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# Added value of TST, IGRAS and IP-10 to identify children with TB infection

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**Abstract**

**Background:**

Current tests of Tuberculosis infection (Tuberculin Skin Test (TST), Interferon-Gamma-Release-Assays (IGRAS) and Interferon-gamma-induced protein-10 (IP-10)) have limitations and their value when used consecutively to identify infected children has not been explored.

**Methods**

This study describes TST, IGRA and IP-10 responses in children in contact with adults with TB, the agreement of the tests and whether using multiple tests indentifies more infected children. 330 children (1-15 years) in contact with adults with PTB and 156 controls were studies in Ethiopia.

**Main results**

Children exposed to adults with high bacilli grades in sputum were more likely to have positive TST, INF-γ and IP-10 than controls. The agreement of positive tests was directly associated to the sputum bacilli grades ($p < 0.001$ for all). The agreement of negative tests was higher in control children. The consecutive use of the tests increased the number of children classified as having at least one positive test.

**Interpretation**

Using three tests increases the number of children classified as infected. This increase is associated with the bacilli load of the adults. Using only one test may underestimate the proportion of infected children but the interpretation of the data is difficult due to the lack of reference standards.

## Introduction

Children in contact with adults with smear-positive pulmonary tuberculosis (PTB) are at high risk of infection. Although this risk is associated with the closeness of contact and the concentration of bacilli in the sputum of the adult index case [1], their investigation is often unrewarding because, besides the century old tuberculin skin test (TST), there are very few tests to identify TB infections in children. The Interferon Gamma Release Assays (IGRAS), which are said to be more specific than the TST, as they do not cross-react with most non tuberculous mycobacteria or the antigens present in the BCG vaccine, represented a step change in the diagnosis of infection in recent decades. Their sensitivity however can be compromised in young children, in those with severe malnutrition or HIV co-infection [2] and a negative test does not exclude infection. Most recently a further marker, the Interferon-gamma-induced protein 10 (IP-10), has been suggested as a potential test to identify TB infections. The advantage of IP-10 is that it is produced in high levels following *Mycobacterium tuberculosis* (MTB) antigen-specific stimulation in both active TB and LTBI cases, demonstrating its potential as a biomarker for MTB in children [3]. The performance of IP-10 is also said to be independent of age and less compromised in individuals co-infected with HIV [4], but the test is newer and is less standardised than the TST and IGRAS.

There is limited data on the additional value of combining the TST, IGRAS and IP-10 test results to enhance the identification of infected children and whether IP-10 response patterns vary according to the number of bacilli contained in the sputum of the adult case, as previously demonstrated for TST and IGRA .

We describe here whether the concomitant use of TST, IGRAs and IP-10 in children in contact with adults with smear-positive PTB increases the number of children that could be labelled as infected, and whether these results vary with the concentration of bacilli in the adults' sputum.

**Materials and methods**

This was a cross sectional study of 1 to 15 year old children in contact with adults with smear-positive PTB and community controls without known contact with TB. The study was conducted in Hawassa zone, in the Southern Region of Ethiopia. Adults with a history of cough > 2 weeks who had a diagnosis of sputum smear-positive PTB were identified consecutively in Hawassa and Bushullo Major Health centres and Hawassa Referral Hospital. Patients who had children and resided within a 20 km radius of Hawassa were invited to participate and visited at home. Community *controls* were defined as apparently healthy children without known contact with adults with PTB and were selected from Hawassa and surrounding rural areas after preparing a list of the villages within a 20 Km radius of Hawassa. Villages were selected at random and one household was selected from each village by spinning a pen somewhere between the centre and the edge of the village. One child aged 1-15 years old identified at random from the selected household was invited to participate.

All participant children were applied a TST using 2 units of Purified Protein Derivate (PPD, RT 23, Statens Serum Institute, Copenhagen, Denmark) using the Mantoux method and indurations were measured using the palpation method 48 to 72 hours later. TST results were graded as negative (< 5mm), intermediate (≥ 5 and < 10 mm) and positive (≥10 mm). Blood samples for IGRAS were collected using QuantiFERON-TB Gold In-Tube (QFT-IT) test (Cellestis, Victoria, Australia) following the manufacturer's instructions. Supernatant plasma was harvested from the QFT-IT tubes after centrifugation and stored at -70°C. INF-γ was measured using the QFT-IT ELISA in a Bio-Rad Plate reader (Model n 550), read at 450 nm and classified as positive, negative or indeterminate using the manufacturer's software. IP-10 concentrations were measured in the same supernatants using a Human IP-10 ELISA Construction Kit (Antigenix America Inc, New York,

NY) and classified as positive or negative according to a receiver operating curve, as previously described [5]. HIV status was established using two blood-based ELISA methods.

The number of bacilli in the adults sputum was graded as "scanty", +, ++, or +++ and used to stratify the proportion of children with positive TST, INF-γ or IP-10. The added value to INF-γ and IP-10 were calculated for children with negative TST or both negative TST and INF-γ to describe whether using multiple tests would increase the proportion of children with at least one positive test.

The study protocol was approved by the Health Bureau of the Southern Region, the Research Ethics Committees of the Liverpool School of Tropical Medicine and Hawassa University, and the Ethiopian Sciences and Technology Commission. Children were enrolled after obtaining informed parental consent. The study is registered in the clinicatrials.gov clinical trials register (number NCT00456469)

**Results**

A total of 486 children were enrolled. Of these 330 (median [range] age 8 [1-15] years) were contacts and 156 (median [range] age 6 [1– 15] years) community controls, with similar proportions of contacts (167, 51%) and controls (79, 51%) were male. The adults of 15 (4.5%) children had +++ grades in their sputum smears, 109 (33%) had ++, 188 (57%) + and 18 (5.5%) "scanty" bacilli. Seventeen (3.5%) children did not have TST results and 28 (6%) did not have INF-γ or IP-10 results.

The proportion of children with positive INF-γ or positive IP-10 increased with increasing adult sputum grades, ranging from 17.6% and 23.5% in contacts of adults with scanty grades to 57% and 50% in contacts with adults with +++ , respectively (Chi square for trend, $p < 0.001$ for both), as

shown in Table 1. The proportion of children with positive TST (86.7%) was also highest among children in contact with adults with +++. However children in contact with adults with scanty bacilli also had a high proportion of positive TST results. In contrast, the proportion of children with positive TST, INF-γ and IP-10 was low in controls (12.8%, 13.1% and 5.8%, respectively). Figure 1 describes the percentage of positive test results for each of the three tests by the adults' smear microscopy grades.

Figure 2 describes the agreement of TST, INF-γ and IP10. The proportion of children with positive TST and INF-γ or with positive TST and IP-10 increased with increasing sputum grades in the adults, with lower concordance among controls and higher concordance in children in contact with adults with high sputum grades (+++). Similarly, the negative concordance of TST, INF-γ and IP10 was higher in controls than among children in contact with adults with ++/+++ sputum grades. Finally, the concordance between INF-γ and IP10 did not vary with the adults' sputum grade.

Four hundred thirty five (282 contact and 153 control) children had the three test results. After exclusion of intermediate TST and indeterminate INF-γ results, 313 children had a full set of interpretable results. These included 9 children in contact with adults with +++, 74 with ++, 109 with +, 7 with scanty sputum grades and 114 controls. Figure 3 describes the proportion of children that would be classified as infected by using the TST result alone, by adding the INF-γ to TST-negative children and by adding the IP-10 to TST- and INF-γ-negative children. The results are presented stratified by the adults' sputum grades. All children exposed to adults with +++ were positive by TST or INF-γ and thus IP-10 did not add further positive children. A high proportion of children exposed to adults with + and ++ sputum grades had positive TST or INF-γ (72% and 70%, respectively). These percentages increased to 75% and 78%, respectively, by adding IP-10 as a third marker). Of note, a small but significant number of community controls had negative TST but positive INF-γ (12 of 114) or negative TST and INF-γ but positive IP-10 (3 of 114).

The same proportion of contacts and controls received BCG (249/335 [74.3%] vs 120/156 [76.9%], p>0.5). Although contacts were more likely to have positive TST and QFT than controls, the responses within the group were similar regardless of their BCG status (p>0.5 for both TST and QFT among children with and without BCG). The number of children infected with HIV was higher in contact than control children (27/258 [10%] and 3/156 [2%] children tested, respectively, p < 0.01). The low number of HIV infected children however precludes further analysis of TST and QFT results stratified by HIV (Table 2).

**Discussion**

Children in contact with adults with smear-positive PTB are at high risk of infection and disease progression. The World Health Organization recommends initiating Isoniazid prophylaxis for young children in close contact with an adult with TB without investigating whether the child is infected. However parents are frequently reluctant to provide prophylaxis and often abandon the 6-month recommended course [6-9]. It might be that identifying infected children and targeting prophylaxis to high risk groups could be more acceptable to parents, but the poor sensitivity of TST, its cross reactions with BCG, and its unreliability in children with malnutrition, immunosupression and severe infections [10] limit the applicability of this approach. IGRA's limitations are also becoming apparent with their widespread use in recent years. TB is most endemic in locations where accessibility to diagnostics is a major barrier and thus the applicability of these tests is limited by their costs, unavailability and laboratory requirements. Further, their well documented phenomenon of conversions (from negative to positive) and reversions (from positive to negative) is frequent but poorly understood [11]. False negative responses have also been reported in very young children and those infected with HIV [12], although they occur at a lower frequency than with the TST. In addition, IGRAS have the logistical difficulty of collecting blood from young children, test processing and procurement, which results in a sizable number of children

not having test results available [12]. A recent policy statement from WHO indicated that there was insufficient data on the performance of IGRAs in low- and middle-income countries and that given its higher costs the use of IGRAS in these settings is not recommended (http://www.who.int/tb/features_archive/policy_statement_igra_oct2011.pdf). IGRAS however have high specificity and their use in tandem with TST may have added diagnostic value [2, 13]. Several screening strategies to identify individuals with TB infection are emerging internationally. These include using either TST or IGRAS alone, using both tests independently or using them consecutively, usually the TST followed by IGRAS. The latter approach is considered the most cost-effective scheme in some settings, although there is only limited objective evidence to date [14].

IP-10, an additional marker recently reported to identify TB infections [15], is expressed in larger quantities than INF- γ, making it easier to measure in blood. HIV infected individuals are able to express IP-10 [4], which is likely due to some IP-10-expressing cells being spared by the HIV infection. Although its performance is less well documented than TST and INF-γ, it has been suggested to have added value to identify infections when used together with INF-γ [16, 17].

The risk of infection in most groups is associated to the number of bacilli in the sputum of the adult, as the three markers were increasingly positive with increasing sputum grades. The use of three markers increased the number of individuals with at least one positive test, reaching 100% in children in contact with adults with +++. The agreement of the tests varied with the sputum smear grades, with a higher proportion tests being simultaneously positive among children exposed to adults with +++ and a lower concordance among contact children who had low risk of infection. Unfortunately the number of children in the +++ category was small and the study is underpowered to detect an added value in these children, as this is the group where the previous tests are more likely to be positive, thus leaving a small margin for added value. If all tests are interpreted as true

positives, a very high proportion of children in contact with adults with TB would be infected. The use of the smear grades, as a proxy of the risk of infection suggests that the test responses may be true positives, however this interpretation is problematic as the tests evaluation is hampered by the lack of a suitable reference standard and without this reference standard it is impossible to differentiate correct from incorrect test results. Furthermore, the increasing number of controls with a single positive result might reflect a low false positive test rate, which accumulates when using several tests consecutively. Thus our results may indicate that as significant proportion of children with asymptomatic infections may be missed by the use of a single test and/or that a significant number of children at low risk of infection may have at least one positive test and that their simultaneous used need to be interpreted with caution in low risk populations.

Although the interpretation of the data remains problematic, the TST in combination with INF-γ and IP-10 increases the proportion of children with one or more positive test results. This increase is in proportion with the bacilli load in the adults' sputum smear. A high proportion of contacts had at least one marker of infection, suggesting that the proportion of children infected after exposure to adults with PTB may be underestimated by using a single test for diagnosis.

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Table 1: TST, INF-γ and IP-10 results in children by sputum grade of the index case

| | | Bacilli count of the adult | | | | Controls | All |
|---|---|---|---|---|---|---|---|
| | | +++ | ++ | + | Scanty | | |
| | **N** | **15** | **109** | **188** | **18** | **156** | **486** |
| **TST** | **Pos** | 13 (86.7) | 46 (43) | 95 (54.6) | 14 (82.4) | 20 (12.8) | 469 |
| | **Int** | 0 | 19 (17.8) | 28 (16.1) | 1 (5.9) | 15 (9.6) | |
| | **Neg** | 2 (13.3) | 42 (39.3) | 51 (29.3) | 2 (11.8) | 121 (77.6) | |
| **INF-γ** | **Pos** | 8 (57.1) | 53 (50.5) | 74 (43.8) | 3 (17.6) | 20 (13.1) | 458 |
| | **Ind** | 5 (35.7) | 13 (12.4) | 24 (14.2) | 10 (58.8) | 25 (16.3) | |
| | **Neg** | 1 (7.1) | 39 (37.1) | 71 (42) | 4 (23.5) | 108 (70.6) | |
| **IP-10*** | **Pos** | 7 (50) | 60 (59.4) | 96 (55.8) | 4 (23.5) | 9 (5.8) | 458 |
| | **Neg** | 7 (50) | 41 (40.6) | 76 (44.2) | 13 (76.5) | 145 (94.2) | |

*IP-10 was graded positive using a cut-off of 3022 pg/ml as calculated in the ROC curve

Table 2: TST and INF-γ results in children by BCG and HIV status

| | | Contacts | | | | Controls | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **BCG +** | **BCG -** | **HIV +** | **HIV -** | **BCG +** | **BCG -** | **HIV +** | **HIV -** |
| **TST** | **Pos** | 135 (56) | 29 (47.5) | 12 (48) | 123 (56.7) | 16 (13.3) | 2 (5.9) | 0 | 20 (13.1) |
| | **Int** | 38 (15.8) | 9 (14.8) | 5 (20) | 24 (11) | 12 (1) | 3 (8.8) | 0 | 15 (9.8) |
| | **Neg** | 68 (28.2) | 23 (37.7) | 8 (32) | 70 (32.3) | 92 (76.6) | 29 (85.3) | 3 (100) | 118 (77.1) |
| **INF-γ** | **Pos** | 105 (46) | 24 (36.4) | 10 (41.6) | 93 (41.5) | 13 (10.9) | 6 (18.2) | 0 | 20 (13.3) |
| | **Ind** | 32 (14) | 20 (30.3) | 4 (16.7) | 44 (19.6) | 23 (19.3) | 2 (6) | 0 | 25 (16.7) |
| | **Neg** | 91 (40) | 22 (33.3) | 10 (41.6) | 87 (38.8) | 83 (69.8) | 25 (75.8) | 3 (100) | 105 (70) |

Figure 1: Proportion of children with positive TST, INF-γ and IP-10 results by sputum grade of the index case

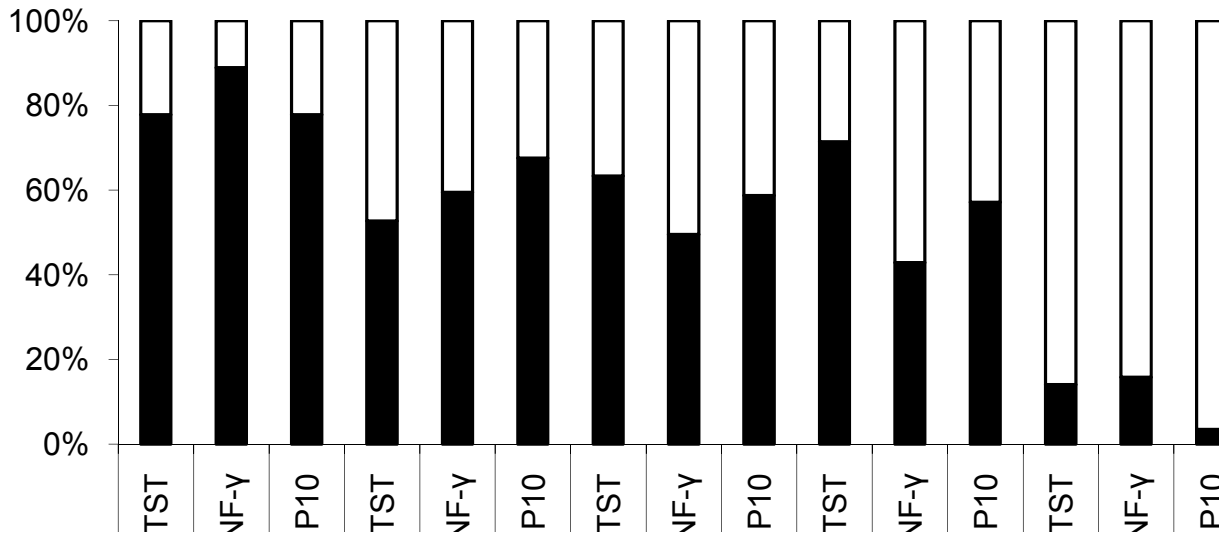

Figure 2: Agreement between TST, INF-γ and IP-10 by sputum grade of the index case.

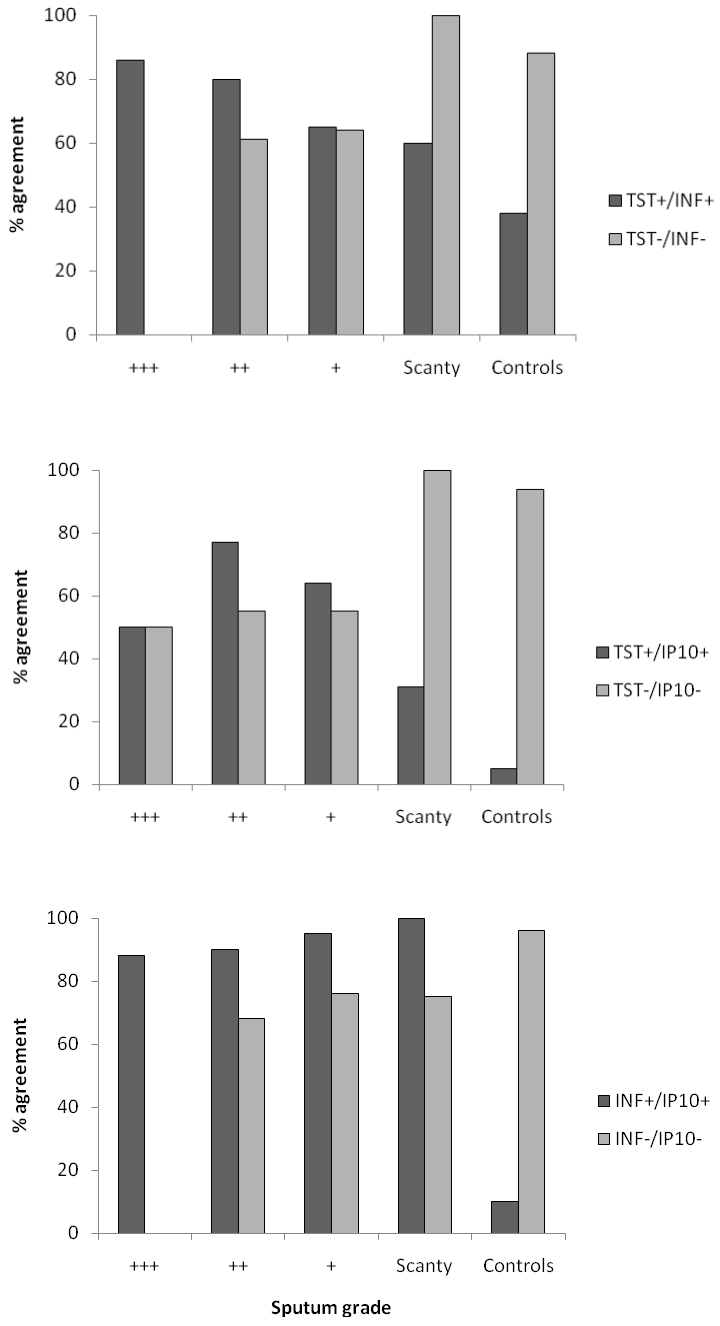

Figure 3: Proportion of children with positive TST, positive INF-γ/negative TST and positive IP-10/negative TST and INF-γ by sputum grade of the index case