OPEN

# Influence of Age and Nutritional Status on the Performance of the Tuberculin Skin Test and QuantiFERON-TB Gold In-Tube in Young Children Evaluated for Tuberculosis in Southern India

*Synne Jenum, MD,* Sumithra Selvam, MSc,† Diana Mahelai, Mphil,‡ Nelson Jesuraj, MD,‡ Vicky Cárdenas, PhD, JD,§ John Kenneth, MD,‡ Anneke C. Hesseling, MD, PhD,¶ Timothy Mark Doherty, PhD,‖ Mario Vaz, MD,** and Harleen M.S. Grewal, MD, PhD, DTMH††*

**Background:** Reliable identification of *Mycobacterium tuberculosis* infection or tuberculosis (TB) disease in young children is vital to assure adequate preventive and curative treatment. The tuberculin skin test (TST) and IFNγ-release assays may supplement the diagnosis of pediatric TB as cases are typically bacteriologically unconfirmed. However, it is unclear to what extent the performance of TST and QuantiFERON-TB Gold In-Tube (QFT; Cellestis' IFNγ-release assay test) depends on the demographic, clinical and nutritional characteristics of children in whom they are tested.

**Methods:** During a 2-year prospective observational study of 4382 neonates in Southern India, children with suspected TB were investigated and classified by a standard TB diagnostic algorithm.

**Results:** Clinical TB was diagnosed in 13 of 705 children referred for case verification with suspected TB. TST and QFT had a susceptibility for clinical TB of 31% and 23%, respectively, in this group. Children <2 years were more likely to test QFT indeterminate. A height-for-age Z score within the lowest quartile increased the odds ratio (OR) for a positive or indeterminate QFT result [OR 2.46 (1.19–5.06), OR 3.08 (1.10–8.58)], whereas the OR for a positive TST was reduced with a weight-for-height Z score within the lowest quartile [OR 0.17 (0.06–0.47)].

**Conclusion:** The sensitivities of the TST and QFT for clinical TB in children <3 years of age were equally poor in this population. Stunted children were more susceptible to *Mycobacterium tuberculosis* infection and more prone to indeterminate QFT results. TST was less reliable in children with wasting.

**Key Words:** tuberculosis, tuberculin skin test, interferon gamma release assay, malnutrition, child. multivariate analyses

(*Pediatr Infect Dis J* 2014;33:e260–e269)

Children <2 years of age at the time of infection typically develop tuberculosis (TB) disease because of primary infection and not as a result of reactivation. Their risk of progression to TB and for developing severe disease is high. Therefore, reliable identification of *Mycobacterium tuberculosis* (MTB) infection or TB in young children is vital to assure adequate preventive and curative treatment. Pediatric TB often remains unconfirmed because of paucibacillary disease and technical difficulties in obtaining adequate specimens. Therefore, evidence of MTB infection by a positive tuberculin skin test (TST) or IFNγ-release assay (IGRA) may be used as a supplement in diagnosis.[1] World Health Organization (WHO) recommends the use of TST over IGRAs in children in low- to middle-income countries as TST is cheaper and evidence suggests little difference in sensitivity.[2] A trend towards lower sensitivity of all tests in children <5 years was highlighted in a recent meta-analysis, but the conclusion was limited by few included subjects.[3] In addition, it is unclear to what extent the performance of TST and IGRAs depends on demographic, clinical and nutritional characteristics in young children.

In this study, we first evaluated the performance of TST and QFT in diagnosing clinical TB, in a cohort of children <3 years in a rural setting in Southern India. We then assessed the associations between TST and QFT results and sociodemographic, clinical and nutritional (intrauterine and postnatal) characteristics in the same cohort.

## METHODS

### Study Design and Setting

This study was nested within the Neonatal Cohort Study (NCS), a population-based prospective study of 4382 Calmette-Guérin bacillus-vaccinated neonates cluster randomized to active (2215) or passive (2167) surveillance for 2 years, in the Palamaner Taluk, India (3.200°N, 72.7500°E, altitude 683 m), from April 2007 to September 2010. Enrollment and consent procedures are previously described.[4] The NCS covered a total population of ~400,000, with an estimated TB incidence of 136/100,000 (Andhra Pradesh, 2010).[5]

The present study is cross-sectional and included children enrolled in the NCS and evaluated for TB at the Case Verification Ward (CVW) at Emmaus Swiss Hospital, during the follow-up period. Referral criteria were either (1) exposure to a known TB case within the last year; (2) respiratory/infectious symptoms or (3) failure to thrive (FTT) defined as any of the following: (i) loss of weight or no weight gain for 2 consecutive visits; (ii) downward crossing of 2 percentile lines on the weight-for-age growth chart or (iii) weight that tracked consistently below the 3rd percentile in the weight-for-age growth chart[6] or (4) a TST ≥10 mm at study closeout.

Accepted for publication April 16, 2014.

From the *Center for Immune Regulation, Oslo University Hospital, Rikshospitalet-Radiumhospitalet Medical Center and the University of Oslo, Oslo, Norway; †Division of Epidemiology, Biostatistics and Population Health; ‡Division of Infectious Diseases, St. John's Research Institute, Bangalore, India; §Aeras, Rockville, MD; ¶Department of Paediatrics and Child Health, Desmond Tutu TB Center, Stellenbosch University, Cape Town, South Africa; ‖GlaxoSmithKline Pharma, Vaccines, Copenhagen, Denmark; **Division of Health and Humanities, St. John's Research Institute, Bangalore, India; and ††Department of Clinical Science, University of Bergen and Department of Microbiology, Haukeland University Hospital, Bergen, Norway.

This work was supported by the Research Council of Norway [grants 179342, 192534 and 196362]; the University of Bergen (Norway); Aeras (MD) and St. John's Research Institute (India). QFT kits were supported in part, by Foundation for Innovative New Diagnostics.

The authors have no other funding or conflicts of interest to disclose.

Address for correspondence: Harleen M.S Grewal, MD, PhD, DTMH, Professor and Senior Consultant. Department of Clinical Science, Faculty of Medicine and Dentistry, University of Bergen, Norway and Department of Microbiology, Haukeland University Hospital, Bergen 5021, Norway. E-mail: Harleen.Grewal@K2.uib.no.



ISSN: 0891-3668/14/3310-e260
DOI: 10.1097/INF.0000000000000399

The study was approved by the institutional review board at St. John's Medical College, Bangalore, India, an independent ethics review committee and the Ministry of Health Screening Committee, Government of India (No. 5/8/9/60/20006-ECD-1 dt.10.11.2006).

## Diagnostic Assessment

Clinical and anthropometric data were recorded. A chest radiograph (CXR), anterioposterior view, was interpreted by 3 independent radiologists. Agreement by 2 radiologists was required to classify a CXR as consistent with TB. A TST was performed by a trained nurse/doctor (2 TU/0.1 mL tuberculin; Span Diagnostics Ltd, Gujarat, India) and read after 48 hours. Peripheral blood (3 mL) was drawn for the QFT (Cellestis/Qiagen, Carnegie, Victoria, Australia) which was performed according to the manufacturer's instructions.

Gastric aspirates and induced sputa were collected on 2 consecutive days for fluorescent microscopy (Auramine) and culture on solid (Löwenstein-Jensen) and liquid (Mycobacterial Growth Indicator Tube, BD) medium. Positive results were confirmed by Ziehl-Neelsen staining and speciated using the HAIN kit (GenoType MTBC, Ver1, Hain Life Sciences, Germany). Direct PCR (The COBAS TaqMan MTB Test, Roche 2007) was done on all culture-negative specimens in children with CXR consistent with TB. The HIV status was not assessed, but data from a household contact study conducted in the same study area during the period 2010–2012 found a HIV prevalence <1% (TB Trials Study Group, unpublished data).

## Clinical Outcomes

A structured diagnostic algorithm including TST result (but not QFT), classified the children as having definite TB, probable TB or not TB. Clinical TB included definite TB [positive culture or polymerase chain reaction (PCR)] and probable TB (CXR suggestive of TB and ≥1 of the following: known TB exposure, fever and/or cough ≥2 weeks, FTT and TST ≥10 mm).

## Statistical Analysis

Low birth weight (<2500 g) and being small for gestational age (SGA: Weight-for-gestational age <10th percentile)[7] were used as indicators of intrauterine nutritional status. The WHO child growth standard Z scores were dichotomized using the cutoff <−2, which defines wasting 2 [weight-for-height Z-score (WHZ)], stunting [height-for-age Z-score (HAZ)] and underweight [weight-for-age Z score (WAZ) and BMI-for-age Z score (BAZ)].[6] Equal to the WHO definition, hemoglobin <10 g/100 mL defined the cutoff for moderate-severe anemia.[8] TST and QFT were handled as categorical variables; TST (binary), cutoff ≥10 mm,[9] QFT in 3 categories: positive (≥0.35 IU/mL), negative (<0.35 IU/mL) and indeterminate. Categorical data were compared by Pearson's $\chi^2$ with Yates Continuity Correction or Fisher's exact test, where appropriate. Kappa statistics were used in the concordance analyses of TST and QFT result and evaluated according to McGinn et al.[10]

Because malnutrition was left-shifted in the study population, children within the lowest quartile were considered malnourished in new dichotomous variables that were included in the univariate and multivariate analyses (cutoffs: WHZ <−2.59, HAZ <−2.14, WAZ <−2.63 and BAZ <−2.51). Univariate associations between positive TST and/or QFT outcomes (indeterminate QFT results excluded) and surveillance arm (active or passive), gender, birth weight, SGA, age, socioeconomic factors (mothers/fathers education, housing, cooking fuel), known TB exposure, symptoms, nutritional status (WHZ, HAZ, WAZ, BAZ, anemia) and isolated Non-tuberculous mycobacteria (NTMs), were assessed by logistic regression. Variables with significant univariate associations with the TST and/or QFT outcomes were considered for inclusion in the multivariate model if they could be assumed to have a casual impact on the outcomes. These assumed that causal relationships were based on the literature and drawn by principles of a directed acyclic graph (DAG).[11] Notably, as the positive TST and/or QFT outcomes are imperfect readouts of the clinical TB outcome (clinical TB disease, MTB infection or no infection/disease) for which there is no gold standard, we chose to illustrate the variables relationship both to the clinical TB outcome and the positive TST and/or QFT outcomes (Fig. 1; causal relationships are indicated with arrows). The variables impact on clinical TB influence on the child's susceptibility for MTB infection and disease, whereas the variables impact on a positive TST and/or QFT result influence on the ability of the tests to correctly reflect the clinical TB outcome (test performance). Following the DAG principle, ancestor variables (variables that is a cause of another

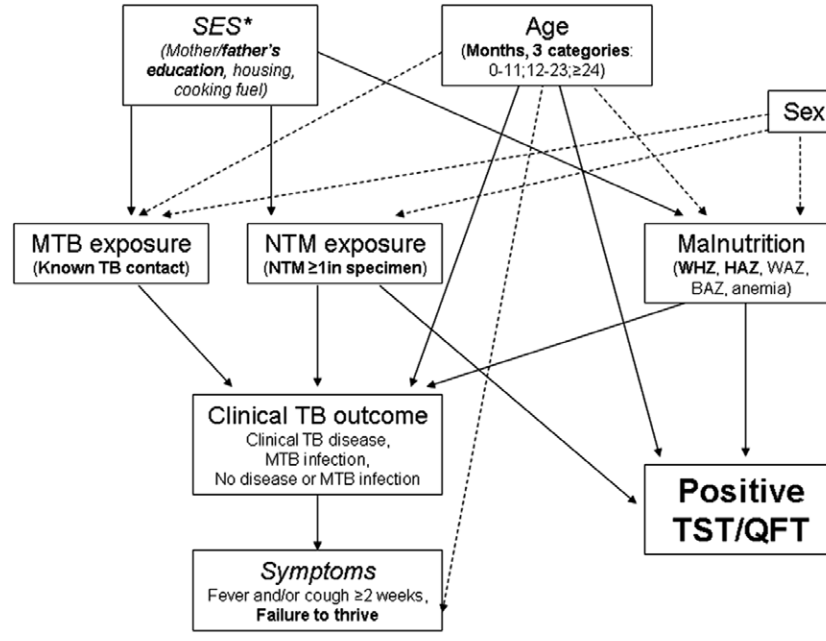


**FIGURE 1.** A DAG illustrating the causal relationships between the determinants for the outcomes clinical TB and positive TST and/or QFT. A causal relationship between a determinate (variable names listed in parenthesis) and an outcome is indicated with arrows. Ancestors (variables that is a cause of another variable more closely linked to the outcome) and descendants of the outcome not included in the multivariate models are in italic. Variables significantly associated to the outcomes in univariate analysis are in bold. Dashed arrows indicate more uncertain relationships.

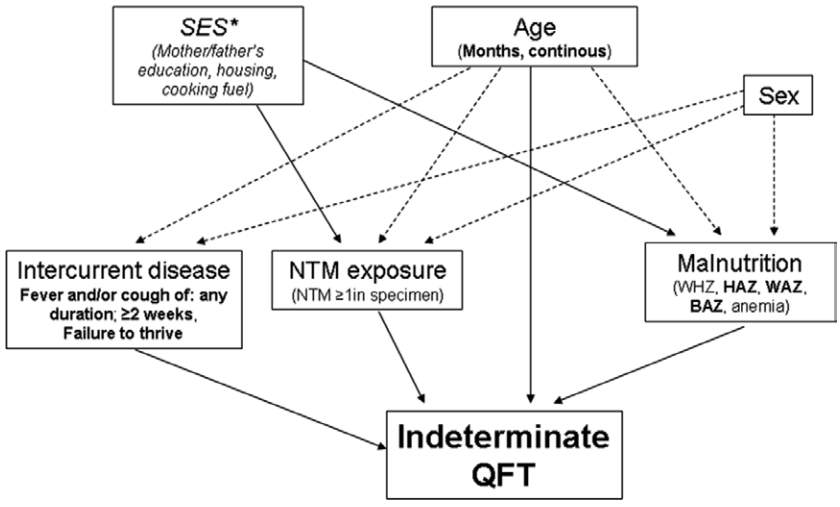


**FIGURE 2.** A DAG illustrating the causal relationships between the determinants for the outcome indeterminate QFT. A causal relationship between a determinate (variable names listed in parenthesis) and an outcome is indicated with arrows. Ancestors (variables that is a cause of another variable more closely linked to the outcome) and descendants of the outcome not included in the multivariate models are in italic. Variables significantly associated to the outcomes in univariate analysis are in bold. Dashed arrows indicate more uncertain relationships.

variable more closely linked to the outcome) and descendants of the outcome were not included in the multivariate models.[11] These considerations left age, TB exposure, WHZ, HAZ and NTM presence in the multivariate model together with gender that is routinely included. WHZ and HAZ represent different entities of malnutrition and important collinearity or interaction was not found when all possible 2-way interactions in each of the multivariate models were tested. Regarding the indeterminate QFT outcome, the assumed causal relationship is different (Fig. 2). Being primarily the result of inadequate mitogen responses, this outcome is not specific for clinical TB outcome, but rather affected by all physiological conditions interfering with T-cell responses in general (Fig. 2). Accordingly, known TB was not considered relevant whereas symptoms of any duration (categories defined in Table 1) were included because of previous reports.[12] Missing data for each variable can be deduced from Table 2.

All $P$ values were calculated using 2-tailed tests and $P < 0.05$ was considered significant. Analyses were conducted using PASW Statistics version 18.0, 2009. SPSS Inc. Chicago, IL.

## RESULTS

### Characteristics of the Study Population

Of 746 children investigated at the CVW, 86 children (11.5%) had known TB exposure. Two or more referral criteria were present in 123 (16.5%). Of 623 children with a single referral criteria present,

**TABLE 1.** Unadjusted and Adjusted OR Estimates and 95% CI for the Significant Associations Between a Positive TST and QFT as Dependant Variable and Sociodemographic, Clinical, Nutritional and Mycobateriological Factors (Not M. *Tuberculosis*)*

| | TST ≥ 10 mm and QFT ≥ 0.35 IU/mL vs. Other Combinations of TST and QFT Test Results (N = 666) | |
|---|---|---|
| | Unadjusted OR (95% CI) | Adjusted OR (95% CI) |
| Surveillance | | |
| Active | **0.14 (0.05–0.37)** | |
| Gender | | |
| Male | 1.16 (0.45–2.98) | 1.42 (0.51–3.95) |
| Age categories | | |
| 0–11 months | **0.12 (0.03–0.42)** | **0.10 (0.02–0.34)** |
| 12–23 months | **0.08 (0.02–0.24)** | **0.07 (0.02–0.25)** |
| ≥24 months† | | |
| Known contact with TB case | | |
| Yes | **5.34 (2.00–14.23)** | **9.41 (2.93–30.2)** |
| FTT | | |
| Yes | **0.17 (0.07–0.45)** | |
| WHZ | | |
| <−2.59 (n = 173) | 2.19 (0.92–5.23) | 2.48 (0.98–6.28) |
| HAZ | | |
| <−2.14 (n = 176) | **3.17 (1.24–8.12)** | **3.46 (1.19–10.0)** |
| NTM | | |
| ≥1 positive specimen | 0.70 (0.23–2.09) | 0.69 (0.21–2.24) |

Adjusted OR estimates and 95% CI are shown for all variables included in the multivariate model. OR estimates and 95% CI for variables not included in the table are available on request.

*Binary logistic regression was applied. CI that do not overlap the null value OR = 1 are shown in bold.

†Reference group.

**TABLE 2.** Characteristics of Children Referred to the CVW Classified According to the Diagnostic TB Algorithm (n = 705)*

| | Total | Clinical TB† | No TB | |
|---|---|---|---|---|
| | n = 705 (%) | n = 13 (%) | n = 692 (%) | *P* Value |
| Surveillance arm (N = 705) | | | | |
| Active | 609 (86.4) | 10 (76.9) | 599 (86.6) | 0.40 |
| No of visits to the CVW (N = 705) | | | | |
| 1 visit | 663 (94.0) | 10 (76.9) | 653 (94.4) | 0.07 |
| 2 visits | 40 (5.7) | 3 (23.1) | 37 (5.3) | |
| 3 visits | 2 (0.3) | — | 2 (0.3) | |
| Sex (N = 705) | | | | |
| Male | 374 (53.0) | 7 (53.8) | 367 (53.0) | 1.00 |
| Birth weight (g; N = 703) | | | | |
| Mean | 2807 | 2735 | 2809 | 0.13 |
| Min-max | 1500–4500 | (1500–4000) | (2000–4500) | |
| SGA | 72 (10.2) | 3 (23.1) | 69 (10.0) | 0.14 |
| Age (months; N = 705) | | | | |
| Mean | 14.8 | 16.8 | 14.8 | 0.84 |
| Min-max | (1–35) | (8–28) | (1–35) | |
| 0–11 months | 220 (31.2) | 4 (30.8) | 216 (31.2) | 0.53 |
| 12–23 months | 392 (55.6) | 6 (46.2) | 386 (55.8) | |
| ≥ 24 months | 93 (13.2) | 3 (23.1) | 90 (13.0) | |
| Mothers education (N = 705) | | | | |
| High school or higher | 143 (20.3) | 1 (7.7) | 142 (20.5) | 0.37 |
| Primary, secondary | 351 (49.8) | 9 (69.2) | 342 (49.4) | |
| Illiterate | 211 (29.9) | 3 (23.1) | 208 (30.1) | |
| Fathers education (N = 701) | | | | |
| High school or higher | 195 (27.7) | 1 (7.7) | 194 (28.0) | 0.18 |
| Primary, secondary | 372 (52.8) | 8 (61.5) | 364 (52.6) | |
| Illiterate | 134 (19.0) | 4 (30.8) | 130 (18.8) | |
| Housing (wall; N = 705) | | | | |
| Bricks | 540 (76.6) | 11 (84.6) | 529 (76.4) | 0.74 |
| Other | 165 (23.4) | 2 (15.4) | 163 (23.6) | |
| Cooking fuel (N = 705) | | | | |
| Electricity (none) or gas | 49 (7.0) | — | 49 (7.1) | 1.00 |
| Other | 656 (93.0) | 13 (100.0) | 643 (92.9) | |
| Known contact with TB case (N = 705) | | | | |
| Yes | 85 (12.1) | 2 (15.4) | 83 (12.0) | 0.66 |
| Symptoms (fever and/or cough; N = 705) | | | | |
| 2 of 2 symptoms | 145 (20.6) | 3 (23.1) | 142 (20.5) | 1.00 |
| 1 of 2 symptoms | 142 (20.1) | 2 (15.4) | 140 (20.2) | |
| None | 418 (59.3) | 8 (61.5) | 410 (59.2) | |
| Fever and/or cough ≥ 2 weeks (N = 705) | | | | |
| Yes | 175 (24.8) | 4 (30.8) | 171 (24.7) | 0.75 |
| FTT (N = 705) | | | | |
| Yes | 537 (76.2) | 8 (61.5) | 529 (76.4) | 0.20 |
| CXR (AP; N = 704) | | | | |
| Consistent with TB | 11 (1.6) | 11 (84.6) | — | **<0.0005** |
| Abnormal, not TB | 12 (1.7) | — | 12 (1.7) | |
| Normal | 681 (96.6) | 2 (15.4) | 679 (98.1) | |
| NTM (N = 705) | | | | |
| ≥1 positive specimen | 166 (23.5) | 2 (15.4) | 164 (23.7) | 0.74 |
| WHZ (N = 703) | | | | |
| Mean | −1.80 | −1.86 | −1.80 | 0.38 |
| Min-max | −4.93 to 3.61 | (−4.06 to 1.59) | (−4.93 to 3.61) | |
| <−2 (wasting) | 305 (43.3) | 7 (53.8) | 298 (43.1) | 0.57 |
| HAZ (N = 703) | | | | |
| Mean | −1.25 | −2.23 | −1.24 | 0.81 |
| Min-max | −6.32 to 3.13 | (−2.95 to 0.35) | (−6.32 to 3.13) | |
| <−2 (stunting) | 197 (27.9) | 6 (46.2) | 191 (27.6) | 0.21 |
| WAZ (N = 705) | | | | |
| Mean | −1.95 | −2.11 | −1.95 | 0.47 |
| Min-max | −5.00 to 1.67 | (−3.99 to 1.33) | (−5.00 to 1.67) | |
| <−2 (underweight) | 354 (50.2) | 8 (61.5) | 346 (50.0) | 0.58 |
| BAZ (N = 703) | | | | |
| Mean | −1.70 | −1.70 | −1.70 | 0.44 |
| Min-max | −4.95 to 4.63 | −3.86 to 1.55 | −4.95 to 4.63 | |
| <−2 (underweight) | 284 (40.3) | 7 (53.8) | 277 (40.0) | 0.40 |
| Hgb (g/100 mL; N = 601) | | | | |
| Mean | 10.7 | 10.0 | 10.7 | 0.16 |
| Min-max | 4.0–18.9 | 8.0–11.6 | 4.0–18.9 | |
| <10 g/100 mL (moderate-severe anemia) | 197 (32.8) | 6 (54.5) | 191 (32.4) | 0.11 |

(*Continued*)

**TABLE 2.** (Continued)

| | Total | Clinical TB† | No TB | |
|---|---|---|---|---|
| | n = 705 (%) | n = 13 (%) | n = 692 (%) | *P* Value |
| TST ( N = 702) | | | | |
| Median | 4.0 | 7.5 | 4.0 | 0.07 |
| Positive (cutoff ≥10 mm) | 69 (9.8) | 4 (30.8) | 65 (9.4) | **0.031** |
| QuantiFERON Gold In-Tube (N = 691) | | | | |
| Positive (≥ 0.35 IU/mL) | 36 (5.1) | 3 (23.1) | 33 (4.8) | **0.003** |
| Negative (< 0.35 IU/mL) | 633 (89.8) | 8 (61.5) | 625 (90.3) | |
| Indeterminate | 22 (3.1) | 2 (15.4) | 20 (2.9) | |
| Mitogen minus nil (median) | 10 | 10 | 10 | 0.40 |
| TB-antigen minus nil (median) | 0.01 | 0.02 | 0.01 | 0.29 |

*Comparison of distribution between infants with and without clinical TB disease performed by *t* test, Mann-Whitney U test or Fisher's Exact test where appropriate. Two-sided *P* values < 0.05 are shown in bold.

†No missing data among infants with clinical TB disease.

452 (60.6%) had FTT, 80 (10.7%) had persistent cough and/or fever and 40 (5.4%) had known TB exposure. Among the 51 (6.8%) children admitted based on TST ≥ 10 mm alone, 44 (86.3%) were referred at study closure. Of the 746 children, 41 were excluded from further analyses as missing data prevented classification by the diagnostic algorithm (40) and 1 child was lost to follow up[1] (Fig. 3). None of these children had a CXR suggestive of TB, and only 1 child had known TB exposure. Subsequently, 705 children were included in further analyses. Their clinical characteristics are provided in Table 2. Except for CXR changes, TST and QFT test results, there were no significant differences in sociodemographic, clinical or nutritional variables between children with or without clinical TB. The mean age was 14.8 months, about 50% of the mothers and fathers had primary or secondary school, 76.6% lived in brick houses but only 7% of the families used gas to cook. Known TB exposure was present in 12.1%. A striking 76.2% had FTT. Poor nutritional status was also reflected in the WHO growth chart Z scores (40–50% had wasting/underweight and 30% had stunting) and in the proportion of children with moderate-severe anemia (32.8%).

## Performance of TST and QFT in Clinical TB Cases

Thirteen clinical TB cases, (4 definite and 9 probable) were identified; 4 were TST positive and 3 QFT positive. The sensitivity of TST and QFT for clinical TB was 31% and 23%, respectively (Table 3). Among children with definite TB, 3 had negative smears but positive cultures and were TST and QFT positive. The 4th child with definite TB was TST and QFT negative (sensitivity for definite TB: 75%). This 18-month-old boy was the only child in our study with a positive smear (gastric aspirate grade 3+), illustrating the shortcomings of TST and QFT also in definite TB cases despite an adequate T-cell response to mitogen (>10). He had been initiated on anti-TB treatment 2 weeks before admission, which likely explains the negative culture, but MTB was confirmed by PCR. Of 9 children classified as probable TB, 1 infant was TST positive, 7 were QFT negative and 2 QFT indeterminate (Table 4).

## Overall TST and QFT Results

In the 705 children, 69 of 702 (9.8%) were TST positive. QFT was positive in 36 and indeterminate in 22 of 691 (5.1% and 3.1%, respectively). Of the children with an indeterminate QFT, 19 (86.4%)

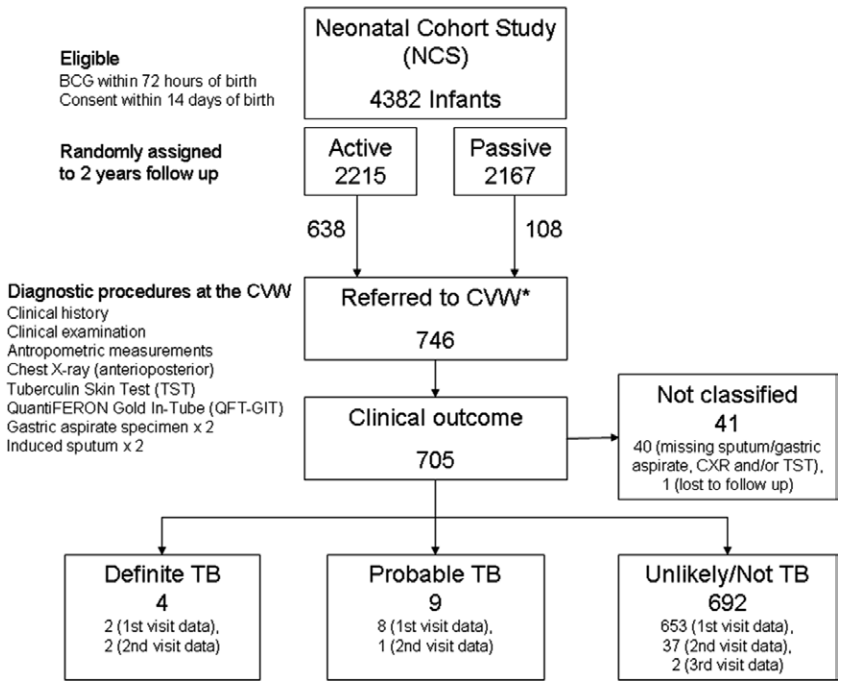


**FIGURE 3.** Flow-chart for the 746 study participants. The diagnostic procedures applied at the *CVW are listed.

**TABLE 3.** The Performance of the TST and the QFT in the Diagnosis of Clinical TB Disease

| | TB Status | | | | | | |
|---|---|---|---|---|---|---|---|
| | Clinical TB | No TB | | | | | |
| | N | N | Sensitivity | Specificity | PPV† | NPV‡ | κ (95% CI)* |
| TST | | | | | | | |
| ≥10 mm | 4 | 65 | 30.8% | 90.6% | 5.8% | 98.6% | **0.07** |
| <10 mm | 9 | 624 | (4/13) | (624/689) | (4/69) | (624/633) | **(−0.02 to 0.16)** |
| QFT | | | | | | | |
| ≥0.35 IU/mL | 3 | 33 | 23.1% | 92.2% | 8.3% | 98.7% | **0.11** |
| <0.35 IU/mL | 8 | 625 | (3/13) | (625/678) | (3/36) | (625/633) | **(−0.02 to 0.27)** |
| Indeterminate | 2 | 20 | | | | | |

*Indeterminate QFT results are excluded from analysis of kappa agreement.
†Positive predictive value.
‡Negative predictive value.
CI that do not overlap the null value κ = 1 are shown in bold.

were aged <12 months and the remaining 3 (13.6%) were aged 12–23 months. The difference in the distribution of indeterminate results within the 3 age categories was thus highly significant (Fisher's Exact test, $P < 0.0005$). Low mitogen response was the predominant cause of an indeterminate QFT result seen in 21 children (95.5%). Notably, indeterminate QFT was more frequent in children with clinical TB (15%) compared with children without (3%).

## TST and QFT Agreement

Valid results for both TST and QFT were obtained in 666 children. The 39 children without corresponding TST and QFT results were thus omitted from this analysis. Agreement between the tests was only fair [$\kappa = 0.30$, 95% confidence intervals (CI): 0.18–0.42], indicating that exchanging 1 test for the other is unlikely to provide the same information. When restricted to those with known TB exposure, which increases the pretest probability of the tests, the agreement was substantial ($\kappa = 0.75$, 95% CI: 0.42–0.95; Table 5). This highlights the importance of focusing on TB exposure rather than immunological tests in young pediatric populations.

## Determinants for a Positive TST and/or QFT

Unadjusted odds ratio (OR) estimates for the variables: surveillance arm (active or passive), gender, birth weight, SGA, age, socioeconomic factors (mothers/fathers education, housing, cooking fuel), known TB exposure, symptoms, nutritional status (WHZ, HAZ, WAZ, BAZ and anemia) and isolated NTMs were performed by logistic regression. If significant, these estimates, with 95% CIs, are presented in Tables 1 and 6 together with unadjusted and adjusted analyses for all variables included in the final multivariate model: gender, age (categorical), known TB exposure, nutritional status (WHZ, HAZ) and NTM isolated from culture. Data not shown are available on request.

Adjusted for the other factors, boys had increased OR for a positive TST. Younger age associated negatively with a positive TST and/or QFT, more prominently with TST than QFT, both in children <12 and 12–23 months compared with children ≥24 months. TB exposure was a determinant for a positive TST and/or QFT. Within the study population, having a WHZ < −2.59 reduced the OR for a positive TST whereas having a HAZ < −2.14 increased the OR for a positive QFT and positivity for both tests, but not for TST alone. Birth weight < 2500 g and SGA were included in the models one at a time, but no significant associations was present and the other coefficients remained unchanged (data not shown). All possible 2-way interactions in the models were tested, but only a slight interaction between WHZ and HAZ ($P = 0.048$) for a positive QFT was found. The model including the interaction gave a better fit (Likelihood Ratio test Statistics: $\chi^2 = 5.34$, $P < 0.025$), but did not change the coefficients. Stratified analyses indicated that the effect of having a HAZ <−2.14 on a positive QFT was not evident in children with WHZ <− 2.59 (OR: 0.55, 95% CI: 0.06–5.13 vs. OR: 4.0, 95% CI: 1.71–9.35).

## Determinants for an Indeterminate QFT

Results from unadjusted and adjusted analyses are given in Table 7. The multivariate model included gender, age (continuous), fever and/or cough of any duration, nutritional status (WHZ and HAZ) and NTM isolated from culture. Adjusted for the other factors, the OR of an indeterminate result was reduced by 0.83 with every month of age (95% CI: 0.76–0.90). Fever and cough as well as a HAZ within the lowest quartile increased the OR for an indeterminate QFT. Neither birth weight <2500 g nor SGA showed any association or changed the other coefficients and were therefore omitted in the final model.

## The Impact of Alternative Nutritional Variables on TST and QFT Outcomes

WAZ and BAZ are alternative markers of nutritional status and indicate underweight if the Z score is <−2.[6] Exchanging WHZ and HAZ for WAZ or BAZ in the multivariate models, WAZ (cutoff <−2.63) and BAZ (cutoff<−2.51) acted similarly by reducing the OR for a positive TST (ORWAZ 0.35, 95% CI: 0.16–0.77; ORBAZ 0.20, 95% CI: 0.07–0.54) and increased the OR for an indeterminate QFT result (ORWAZ 4.00, 95% CI: 1.61–9.95; ORBAZ 3.02, 95% CI: 1.23–7.44) in children with Z scores below the cutoff.

# DISCUSSION

To our knowledge, this is the first extensive study of TST and QFT performance in young Indian children. The sensitivities of TST and QFT test for clinical TB were equally poor, which may be attributable to the influence of younger age and malnutrition. QFT results did not add significantly to the information already provided by TST, supporting current WHO guidelines in high-burden TB settings.[2] We report for the first time that a low HAZ score, which indicates stunting, a result of chronic malnutrition, is a determinant for an indeterminate QFT result when corrected for other factors.

The sensitivities of TST and QFT for clinical TB were 31% and 23%, which is comparable with studies conducted in young African children.[13–16] The diagnostic criteria in this study are slightly less stringent than recently recommended by an expert panel,[17] but the proportion of confirmed TB cases (30%) is similar to other studies of pediatric TB.[18] The lack of a gold standard for MTB infection and the diagnosis of smear and culture-negative TB complicates the evaluation of TST and QFT performance.

**TABLE 4.** Characteristics of Clinical TB Cases

| | Surveillance | Gender | Age (Months) | Data From Visit | TST (mm) | QFT | | | | CXR Result | Known TB Exposure | Fever/Cough ≥2 Weeks | FTT | WHZ | HAZ | WAZ§ | SGA | Hgb (g/dL) | Smear‖ | Culture |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Test Result | Nil | Test Value (IU/mL) | Mitogen Minus Nil (IU/mL) | | | | | | | | | | | |
| **Definite TB** | Passive | M | 18 | 2nd | 9 | – | 0.15 | 0.11 | >10 | Abnormal, TB | + | + | + | −2.35 | −1.21 | −2.26 | – | 11.3 | +++ (confirmed by PCR) | – |
| | Active | M | 9 | 1st | 10 | + | 0.32 | 8.41 | >10 | Normal | – | + | + | −2.93 | −0.83 | −2.58 | – | 11.3 | – | + (3/4) |
| | Active | M | 28 | 2nd | 13 | + | 0.25 | >10 | >10 | Normal | – | – | – | −1.94 | −2.7 | −2.84 | – | 9.4 | – | + (3/4) |
| | Passive | M | 26 | 1st | 20 | + | 0.4 | >10 | >10 | Abnormal, TB | – | – | + | 0.36 | −2.61 | −1.14 | – | Not done | – | + (2/4) |
| Probable TB | Active | F | 18 | 1st | 0 | – | 0.08 | −0.01 | >10 | Abnormal, TB | – | + | + | −2.74 | −2.04 | −2.99 | + | 9.5 | – | – |
| | Active | F | 10 | 1st | 2 | – | 0.13 | 0.02 | 1.18 | Abnormal, TB | – | + | + | −2.16 | −1.65 | −2.49 | + | 8.0 | – | – |
| | Active | M | 14 | 2nd | 4 | - | 0.96 | −0.6 | >10 | Abnormal, TB | + | + | – | −4.06 | −2.44 | −3.99 | – | 11.1 | – | – |
| | Active | F | 16 | 1st | 4 | – | 0.06 | −0.02 | 3.92 | Abnormal, TB | – | + | – | −2.65 | 0.18 | −1.81 | – | 11.6 | – | – |
| | Active | M | 15 | 1st | 4 | – | 0.18 | 0.09 | >10 | Abnormal, TB | – | + | + | −2.99 | −2.36 | −3.23 | – | 9.1 | – | – |
| | Active | F | 13 | 1st | 5 | – | 0.08 | 0.01 | >10 | Abnormal, TB | –§ | – | + | −1.46 | −1.72 | −1.91 | + | 10.2 | – | – |
| | Passive | F | 24 | 1st | 16 | – | 0.15 | 0.23 | >10 | Abnormal, TB | – | + | – | −1.26 | −2.95 | −2.54 | – | 9.5 | – | – |
| | Active | M | 8 | 1st | 5 | indet† | 0.29 | −0.12 | 0.02 | Abnormal, TB | – | + | – | −1.57 | 0.35 | −1.03 | – | 8.8 | – | – |
| | Active | F | 10 | 1st | 6 | indet† | 0.1 | −0.01 | 0.49 | Abnormal, TB | – | + | – | 1.59 | 0.34 | 1.33 | – | not done | – | PCR indet† |

*From gastric aspirate and/or induced sputum.
†indeterminate.

Determining the clinical relevance of bacteriologically unconfirmed TB is an important issue in pediatric TB. Because pediatric TB is often paucibacillary,[1] it can be argued that false negative QFT results could be expected, as increasing evidence supports a positive association between the antigen load and the magnitude of MTB-specific IFNγ responses.[19,20] Accordingly, children with sputum-negative TB could simply be in the early stages of TB. Given that cases in this study were identified by active case finding, this is very likely.[1] Although published studies indicate an increased risk of TB disease in subjects with TST indurations ≥12 mm[21] or high IFNγ-responses after recent infection,[19,20] these studies were conducted almost entirely in adults. Interestingly, adults with discordant TST and IGRA results had the lowest rates of TB in a household contact study in the Gambia.[20] From this perspective, one could argue that there is an over diagnosis of TB in young children. However, epidemiological estimates suggest the opposite[1] and TST and/or QFT negativity is not restricted to unconfirmed TB cases,[13,15,16,22] as shown in this study. Furthermore, we report increased OR for indeterminate QFT in children with intercurrent disease (fever and/or cough of any duration). This questions the validity of extrapolation of immune responses in asymptomatic, latently infected adults, to immune responses in sick children. Moreover, unlike adults, children <2 years at time of infection who progress to TB can almost universally be assumed to do so after a primary infection.[1] The nature of a primary and/or immature immune response[23] could cause MTB-specific, IFNγ-producing T-cells to be present in lower numbers in young children.

Ideally, determinants for TST and QFT outcomes should be evaluated in clinical TB cases, but this is possible only in prospective community-based settings where the TB incidence is high. Therefore, we evaluated associations between determinants and TST/QFT results regardless of TB disease status. As expected, known TB exposure associated with a positive TST and/or QFT when corrected for other factors. Our study indicates that there might be factors which mask the true relationship between known TB exposure and a positive TST since this association was evident only in adjusted analysis.

Boys were more likely to be TST positive than girls when corrected for other factors. Basu et al[24] recently reported that boys were more likely to be QFT positive, but found no such association with TST. There are well-known gender differences in TB epidemiology, but it is unclear to which extent these are attributable to genetic as opposed to socioeconomic and cultural factors.[25]

Children <2 years were less likely to be TST and/or QFT positive and more likely to have an indeterminate QFT result than children aged 2–3 years. This is not surprising as the immune responses in infancy are considered immature and evolving.[23] Previous findings in young children are contradictory with regard to a positive association between age and a positive TST.[14,24] Regarding QFT, some studies reported no association with age, but few young children were included.[16,26] In a large study of children <5 years, age associated positively with a positive TST and QFT after adjusting for Calmette-Guérin bacillus vaccination and gender.[24] Many studies have suggested indeterminate QFT to be more frequent in young individuals.[16,22,24]

The burden of malnutrition in this study was considerable. Malnourished children are more susceptible to infections because of altered immune responses[27] and would thus be expected to have increased susceptibility to MTB infection and subsequently an increased rate of positive TST and/or QFT. On the other hand, blunted immune responses by malnutrition are likely to cause suboptimal performance of immunological tests as reported for immunocompromised children.[28] We report that HAZ <−2.16 increased the OR for a positive TST and/or QFT, when corrected for other factors. A HAZ <−2 is a marker of stunting, the result of chronic malnutrition. Our findings therefore confirm the increased susceptibility to MTB infection in children with chronic malnutrition. At

**TABLE 5.** Kappa Agreement (κ)* Between the QFT and the TST in A: All Children Tested With Both Tests; B: Children With Known TB Exposure

| | A: TST, All (N = 688) | | B: TST, Children With Known TB Exposure (N = 79) | |
|---|---|---|---|---|
| | ≥10 mm, N (%) | <10 mm, N (%) | ≥10 mm, N (%) | <10 mm, N (%) |
| QFT | | | | |
| ≥0.35 IU/mL | 18 (26.9) | 18 (2.9) | 7 (63.6) | 1 (1.5) |
| <0.35 IU/mL | 48 (71.6) | 582 (93.7) | 3 (27.3) | 65 (95.6) |
| Indeterminate | 1 (1.5) | 21 (3.4) | 1 (9.1) | 2 (2.9) |
| κ (95% CI) | **0.30 (0.18–0.42)** | | **0.75 (0.42–0.95)** | |

*Indeterminate QFT results are excluded from analysis of kappa agreement. CI that do not overlap the null value κ = 1 are shown in bold.

**TABLE 6.** Unadjusted OR Estimates and 95% CI for Significant Associations Between a Positive TST or QFT as Dependant Variables and Sociodemographic, Clinical, Nutritional and Mycobacteriological Factors (Not *M. tuberculosis*)*

| | TST ≥ 10 mm vs. TST < 10 mm (N = 702) | | QFT ≥ 0.35 IU/mL vs. QFT < 0.35 IU/mL (N = 669) | |
|---|---|---|---|---|
| | Unadjusted | Adjusted | Unadjusted | Adjusted |
| | OR (95% CI) | OR (95% CI) | OR (95% CI) | OR (95% CI) |
| Surveillance | | | | |
| Active | **0.12 (0.07–0.21)** | | **0.33 (0.16–0.70)** | |
| Gender | | | | |
| Male | 1.54 (0.92–2.56) | **1.91 (1.03–3.53)** | 1.47 (0.74–2.93) | 1.55 (0.76–3.17) |
| Age categories | | | | |
| 0–11 months | **0.05 (0.02–0.10)** | **0.04 (0.02–0.08)** | **0.31 (0.13–0.75)** | **0.34 (0.13–0.86)** |
| 12–23 months | **0.05 (0.03–0.09)** | **0.05 (0.02–0.09)** | **0.23 (0.11–0.51)** | **0.25 (0.11–0.56)** |
| ≥ 24 months† | | | | |
| Fathers education | | | | |
| High school or higher | 2.06 (0.84–5.01) | | 0.83 (0.32–2.17) | |
| Primary, secondary | **2.31 (1.01–5.27)** | | 0.79 (0.34–1.8) | |
| Illiterate† | | | | |
| Known contact with TB case | | | | |
| Yes | 1.62 (0.83–3.15) | **2.60 (1.14–5.93)** | **2.37 (1.04–5.42)** | **3.03 (1.26–7.28)** |
| FTT | | | | |
| Yes | **0.16 (0.10–0.27)** | | **0.48 (0.24–0.98)** | |
| WHZ | | | | |
| <−2.59 (n = 173) | **0.22 (0.09–0.55)** | **0.17 (0.06–0.47)** | 0.90 (0.40–2.02) | 0.94 (0.41–2.15) |
| HAZ | | | | |
| <−2.14 (n = 176) | **1.71 (1.01–2.90)** | 1.42 (0.74–2.71) | **2.57 (1.30–5.08)** | **2.46 (1.19–5.06)** |
| BAZ | | | | |
| <−2.51 (n = 175) | **0.21 (0.09–0.54)** | | 0.75 (0.32–1.76) | |
| NTM | | | | |
| ≥1 positive specimen | **0.46 (0.22–0.95)** | 0.46 (0.20–1.04) | 1.05 (0.48–2.27) | 1.12 (0.78–1.12) |

Adjusted OR estimates and 95% CI are shown for all variables included in the multivariate model. Odds ratio estimates and 95% CI for variables not included in the table are available on request.

*Binary logistic regression was applied. CI that do not overlap the null value OR = 1 are shown in bold.

†Reference group.OR

the same time, chronic malnutrition makes these children more prone to indeterminate QFT, overwhelmingly because of lower mitogen-driven responses. Together with our finding of a substantially reduced OR for a positive TST when WHZ was <−2.61, our study provides strong evidence of a blunted immune response in malnourished children. The clinical consequences of blunted immunity are demonstrated by a higher mortality in hospitalized children with indeterminate QFT, regardless of the underlying disease.[16] Studies on the effect of malnutrition on TST are conflicting,[29,30] but reduced sensitivity of TST for clinical TB in children with WAZ <−2 have been reported.[13] Few studies (all evaluating WAZ <−2 without considering other Z scores) have evaluated malnutrition as a determinant for QFT results.[16,26] The definitions of malnutrition in studies vary considerably, and conflicting results might be attributed to different effects of chronic and subacute malnutrition. Furthermore, the standard Z score cutoff <−2 has not been evaluated with regard to immunological changes related to malnutrition. We have demonstrated that the effect of malnutrition depends on the variable used. When WAZ or BAZ replaced WHZ and HAZ in our adjusted models, the effect of chronic malnutrition on a positive QFT result was lost.

Children with at least 1 NTM isolated from culture (23.5%) had no increased OR for being TST positive when corrected for other factors, suggesting that reduced specificity of TST by NTM cross-reactivity plays little role in this setting. Similarly, no apparent impact of NTM on TST was reported in Gambian children.[31]

**TABLE 7.** Unadjusted and Adjusted OR Estimates and 95% CI for the Significant Associations Between an Indeterminate QFT Assay as Dependant Variables and Demographic, Clinical, Nutritional and Mycobateriological Factors*

| | Indeterminate QFT vs. Valid QFT (N = 691) | |
|---|---|---|
| | Unadjusted | Adjusted |
| | OR (95% CI) | OR (95% CI) |
| Surveillance | | |
| Active | 3.35 (0.45–25.2) | |
| Gender | | |
| Male | 1.61 (0.66–3.88) | 1.41 (0.56–3.58) |
| SGA | | |
| <10th percentile (WHO) | 2.02 (0.67–6.16) | |
| Age (months) | | |
| | **0.84 (0.77–0.91)** | **0.83 (0.76–0.90)** |
| Symptoms (fever and/or cough) | | |
| 2 of 2 symptoms | **3.48 (1.32–9.20)** | **3.20 (1.14–8.95)** |
| 1 of 2 symptoms | 1.90 (0.61–5.92) | 1.34 (0.40–4.50) |
| None† | | |
| Fever and/or cough ≥ 2 weeks | | |
| Yes | **2.70 (1.14–6.36)** | |
| FTT | | |
| Yes | **0.34 (0.15–0.81)** | |
| WHZ | | |
| <−2.59 (n = 173) | 2.19 (0.92–5.23) | 2.48 (0.98–6.28) |
| HAZ | | |
| <−2.14 (n = 176) | 1.41 (0.57–3.51) | **3.08 (1.10–8.58)** |
| WAZ | | |
| <−2.63 (n = 179) | **3.03 (1.29–7.12)** | |
| BAZ | | |
| <−2.51 (n = 175) | **3.17 (1.35–7.45)** | |
| NTM | | |
| ≥1 positive specimen | 0.70 (0.23–2.09) | 0.69 (0.21–2.24) |

Adjusted OR estimates and 95% CI are shown for all variables included in the multivariate model. OR estimates and 95% CI for variables not included in the table are available on request.

*Binary logistic regression was applied. CI that do not overlap the null value OR = 1 are shown in bold.

†Reference group.

QFT (positive/indeterminate) was not influenced by the presence of NTM, and all children with RD1-positive NTM (*M. kansasii*) were QFT negative. No child fulfilled the ATS/IDSA criteria[32] for NTM disease, suggesting that NTMs were commensals in this setting.

The strength of this study is the community-based prospective design with a considerable number of enrolled children in a country with high TB burden but low HIV prevalence (<1%; TB Trials Study Group, unpublished data). This "real-life" context makes our findings very clinically relevant. The high prevalence of malnutrition and colonization by NTMs may limit the generalizability to other settings, but these conditions are typical for many areas with high-to-moderate TB burden. The prevalence of helminth infection is presumably high[33] and might interact with host immune responses,[34,35] but we were unable to collect stool samples in this cohort. Well-quantified TB exposure is a reliable surrogate measure of MTB infection,[36] as reflected in this study by the increased kappa agreement between TST and QFT when the analysis was restricted to TB-exposed children. Unfortunately, graded data on TB exposure was not available in this study. TST ≥ 10 mm outcome was both a referral criteria and 1 of 4 diagnostic criteria (≥1 criteria in addition to characteristic CXR changes) required for a diagnosis of clinical TB. The TST estimates could therefore be subject to selection bias, which is a limitation in our study. This compromise in design was required to avoid losing TB cases; the primary aim of the NCS was to establish the true incidence of TB in this cohort as part of preparing the site for future vaccine trials. This selection bias could result in an overestimation of the TST's sensitivity for clinical TB, but as none of the probable TB cases depended on a positive TST for their diagnosis, this does not affect the TST sensitivity estimate. Furthermore, compared with the OR estimates of the associations between a positive TST and the assessed variables, the OR estimates for a positive QFT outcome may be overestimated because TST−QFT+ discordant positives children within the NCS were only referred if they had known TB exposure or were symptomatic, whereas TST+QFT− children might have had no other referral criteria. Notably, referral based on a positive TST only was present in 51 of 746 children, which limits this effect.

## CONCLUSIONS

This study highlights that in assessing children with suspected TB, it is vital to keep in mind the potential for poorer TST and QFT performance in the most vulnerable, namely the young and/or malnourished. Accordingly, the implementation of WHO's treatment recommendations should be intensified.[37] Withholding treatment in exposed and/or TST/IGRA positive but otherwise healthy children can only be justified if careful clinical follow up is possible. TST and QFT are immunological tests of cellular immunity, which provide indirect evidence of MTB infection. Currently, extensive research is aimed at identifying immunologic biomarkers for diagnostic purposes, with a strong emphasis on cellular immunity. Better diagnostic accuracy in this population is badly needed, but based on the results of this study, we suggest that age and malnutrition are likely to influence the performance of all cellular immunological markers. Future research on other approaches than host cell-mediated immune markers might prove more fruitful in this population.

## ACKNOWLEDGMENTS

*The authors thank Geojith George and Naveen Kumar Kellengere at St. John's Research Institute, Bangalore. The authors also thank Lien M Diep of the Department of Biostatistics, Oslo University Hospital, for statistical assistance. The authors are grateful to Foundation for Innovative New Diagnostics for their partial contribution of QFT kits for the study.*

*TB Trials Study Group: Doherty TM (GlaxoSmithKline, Copenhagen, Denmark); Grewal HMS (Department of Clinical Science, Faculty of Medicine and Dentistry, University of Bergen, and Department of Microbiology, Haukeland University Hospital, Bergen, Norway); Lindtjørn B (University of Bergen, Norway); Hesseling AC (Department of Paediatrics and Child Health, Stellenbosch University, Cape Town, South Africa); Jacob A (Emmaus Swiss Leprosy Project, Palamaner, India); Jahnsen FL (Center for Immune Regulation and the Department of Pathology, Oslo University Hospital, Rikshospitalet-Radiumhospitalet Medical Center, Oslo, Norway); Kenneth J, Kurpad AV, Macaden R, Nelson J, Sumithra S, Vaz M (St. John's Research Institute, Bangalore, India) and Cárdenas V (Aeras, Rockville, MD).*

## REFERENCES

1. Perez-Velez CM, Marais BJ. Tuberculosis in children. *N Engl J Med*. 2012;367:348–361.
2. World Health Organization. Report of the Tenth Meeting of the Strategic and Technical Advisory Group for Tuberculosis (STAG-TB), 2010. [WHO, Geneva, Switzerland]. 2010.
3. Mandalakas AM, Detjen AK, Hesseling AC, et al. Interferon-gamma release assays and childhood tuberculosis: systematic review and meta-analysis. *Int J Tuberc Lung Dis*. 2011;15:1018–1032.

4. Rajaraman D, Jesuraj N, Geiter L, et al.; TB Trials Study Group. How participatory is parental consent in low literacy rural settings in low income countries? Lessons learned from a community based study of infants in South India. *BMC Med Ethics*. 2011;12:3.
5. Government of India, Central TB Division. TB India 2011, Revised National TB Control Programme, Annual Status Report. 2011. Available at: http://planningcommission.nic.in/reports/genrep/health/RNTCP_2011.pdf Accessed April 6, 2014.
6. World Health Organization. Child Growth Standards. [WHO, Geneva, Switzerland]. 2006.Available at: http://www.who.int/childgrowth/standards/Technical_report.pdf. Accessed April 6, 2014.
7. Mikolajczyk RT, Zhang J, Betran AP, et al. A global reference for fetal-weight and birthweight percentiles. *Lancet*. 2011;377:1855–1861.
8. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. [WHO, Geneva, Switzerland]. 2011. Available at: http://apps.who.int/iris/bitstream/10665/85839/3/WHO_NMH_NHD_MNM_11.1_eng.pdf?ua=1. Accessed April 6, 2014.
9. Consensus statement on childhood tuberculosis. *Indian Pediatr*. 2010;47:41–55.
10. McGinn T, Wyer PC, Newman TB, et al.; Evidence-Based Medicine Teaching Tips Working Group. Tips for learners of evidence-based medicine: 3. Measures of observer variability (kappa statistic). *CMAJ*. 2004;171:1369–1373.
11. Shrier I, Platt RW. Reducing bias through directed acyclic graphs. *BMC Med Res Methodol*. 2008;8:70.
12. Zrinski Topić R, Zoričić-Letoja I, Pavić I, et al. Indeterminate results of QuantiFERON-TB Gold In-Tube assay in nonimmunosuppressed children. *Arch Med Res*. 2011;42:138–143.
13. Liebeschuetz S, Bamber S, Ewer K, et al. Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. *Lancet*. 2004;364:2196–2203.
14. Nicol MP, Davies MA, Wood K, et al. Comparison of T-SPOT.TB assay and tuberculin skin test for the evaluation of young children at high risk for tuberculosis in a community setting. *Pediatrics*. 2009;123:38–43.
15. Moyo S, Isaacs F, Gelderbloem S, et al. Tuberculin skin test and QuantiFERON® assay in young children investigated for tuberculosis in South Africa. *Int J Tuberc Lung Dis*. 2011;15:1176–81, i.
16. Rose MV, Kimaro G, Nissen TN, et al. QuantiFERON®-TB gold in-tube performance for diagnosing active tuberculosis in children and adults in a high burden setting. *PLoS One*. 2012;7:e37851.
17. Graham SM, Ahmed T, Amanullah F, et al. Evaluation of tuberculosis diagnostics in children: 1. Proposed clinical case definitions for classification of intrathoracic tuberculosis disease. Consensus from an expert panel. *J Infect Dis*. 2012;205 Suppl 2:S199–S208.
18. Dodd LE, Wilkinson RJ. Diagnosis of paediatric tuberculosis: the culture conundrum. *Lancet Infect Dis*. 2013;13:3–4.
19. del Corral H, París SC, Marín ND, et al. IFNgamma response to *Mycobacterium tuberculosis*, risk of infection and disease in household contacts of tuberculosis patients in Colombia. *PLoS One*. 2009;4:e8257.
20. Lienhardt C, Fielding K, Hane AA, et al. Evaluation of the prognostic value of IFN-gamma release assay and tuberculin skin test in household contacts of infectious tuberculosis cases in Senegal. *PLoS One*. 2010;5:e10508.
21. Radhakrishna S, Frieden TR, Subramani R; Tuberculosis Research Centre (ICMR). Association of initial tuberculin sensitivity, age and sex with the incidence of tuberculosis in south India: a 15-year follow-up. *Int J Tuberc Lung Dis*. 2003;7:1083–1091.
22. Ferrara G, Losi M, D'Amico R, et al. Use in routine clinical practice of two commercial blood tests for diagnosis of infection with *Mycobacterium tuberculosis*: a prospective study. *Lancet*. 2006;367:1328–1334.
23. Basu Roy R, Whittaker E, Kampmann B. Current understanding of the immune response to tuberculosis in children. *Curr Opin Infect Dis*. 2012;25:250–257.
24. Basu Roy R, Sotgiu G, Altet-Gómez N, et al. Identifying predictors of interferon-γ release assay results in pediatric latent tuberculosis: a protective role of bacillus Calmette-Guerin?: a pTB-NET collaborative study. *Am J Respir Crit Care Med*. 2012;186:378–384.
25. Neyrolles O, Quintana-Murci L. Sexual inequality in tuberculosis. *PLoS Med*. 2009;6:e1000199.
26. Dogra S, Narang P, Mendiratta DK, et al. Comparison of a whole blood interferon-gamma assay with tuberculin skin testing for the detection of tuberculosis infection in hospitalized children in rural India. *J Infect*. 2007;54:267–276.
27. Cunningham-Rundles S, McNeeley DF, Moon A. Mechanisms of nutrient modulation of the immune response. *J Allergy Clin Immunol*. 2005;115:1119–28; quiz 1129.
28. Haustein T, Ridout DA, Hartley JC, et al. The likelihood of an indeterminate test result from a whole-blood interferon-gamma release assay for the diagnosis of *Mycobacterium tuberculosis* infection in children correlates with age and immune status. *Pediatr Infect Dis J*. 2009;28:669–673.
29. Gofama MM, Garba AM, Mohammed AA. Mantoux test reactions among children managed for tuberculosis in Maiduguri, Nigeria. *Scand J Infect Dis*. 2011;43:15–18.
30. Piñeiro R, Cilleruelo MJ, García-Hortelano M, et al. Effect of nutritional status on Tuberculin skin testing. *Indian J Pediatr*. 2013;80:271–275.
31. Burl S, Adetifa UJ, Cox M, et al. The tuberculin skin test (TST) is affected by recent BCG vaccination but not by exposure to non-tuberculosis mycobacteria (NTM) during early life. *PLoS One*. 2010;5:e12287.
32. Griffith DE, Aksamit T, Brown-Elliott BA, et al.; ATS Mycobacterial Diseases Subcommittee; American Thoracic Society; Infectious Disease Society of America. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med*. 2007;175:367–416.
33. Ragunathan L, Kalivaradhan SK, Ramadass S, et al. Helminthic infections in school children in Puducherry, South India. *J Microbiol Immunol Infect*. 2010;43:228–232.
34. Lucas M, Nicol P, McKinnon E, et al. A prospective large-scale study of methods for the detection of latent *Mycobacterium tuberculosis* infection in refugee children. *Thorax*. 2010;65:442–448.
35. Wassie L, Aseffa A, Abebe M, et al. Parasitic infection may be associated with discordant responses to QuantiFERON and tuberculin skin test in apparently healthy children and adolescents in a tuberculosis endemic setting, Ethiopia. *BMC Infect Dis*. 2013;13:265.
36. Mandalakas AM, Kirchner HL, Lombard C, et al. Well-quantified tuberculosis exposure is a reliable surrogate measure of tuberculosis infection. *Int J Tuberc Lung Dis*. 2012;16:1033–1039.
37. Hill PC, Rutherford ME, Audas R, et al. Closing the policy-practice gap in the management of child contacts of tuberculosis cases in developing countries. *PLoS Med*. 2011;8:e1001105.