Two Interferon Gamma Release Assays and Tuberculin Skin Test in the diagnosis of *Mycobacterium tuberculosis* infection and disease in The Gambia

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BACKGROUND

- The tuberculin skin test until recently was the only diagnostic test for latent TB infection (LTBI)

- In recent years, the interferon gamma release assays (IGRA) that measure interferon gamma released by sensitized T-cells, have been developed for the diagnosis of LTBI and provides a means of identifying and tracking short lived effector T-cells responding to specific TB antigens.

- IGRAs differ from each other mainly with respect to the technique of IFN-γ detection (enzyme linked immunospot; ELISPOT vs. enzyme linked immunosorbent assay; ELISA) and the samples utilized (peripheral blood mononuclear cells vs. whole blood)

- Two interferon gamma release assays (IGRAs) are now licensed for the diagnosis of LTBI

- The T.SPOT.TB® is ELISPOT-based and uses PBMCs while QuantiFERON-TB Gold® is a whole-blood ELISA test.

- The IGRAs, now available as standardized assays are being evaluated in a variety of settings leading to an increasing body of literature supporting their use

- But there remains insufficient data on test performance in high risk groups such as children

- We had previously compared an in-house IGRA to TST in children across a sleeping gradient of exposure to an index TB case and found it slightly less sensitive than TST in diagnosis of LTBI from recent exposure.

  [Hill,et al. Pediatrics 2006;117: 1542-1548]

Objectives

- **Hypothesis-**
  - The diagnostic performance of 2 commercial IGRA assays compared to the TST across a TB exposure gradient is equivalent in Gambian adult and childhood TB contacts.

- **Objectives-**
  - To evaluate the response of the TST, T-SPOT.TB and QuantiFERON TB Gold In Tube (QFT-GIT) tests in childhood TB contacts across a gradient of sleeping proximity to an index case

  - To estimate the sensitivity of all tests in smear positive TB cases

Methods (1)

- Sputum smear positive TB cases aged ≥15 years were consecutively recruited.
- Contacts aged 0.5-14 years who have lived for ≥3 months in the same compound as the case were also recruited
- They were excluded if they had been treated for TB in the past year or diagnosed with TB within a month of recruitment
- Written informed consent was obtained from all subjects

Methods (2)

- Blood samples taken for both IGRAs, HIV testing and TST given.

- Ascertainment of exposure
  - Tuberculosis contacts were categorized according to where they slept:
    - in the same bedroom as the case,
    - A different bedroom in the same house, or
    - in a different house in the same compound.

- Procedures
  - TST was done with 2 TU PPD RT-23A TST, A 10mm cut off was used. Fieldworkers who gave this test were blinded to lab results

  - All commercial assays were performed and results interpreted according to the manufacturers instructions. Lab personnel were blinded to subjects status and TST results.

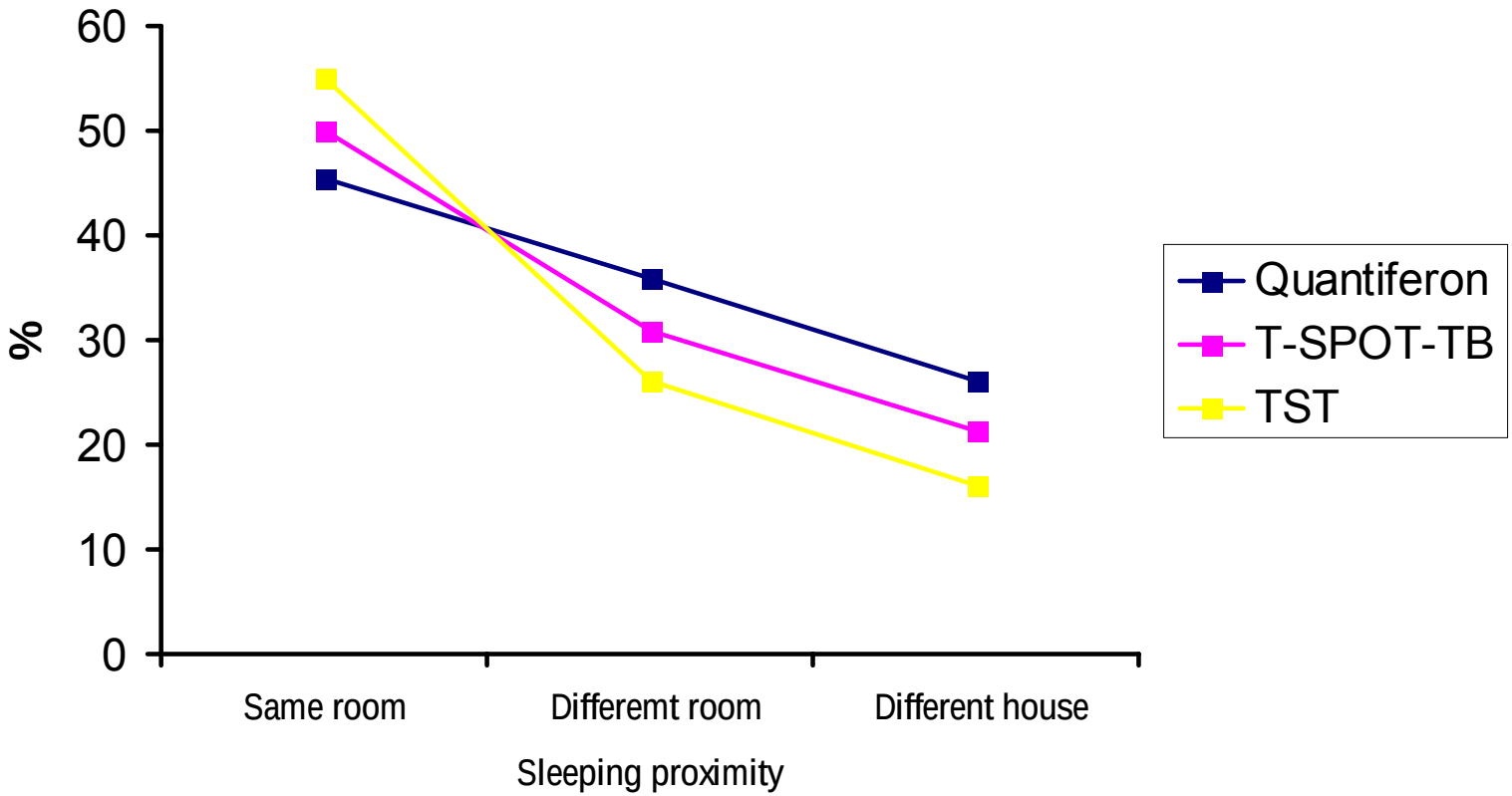
Preliminary Results (1)

- 385 subjects recruited, 100 cases and 285 contacts

- The sensitivities all tests in TB cases were
  - 82.8%[95%CI 81.5-94.9%] for T-SPOT.TB,
  - 85.4%[95%CI 81.4-95.8%] for QFT-GIT
  - 66.7%[95%CI 46.3-87.0%] for TST

- The prevalence of LTBI by
  - TST, 26.5% [95%CI 21.0-32.0%]
  - T-SPOT-TB 27.3% [95%CI 24.2-36.1%]
  - QFT-GIT 34.1% [95%CI 27.0-41.5%]

Percentage of contacts positive for TST, T-SPOT.TB and QFT-GIT by *M. tuberculosis* exposure



Univariable and multivariable odds ratios determined by logistic regression for sleeping proximity as a surrogate marker of exposure to *M. tuberculosis*

	T-SPOT.TB (n=231)				QuantiFERON (n=171)				TST (n=248)			
	Positive results No.(%) of contacts	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	p-value	Positive results No. (%) of contacts	Unadjusted OR (95% CI)	Adjusted OR(95% CI)	p-value	Positive results No.(%) of contacts	Unadjusted OR(95% CI)	Adjusted OR(95% CI)	p-value
Sleep proximity												
Different house	16 (21.3)	1	1		14(25.9)	1			12(16.2)	1	1	
Different room	40 (30.8)	2.9 (1.0-8.2)	3.4 (1.1-10.3)		34(35.8)	1.8 (0.7-4.9)	1.6 (0.7-3.9)		37(25.9)	1.8 (0.8-4.1)	2.4 (1.1-5.1)	
Same room	13 (50.0)	7.4 (2.0-28.2)	10.0 (2.4-41.4)	0.007**	10(45.5)	2.6 (0.7-9.4)	3.5 (1.0-11.7)	0.13**	17(54.8)	3.2 (1.2-8.9)	9.2 (3.3-25.8)	0.0001**

TST ≥10mm defined as positive, **Test for trend

Results (2)
Agreement/Discordance Analysis

- The agreement in contacts between
  - T-SPOT.TB&QFT was 83% (κ=0.60,discordance p=0.05),
  - TST&QFT-GIT 75.5% (κ=0.44, discordance p=0.006),
  - TST&T-SPOT-TB 73.3% (κ=0.43,discordance p=0.0003),

- The agreement in index cases between
  - T-SPOT.TB&QFT was 85.3% (κ=0.27,discordance p=0.76),
  - TST&QFT-GIT 88.9% (κ=0.72, discordance p=0.37),
  - TST&T-SPOT-TB 59.1% (κ=0.1, discordance p=0.09)

Results

- Effect/influence of BCG vaccination
  - T-SPOT.TB: OR 1.3 (0.7-2.4), p=0.43
  - QFT: OR 1.1 (0.6-2.2), p=0.77
  - TST: OR 0.7 (0.4-1.4), p=0.35

- Effect/influence of sputum smear grade in TB case
  - T-SPOT.TB: OR 1.0 (0.5-2.2), p=0.97
  - QFT: OR 1.6 (0.7-3.9), p=0.28
  - TST: OR 0.9 (0.4-1.7), p=0.67

Conclusions

- The detection of LTBI was similar with all 3 tests although the QFT tended towards more positive results

- All 3 tests responded to the *M.tuberculosis* exposure gradient but significantly so for TST and TSPOT compared to QFT.

- There was good concordance between T-SPOT.TB and QFT but significant discordance between TST&T-SPOT-TB and between the TST&QFT-GIT

CONCLUSIONS

- The IGRAs have much better sensitivity in TB cases compared to TST but sensitivity in all tests remain suboptimal for the diagnosis of TB

- These results do not support the replacement of TST by IGRAs for diagnosis of LTBI in The Gambia

Future perspectives

- Need to understand the nature and reason for discordance between IGRAs and TST
- Evaluate the utility of IGRAs as biomarkers for treatment or vaccine efficacy
- What is the value of IGRAs in predicting progression from LTBI to TB disease?
- To understand test and biologic variability

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