

Rapid site-specific immunodiagnosis of tuberculosis: Is it useful in clinical practice in South Africa?

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Introduction:

Delayed diagnosis of smear negative pleural TB, pulmonary TB, and TB meningitis is associated with significant mortality. Rapid T-cell immunodiagnosis using the antigen-specific IFN- γ release assays (IGRAs), with cells from the site of disease, holds promise to rapidly detect TB.

Methods:

We prospectively evaluated the performance of the two standardized IGRAs QuantiFERON[®]-TB-Gold-In-Tube (QFT-GIT) & T-SPOT[®]-TB, unstimulated IFN- γ & PCR in 294 pleural (PLF), TB meningitis (TBM) and smear negative pulmonary TB (PTB) suspects, using pleural, cerebrospinal and pulmonary alveolar mononuclear cells. Histology and culture positivity for *M.tb* served as the gold standard.

Results:

74 subjects with suspected TB pleural effusions (27% HIV+) were recruited (48, 19 and 8 with definite, probable and non-TB, respectively). 11/74 (15%) of pleural samples had total cell counts that were inadequate for T-cell processing. The sensitivity and specificity of T-SPOT[®]-TB was 85% & 54% and for QFT-GIT was 61% & 88%. This was comparable to ADA (94% & 64%) but significantly poorer than unstimulated IFN- γ (97% & 100%; p = 0.002).

85 subjects with suspected pulmonary TB (28% HIV+) were recruited (24, 11, 48, and 2 with definite, probable, non-TB and an uncertain diagnosis, respectively). 34% of T-SPOT[®]-TB and 41% of QFT-GIT test results were inconclusive. The sensitivity & specificity for T-SPOT[®]-TB and QFT-GIT, was [86, 94 (n=55)] and [55, 86 (n=46)], respectively. Rapid diagnosis of TB was achieved more frequently with T-SPOT[®]-TB than smear-microscopy [14/24(58%) vs. 7/24(29%) of definite TB cases; p=0.02].

135 subjects with suspected TB meningitis were recruited (26, 61 and 48 with definite, probable and non-TB, respectively). The optimum cut-point derived from the ROC yielded a sensitivity & specificity for ESAT-6 and CFP-10 of [69% & 98%] and [69% & 96%] respectively.

Conclusions:

(i) In a high burden setting, IGRAs have limited utility for the diagnosis of pleural TB. Unstimulated IFN- γ was the most accurate biomarker to distinguish TB from non-TB effusions. (ii) The RD-1 ELISPOT assay is an accurate test for the diagnosis of smear-negative PTB. However it is limited by a high proportion of inconclusive reactions. (iii) The RD-1 ELISPOT assay is a good rapid rule-in test for TB meningitis.

Introduction

The diagnosis of tuberculosis is frequently delayed due to the inability to rapidly identify Acid Fast Bacilli (AFB's) in clinical material.

The delay in diagnosis may lead to significant delays in instituting appropriate therapy and resulting increased morbidity and mortality.

Smear negative pulmonary TB, pleural TB and TB meningitis are particularly difficult to diagnose.

New rapid immuno-diagnostic assays hold promise to rapidly detect TB, however they require clinical validation.

Methods

294 TB suspects were prospectively recruited

- 74 Pleural TB
- 85 Smear negative pulmonary TB
- 135 TB meningitis

Diagnostic criteria

Definite TB:

- Positive *M. TB* culture
- Histology in keeping with tuberculosis (granuloma or AFB +ve) and Clinico-radiological picture consistent with TB and a response to anti-TB treatment

Probable TB:

Clinical picture of TB and treated for TB without "definite TB" diagnosis

Non-TB:

- Alternative diagnosis made on histology
- Not treated for TB
- On 3 month follow-up not found to have developed TB

Assays investigated:

- Standardized interferon gamma release assays (IGRA)
 - T-SPOT[®]-TB
 - QuantiFERON[®]-TB-Gold-In-Tube
- Unstimulated Interferon- γ

Pleural Effusions

Results

- 74 pleural TB suspects recruited
 - 48 definite TB, 19 probable TB & 8 non-TB
- 46.4% (26/56) were HIV positive.
- Pleural T-cell assays could only be performed in 63 of the 74 (85%) patients (inadequate cell numbers were isolated from 11 patients)

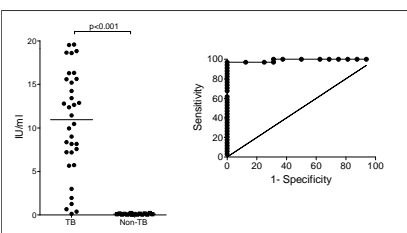


Figure 1 Pleural Fluid Unstimulated Interferon- γ Results

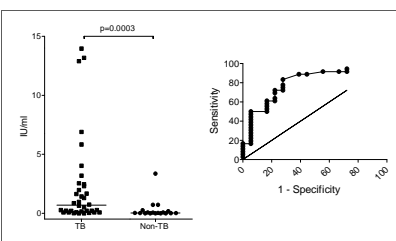


Figure 2 Pleural Fluid QuantiFERON-TB GIT Results

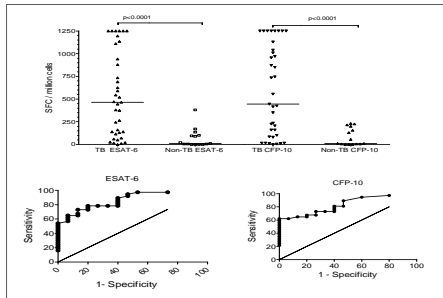


Figure 3 Pleural Fluid T-SPOT[®]-TB Results

Pleural effusion performance characteristics (95% C.I.)				
Diagnostic Test*	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Adenosine Deaminase (ADA)	96% (86-99)	69% (44-86)	98% (85-99)	92% (82-97)
Unstimulated IFN- γ	97% (85-99)	100% (81-100)	100% (90-100)	94% (73-99)
T-SPOT [®] -TB	86% (71-94)	60% (36-80)	84% (69-92)	64% (39-84)
QFT-GIT	57% (41-72)	80% (55-93)	87% (68-96)	44% (28-63)

*Cut-points used: ADA >30 IU/ml, Unstimulated IFN- γ 0.31 IU/ml, T-SPOT[®]-TB > SFC 24 SFC/million pleural mononuclear cells, QFT-GIT >0.35 IU/ml

Broncho-alveolar lavage

Results

- 85 smear negative TB suspects
 - 24 definite TB, 11 probable TB, 48 non-TB and 2 uncertain
- Inconclusive results occurred frequently:
 - 34% for T-SPOT[®]-TB
 - 41% for QFT-GIT
- Inconclusive results resulting from failure of the positive control was more common in the QFT-GIT assay (85% vs.46% in T-SPOT[®]-TB; p=0.001)
- Using SEB as an additional control reduced failure to 3%

- Rapid diagnosis of TB was achieved in 58% of definite TB cases using the T-SPOT[®]-TB assay compared to 29% by smear-microscopy alone.

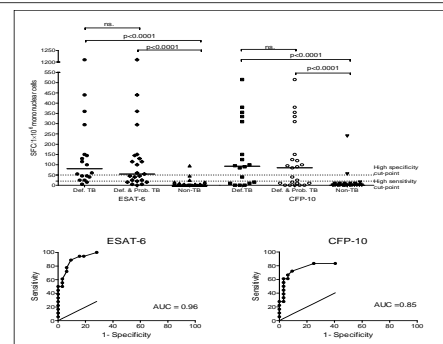


Figure 4 Bronchoalveolar lavage Fluid T-SPOT[®]-TB Results

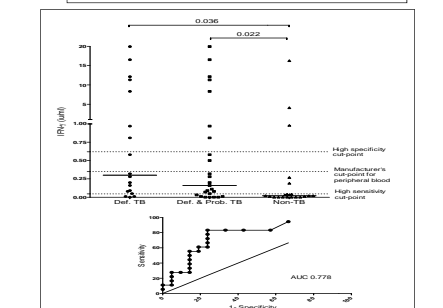


Figure 5 Bronchoalveolar lavage Fluid QuantiFERON[®]-TB GIT Results

Bronchoalveolar lavage performance characteristics (95% C.I.)				
Diagnostic Test*	Sensitivity	Specificity	Positive predictive value	Negative predictive value
T-SPOT [®] -TB	78% (55;91)	94% (80;98)	88% (64;97)	88% (74;95)
QFT GIT	55% (34;75)	86% (65;95)	77% (50;92)	69% (50;84)

*Cut-points used: T-SPOT[®]-TB \geq 30 SFC/ million alveolar mononuclear cells, QFT-GIT >0.35 IU/ml

Cerebrospinal fluid

Results

- 135 subjects with suspected TB meningitis
 - 26 definite TB, 61 probable TB and 48 non-TB
- [Further detailed analysis is in progress]

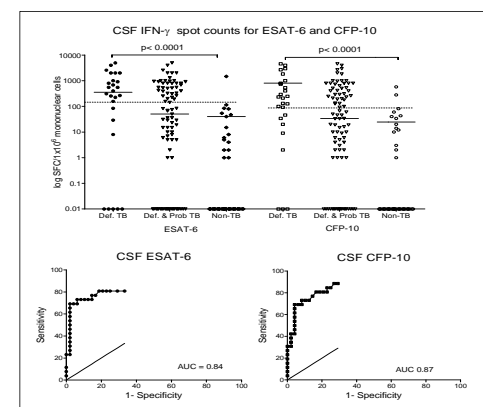


Figure 6 Cerebrospinal Fluid T-SPOT[®]-TB Results

CSF performance characteristics (95% C.I.)				
Diagnostic Test*	Sensitivity	Specificity	Positive predictive value	Negative predictive value
T-SPOT [®] -TB	69% (48-86)	98% (83-99)	86% (65-95)	85% (73-92)
ESAT-6	69% (48-86)	96% (86-99)	90% (70-97)	85% (73-92)

*Cut-points used: T-SPOT[®]-TB ESAT-6 \geq 135 SFC/ million CSF mononuclear cells, CFP-10 \geq 87 SFC/ million CSF mononuclear cells

Conclusions

- In pleural fluid, unstimulated IFN- γ was the most accurate biomarker to distinguish TB from non-TB effusions.
- In BAL, although a rapid diagnosis can be made with the RD-1 ELISPOT assay using alveolar mononuclear cells, usefulness is limited by a high proportion of indeterminate reactions.
- In CSF, IGRAs hold promise as a rapid rule-in test for TB meningitis.
- There are compartment specific differences (PLF vs. CSF vs. BAL) which impacts on the utility of IGRAs.
- The CSF and alveolar compartments are prone to reduced sensitivity.
- The CSF is less prone to inconclusive results.