others have found an inverse correlation between age and quantitative PHA-induced IFN-γ responses in children, and the possibility of introducing an age-specific mitogen cutoff has been discussed.[6,7] However, we were not able to reproduce this association with age, probably because PHA-induced responses were impaired due to the general malaise of the children. Interestingly, we found no significant difference in overall quantitative PHA-induced IP-10 levels in children compared with adults, whereas, as expected, the IFN-γ responses were significantly reduced in children.

There have been reports regarding the compromised accuracy of the QFT-IT in malnourished[33] and HIV-infected[27] children, but we were unable to confirm these findings with either the QFT-IT or IP-10. However, it is possible that the effects of HIV and malnutrition on test outcome were blurred by a great overall morbidity and assumed immunosuppression in our population. Further studies in children who have varying degrees of comorbidity are needed to describe these potential differences between IP-10 and QFT-IT.

Limitations

Comparing studies on TB in children is often complicated by a substantial heterogeneity in study methods.[9,24] In this article, we chose to retrospectively

classify the children according to new consensus guidelines[24] to facilitate future study comparisons. However, the classification algorithm left us with few children in the high-risk TB groups, making statistical conclusions based on these groups linked with substantial imprecision.

We examined the impact of age, HIV infection, malnutrition, and PTB exposure on IP-10 and QFT-IT test outcomes. Many other factors, such as iron deficiency and helminth infection,[31,33] vitamin D deficiency,[37] CD4+ cell count, and HIV viral load,[32] have been shown to affect test outcomes. Unfortunately, we did not have access to such data.

We compared the accuracy of the IP-10 release assay versus one of the commercially available IGRAs, the QFT-IT. We are aware that other IGRA platforms, such as those using standardized peripheral blood mononuclear cell numbers, may perform differently in children. Hence, any conclusions in the present article cannot be extrapolated to IGRAs in general but only to QFT-IT specifically. Finally, cutoff values for positive IP-10 and QFT-IT test results are determined in adults and have not been systematically assessed in children.[38]

Perspectives

In line with current recommendations,[1] we found no indication for the use of either QFT-IT or IP-10 as diagnostic tools for active TB in children in high-endemic areas. We emphasize the need for studies investigating other test modalities. Second, special consideration should be given when testing children $<$2 years of age. Reduced IFN-γ levels in children compared with adults emphasize the importance of investigations into an age-specific mitogen cutoff.[6,7]

CONCLUSIONS

This study is the first to evaluate diagnostic methods in TB-suspect children by using new consensus case definitions. We investigated the accuracy of IP-10 and QFT-IT for diagnosing active TB in severely ill Tanzanian children and found that both tests performed poorly in this setting. History of PTB exposure was associated with positive IP-10 but not positive QFT-IT test results, and children $<$2 years of age had increased risk of indeterminate results in both tests. Thus, IP-10 and QFT-IT offer little diagnostic value in TB-suspect children from high-burden hospital settings.

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