Published in final edited form as:

J Infect. 2011 June ; 62(6): 456–461. doi:10.1016/j.jinf.2011.04.003.

LIMITED ADDED VALUE OF T-SPOT.*TB* BLOOD TEST IN DIAGNOSING ACTIVE TB: A PROSPECTIVE BAYESIAN ANALYSIS

Aileen E Boyd, MRCP MD,

Department of Medicine, Royal Free Hospital, Pond Street, London NW3 2QG, UK. Tel 0776 023 1654. Fax 0208 7731510

Anthony Ashcroft, BSc (Hons),

Department of Microbiology, Homerton University Hospital, Homerton Row, London E9 6SR, UK.

Marc Lipman, FRCP MD, and

Department of Respiratory Medicine, Royal Free Hospital, Pond Street, London NW3 2QG, UK.

Graham H Bothamley, FRCP PhD

Department of Respiratory Medicine, Homerton University Hospital, Homerton Row, London E9 6SR, UK

Aileen E Boyd: aileenboyd@doctors.org.uk; Anthony Ashcroft: ta64@mac.com; Marc Lipman: Marclipman@nhs.net; Graham H Bothamley: Graham.Bothamley@homerton.nhs.uk

Abstract

Objectives—To determine the diagnostic value of a blood interferon-gamma release assay in suspected active tuberculosis (TB)

Methods—136 subjects with suspected pulmonary TB (pTB) at a single London centre with intermediate TB incidence, were clinically graded into low (<25%), medium, or high (>75%) likelihood of active pTB and then tested by T-SPOT.[®]*TB* assay. The diagnosis was confirmed by culture (n=33), treatment response (n=13) or a firm alternative diagnosis (n=90).

Results—Overall, the T-SPOT.*TB* sensitivity was 74% (95% confidence intervals 60–84%), positive predictive value (PPV) 56% (43–68%), negative predictive value (NPV) 83% (71–90%), positive likelihood ratio (PLR) 1.75 and negative likelihood ratio (NLR) 0.45. Results for high pTB likelihood subjects: PPV 100%, NPV 25% (7–60%), PLR >69, NLR 0.31. Results for intermediate pTB likelihood subjects: PPV 67% (41–85%), NPV 88% (65–96%), PLR 2.39, NLR 0.26. Results for low pTB likelihood subjects: PPV 15% (6–34%), NPV 92% (79–97%), PLR

© 2011 The British Infection Society. Published by Elsevier Ltd. All rights reserved.

Correspondence to: Aileen E Boyd, aileenboyd@doctors.org.uk.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Contributors: AB and GB contributed to trial design and wrote the protocol. AB was responsible for recruitment of patients and collected the data. AB, GB and ML provided clinical expertise. AA performed the laboratory tests. AB drafted the first version of the manuscript. GB and ML critically reviewed, revised, and supplemented the manuscript. All authors approved the final version. AB is the guarantor.

Preliminary data from this study was first presented at the British Thoracic Society Winter Meeting, 2005 and the American Thoracic Society Annual Conference, 2006.

All authors have no conflict of interest to declare.

1.23, NLR 0.80. False negatives occurred in 24% of cases of active tuberculosis (4 smear and culture-positive, 3 smear-negative and culture-positive, and 4 culture-negative).

Conclusions—The predictive values and likelihood ratios show the T-SPOT.*TB* test does not assist in confidently confirming or excluding active TB, regardless of the pre-test probability of disease.

Introduction

The 6 kDa early secretory antigen of TB (ESAT-6) and the 10 kDa culture filtrate protein (CFP-10), expressed by *Mycobacterium tuberculosis* but not by *M. bovis*-BCG, have heralded a new era in TB diagnostics (1). The T-SPOT®.*TB* test (Oxford Immunotec, Oxford, UK) and QuantiFERON-TB Gold In Tube (Cellestis Ltd, Carnegie, Victoria, Australia) are commercially available tests incorporating these antigens. These interferon-gamma release assays (IGRA) not only have a higher specificity but a meta-analysis of 124 studies (2) has also shown higher sensitivity than the tuberculin skin test (TST), however, like the TST they remain unable to distinguish active from latent TB (3, 4). The usefulness of these tests as diagnostic aids to rule in or rule out active TB in routine clinical practice has not yet been proven as this depends on the pre-test probability of TB in that situation. Here we evaluate, by Bayesian analysis, the value of the T-SPOT.*TB* test, in the diagnostic work-up of patients attending a hospital in London, UK with suspected pulmonary TB.

Bayesian logic can assist the assessment of diagnostic tests by determining the population where a test is likely to be most helpful. Pre-test probabilities of TB may be determined using scoring systems (5) or clinical estimates (6). The latter have been used to assess predictive values of a nucleic acid amplification test (NAAT) in the diagnosis of TB (6). We used similar techniques to determine predictive values and likelihood ratios of the T-SPOT.*TB* test. Likelihood ratios combine information about sensitivity and specificity, and are considered the best measure of the usefulness of a test as they indicate how much more likely a positive (or negative) test is to be found in someone with (or without) the disease (7). “A likelihood ratio greater than 1 indicates that the test result is associated with the presence of the disease, whereas a likelihood ratio less than 1 indicates that the test result is associated with the absence of disease... Likelihood ratios above 10 and below 0.1 are considered to provide strong evidence to rule in or rule out diagnoses respectively in most circumstances” (8).

Patients and Methods

Patients

Patients were recruited prospectively at the Homerton University Hospital, Hackney, East London, UK between August 2004 and March 2006 as part of a study *Clinical Suspicion of Tuberculosis: A Technology Assessment Tool*. This is an intermediate TB incidence area (rate 60/100,000) with a large immigrant population migrating from areas of high TB incidence. The research ethics committee of the East London and City Health Authority (REC number P/03/285) granted ethical approval and all patients consented to have blood drawn for T-SPOT.*TB* testing in addition to standard investigations including microbiological/histological analysis. 150 patients (demographic details in table 1) were recruited from TB clinics, general respiratory clinics, the emergency department and from medical, surgical, obstetric and psychiatric wards. All patients had been assessed by another consultant or primary care physician, who considered active pulmonary TB a possible diagnosis and requested sputum smear microscopy and mycobacterial culture.

Two other physicians, one in training (AEB, subsequently referred to as physician A) and the other a consultant lead in TB (GHB, subsequently referred to as physician B), estimated the clinical suspicion of TB (CSTB) as a percentage between 1% and 99% based only on clinical history (including HIV test result), clinical examination findings, chest radiograph and sputum smear results using the questionnaire developed for a previous study (6) and being blind to the T-SPOT.TB results. The questionnaire (see supplementary material appendix 1) collected data on year and country of birth; gender; ethnicity; smoking, alcohol and drug use; homelessness or use of hostels; imprisonment; sexual history; immigration; personal and family history of TB; duration of symptoms (cough, fever, sweats, weight loss, hemoptysis, swollen glands); co-morbidities; examination findings; HIV status and CD4 count; chest radiograph appearance; and sputum smear result. The CSTB scores were then stratified into low (<25%), intermediate (25–75%) or high (>75%) estimated pre-test probability of TB. Where estimates differed by category, an independent TB expert (ML, subsequently referred to as physician C) adjudicated (see supplementary material appendix 2). The final diagnosis was obtained through analysis of patients' medical records and laboratory results and confirmed at least three years after entry into the study. A diagnosis of "confirmed TB" was made if sputum was culture positive, or there was microscopic, histological or molecular evidence for TB. A diagnosis of "clinical TB" was made if there was appropriate response to treatment and compatible clinical history and radiographic appearances. Patients classified as having "active TB" had either "confirmed TB" or "clinical TB". A diagnosis of "no TB" was made if there was a confirmed alternative diagnosis or clinical explanation for the patients' symptoms and radiographic changes. The diagnoses were then used to calculate the positive and negative predictive values (PPV and NPV) and likelihood ratios for the T-SPOT.TB test at different degrees of suspicion of TB.

T-SPOT.TB test

The T-SPOT.TB test was performed according to the standard operating procedures for the test either at Oxford Immunotec (n=65) or, after appropriate training of laboratory personnel, at the Homerton University Hospital (n=85). The technician performing the test was blind to clinical information about the patient. Blood was tested separately for ESAT-6 (panel A) and CFP-10 (panel B). The manufacturer's recommendation is that the test is deemed positive if the spot count in either panel A or B minus the negative control spot count is greater than or equal to six, or, if the negative control had six or more spots, and either panel spot count has at least twice the negative control spot count.

Statistics

The formulae for different statistical tests are (abbreviations: TP = true positive, TN = true negative, FP = false positive, and FN = false negative):

Sensitivity = $TP / (TP + FN)$, Specificity = $TN / (TN + FP)$, Positive predictive value = $TP / (TP + FP)$, Negative predictive value = $TN / (TN + FN)$, Accuracy = $(TP + TN) / (TP + FP + FN + TN)$, Positive likelihood ratio = $sensitivity / (1 - specificity)$, Negative likelihood ratio = $(1 - sensitivity) / specificity$.

Results

Figure 1 shows the study flow diagram. T-SPOT.TB was performed on 150 subjects with results available for 136 as 14 were excluded due to processing problems; eight were not processed according to protocol (stored for > 24 hours before processing), four because of laboratory error, one had insufficient white cells, and one was an insufficient sample. Indeterminate results were found in 12 (8.9%); among them, three with HIV infection and one with diabetes mellitus. Overall, for those with an immunologically debilitating condition, 1/4 with TB gave a positive test and 5/9 without TB had a negative result.

The final diagnoses of the 136 patients were as follows: 46 (33.8%) active TB (of which 33 (24.3%) were confirmed TB and 13 (9.6%) were clinical TB), and 90 (66.2%) no TB. The microbiological method of obtaining the diagnosis is shown in table 2 and figure 2 shows the final diagnoses stratified by T-SPOT.*TB* result. The test's overall sensitivity for diagnosing TB was 73.9% (95% CI: 60–84%) (34/46) or 75.8% (95% CI: 59–87%) (25/33) if only confirmed cases were included. The specificity of the test in diagnosing active disease was 57.8% (95% CI: 47–67%) (52/90). The results for patients with an alternative diagnosis are shown in table 3. Table 4 indicates the overall utility of T-SPOT.*TB* stratified by CSTB grade. The overall positive predictive value was 55.7% and was 15%, 67% and 100% in the low, intermediate and high CSTB groups, respectively. The overall negative predictive value was 82.5% and was 92%, 88% and 25% in the respective groups. The overall positive likelihood ratio was 1.75 and was 1.23, 2.39, and >69 by CSTB group. The overall negative likelihood ratio was 0.45 and was 0.8, 0.26 and 0.31 by respective CSTB group.

Conclusions

The aim of this study was to determine the role of the T-SPOT.*TB* test in the diagnosis of active pulmonary TB in routine clinical practice. The T-SPOT.*TB* test had a sensitivity of 73.9% (95% CI: 60–84%) and specificity of 57.8% (95% CI: 47–67%), therefore providing little additional diagnostic certainty either as a screening test as these require high sensitivity, or as a confirmatory assay as the positive predictive value was only 55.7%. It may have been hoped this test could be used to rule out TB but we have shown a disappointing NPV of 82.5%. The impact of disease prevalence on this indicator is shown by the wide ranging NPV (25–92%) albeit with wide confidence intervals although they do not overlap. The overall negative likelihood ratio was 0.45 and did not fall below 0.1 even in the low CSTB group indicating the test cannot rule out TB, even in those with low probability of the disease.

A systematic review of 17 studies (2) assessing the T-SPOT.*TB* test in 837 culture-positive pulmonary TB patients gave a pooled sensitivity of 87.5% (95% CI: 85–90%). Sester et al (4) also performed a meta-analysis of 19 studies and found a pooled sensitivity for confirmed and non-confirmed TB of 81% (78–84%) although this includes one outlying study with a sensitivity of only 35%. It is not clear why our sensitivity was much lower (75.8% (95% CI: 59–87%) for culture-positive cases) other than the possibility that we had more patients with immunologically-debilitating conditions, advanced disease or other co-morbid conditions which may have interfered with the test. Another explanation may be the genetic diversity of our test population which might include individuals who have an impaired response to the antigens. Indeed, a study by Mazurek *et al* (9) revealed that white (non-hispanic) ethnicity can be associated with false negative IGRA results. A UK study (10), in children drawn from a similarly ethnically diverse population, reported a T-SPOT.*TB* sensitivity of only 67% (46–83%) in those with confirmed active TB.

The study by Diel et al (2) investigating 255 healthy participants without known TB exposure found a specificity of 86.3% (95% CI: 81–90%) whereas, Sester et al (4) noted a specificity of 59% (56–62%) in patients with an alternative diagnosis to TB. This is similar to our specificity of 57.8% (47–67%) and we believe this result better reflects clinical reality, as we confined our study to subjects with suspected TB, and did not use healthy controls. Previous work (11) investigating the test in suspected TB had a much higher ratio of “diagnosed TB” to “definitely not TB” than our own (194:154 compared to 46:90, respectively). Here, too, positive and negative likelihood ratios remained outside the targets of 10 and 0.1, despite the addition of another RD1 sequence antigen.

Dosanji et al (11) combined information from an ELISpot assay with tuberculin skin testing to improve the sensitivity. They found that the negative likelihood ratios fell to below 0.1 if both the ELISpot and the skin tests were negative. We did not include information on skin testing as it was not performed routinely on many patients. Future studies could investigate this but there are numerous difficulties when using this test such as lack of international standardisation of tuberculin, potential errors in performing and reading the TST, and the inaccuracy of information often obtained regarding prior BCG vaccination.

Indeterminate results were assessed by Diel et al and Sester et al (2, 4) and were found to be 3.8% (rising to 6.12% in a subgroup analysis of immunosuppressed subjects) and 3.4%, respectively. Our indeterminate rate was much higher at 8.9% (12/136) which included 3 with HIV infection and one with diabetes mellitus. Of the other subjects one had lung cancer, another was taking low dose prednisolone for sarcoidosis and a third had self-reported poor nutrition. The remaining five were unremarkable. It is possible that errors in processing (either at the bedside or in the laboratory) could account for the excess indeterminate results. Diel et al (2) commented that most studies did not state the number of cases with insufficient cells for the test (ie invalid samples) but 80 subjects from the 59 studies which included 12165 patients, were invalid causing the “no result” rate to rise to 4.46 (4.1–4.8%). In our study, an additional two cases were invalid due to insufficient cells or sampling. The rate of indeterminate and invalid tests should be taken into account when considering the usefulness and cost of the test.

We confirm the importance of Bayesian analysis in the assessment of the T-SPOT.TB test as it reveals large variations in predictive values and likelihood ratios for differing TB probabilities. Those of intermediate suspicion of TB have little to gain from the T-SPOT.TB test owing to its low specificity (and hence low PPV and NPV) which is unfortunate as this is the group that would benefit most from a test, with an earlier result than is attainable by culture. Importantly, the test cannot be used to confidently exclude TB even in those with low probability of the disease because in this group although the NPV was 92% the NLR remained above 0.1 at 0.8.

Our study was limited by the relatively small numbers of subjects in each group. There was also considerable variability in clinical judgment - reflecting the subjective nature of estimating risk, differences in clinical expertise and experience of the local population. Future research involving Bayesian analysis could use a greater number of clinical assessors to ensure more accurate pre-test probabilities. However, clinical judgements are rarely made by a team of more than three doctors in practice, and therefore this study represents a pragmatic evaluation of the new test.

In conclusion, in our hospital in London the T-SPOT.TB test added little value regardless of the pre-test probability of TB, to the current methods used to diagnose active TB. We propose similar evaluations are performed at other sites managing TB elsewhere.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the clinicians of Homerton University Hospital for registering the possibility of TB in these patients and Professor Catanzaro for his support. Financial support: Oxford Immunotec Ltd, Oxford, UK provided the T-SPOT.TB kits free of charge (the company agreed at the outset that they would have no role in the preparation of this or any other publications related to use of these free test kits at the Homerton University Hospital NHS Trust, London); US National Institutes for Health (grant number R01 A1053531) and UK National Health Service Research & Development Culyer allocation.

References

1. Richeldi L. An update on the diagnosis of tuberculosis infection. *Am J Respir Crit Care Med*. 2006; 174:736–742. [PubMed: 16799073]
2. Diel R, Loddenkemper R, Nienhaus A. Evidence-Based Comparison of Commercial Interferon-gamma Release Assays for Detecting Active TB: A Metaanalysis. *Chest*. 2010; 137(4):952–968. [PubMed: 20022968]
3. Goletti D, Carrara S, Butera O, Amicosante M, Ernst M, Sauzullo I, et al. Accuracy of immunodiagnostic tests for active tuberculosis using single and combined results: a multicenter TBNET-Study. *PLoS One*. 2008; 3(10):e341.
4. Sester M, Sotgiu G, Lange C, Giehl C, Girardi E, Migliori GB, et al. Interferon- γ release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. *Eur Respir J*. 2011; 37(1):100–111. [PubMed: 20847080]
5. Mylotte JM, Rodgers J, Fassl M, Seibel K, Vacanti A. Derivation and validation of a pulmonary tuberculosis prediction model. *Infect Control Hosp Epidemiol*. 1997; 18(8):554–560. [PubMed: 9276236]
6. Catanzaro A, Perry S, Clarridge JE, Dunbar S, Goodnight-White S, LoBue PA, et al. The role of clinical suspicion in evaluating a new diagnostic test for active tuberculosis: results of a multicenter prospective trial. *JAMA*. 2000; 284:639–645. [PubMed: 10665704]
7. Greenhalgh T. How to read a paper: papers that report diagnostic or screening tests. *BMJ*. 1997; 315:540–543. [PubMed: 9329312]
8. Deeks JJ, Altman DG. Diagnostic tests 4: likelihood ratios. *BMJ*. 2004; 329:168–169. [PubMed: 15258077]
9. Mazurek GH, Weis SE, Moonan PK, Daley CL, Bernardo J, Lardizabal AA, et al. Prospective Comparison of the Tuberculin skin test and 2 Whole-Blood Interferon-gamma Release Assays in Persons with Suspected Tuberculosis. *CID*. 2007; 45:837–845.
10. Bamford ARJ, Crook AM, Clark JE, Nademi Z, Dixon G, Paton JY, et al. Comparison of interferon-gamma release assays and tuberculin skin test in predicting active tuberculosis (TB) in children in the UK: a paediatric TB network study. *Arch Dis Child*. 2010; 95:180–186. [PubMed: 19815937]
11. Dosanjh DPS, Hinks TSC, Innes JA, Deeks JJ, Pasvol G, Hackforth S, et al. Improved Diagnostic Evaluation of Suspected Tuberculosis in Routine Practice. *Ann Intern Med*. 2008; 148(5):325–336. [PubMed: 18316751]

Figure 1.
Flow chart showing numbers of patients grouped by the clinician's suspicion of TB (CSTB) – high (>75% probability), intermediate (25–75% probability) or low (<25% probability); the final diagnosis and the T-SPOT.TB test results

Figure 2.
Flow chart showing numbers of patients grouped by the T-SPOT.TB test results and final diagnosis

Table 1

Demographic characteristics of patient population

Demographic characteristics	Number (%)
Female	65 (43)
Ethnicity	
White - UK	73 (49)
Black African	33 (22)
Indian subcontinent	18 (12)
Afro-Caribbean	13 (9)
Vietnamese	8 (5)
Chinese	2 (1)
Other ^a	3 (2)
Born outside UK	91 (61)
Arrived in UK within preceding 5 years	36 (24)
Smoking history (past or present)	90 (60)
Alcohol excess (self-confessed)	27 (18)
Illegal drug use in past 2 years	37 (25)
Street homeless or hostel for ≥ 6 months (past or present)	30 (20)
Prison in previous 2 years	7 (5)
Tuberculosis history	35 (23)
Close family history of TB	27 (18)
Personal history of active TB	8 (5)
Co-morbidities	
HIV	10 (7)
Diabetes	8 (5)

^a Mauritian, Philippino and mixed Afro-Caribbean/White

Table 2

Method of diagnosis for patients with confirmed active pulmonary tuberculosis and T-SPOT.*TB* results (the brackets indicate the number with an immunologically debilitating condition)

	T-SPOT.<i>TB</i> test result			
	Positive	Negative	Indeterminate	Total
Microbiology results				
Smear positive, culture positive	16	4(2)	1	21
Smear negative, culture/NAAT ^a positive	9	3(1)	0	12
Culture negative but characteristic response to treatment (clinical TB)	9(1)	4	0	13
Total	34	11	1	46

^aNucleic acid amplification test

Table 3T-SPOT.*TB* results for patients with an alternative diagnosis to tuberculosis.

Diagnosis for patients without active TB	Details of diagnosis	T-SPOT. <i>TB</i> positive	T-SPOT. <i>TB</i> negative	Total
Other histological / microbiological diagnoses	Malignancy	1	5	6
	Wegener's granulomatosis	0	1	1
	Other infection	3	5	8
	Allergic bronchopulmonary aspergillosis	0	1	1
Other mycobacterial species	<i>M. xenopi</i>	1	(2 ^a)	1
	<i>M. fortuitum</i>	0	2	2
	<i>M. avium</i>	0	1	1
	<i>M. goodii</i>	0	1	1
Sarcoidosis		1	4	5
Inactive TB		6	4	11
Latent TB		5	0	5
Other clinical diagnoses ^b		10	28	38
Total		27	52	79

^a2 other isolates of *M. xenopi* occurred; one in a patient with a primary diagnosis of malignancy and another in a patient from whom *M. avium* was also isolated.

^bThese include lower respiratory tract infections (13), chronic obstructive pulmonary disease (4), asthma (4), radiological misinterpretation of normal chest x-rays (2), alcohol-related problems (2), asbestos-related lung disease, bronchiectasis, longstanding collapse of right upper lobe of lung, pleural thickening, congestive cardiac failure, eosinophilic pneumonia, haematemesis, hepatitis C, IgG1 deficiency with restrictive lung disease, opiate overdose, thrombocytopenia, typing error (the patient had a normal chest radiograph but the formal report had a "no" missing from "radiological evidence of TB"), urinary tract infection and non-specific viral infection.

Table 4

Summary of diagnostic indices of T-SPOT.*TB* test when stratified by clinician's suspicion of active pulmonary tuberculosis (CSTB)

	All patients	Low CSTB	Intermediate CSTB	High CSTB
Number of patients	136	72	35	29
Sensitivity	34/46	4/7	10/12	20/27
	73.9% (60–84%)	57% (24–84%)	83% (55–95%)	74.1% (55–87%)
Specificity	52/90	35/65	15/23	2/2
	57.8% (47–67%)	53.8% (42–65%)	65.2% (45–81%)	100%
Positive predictive value	34/61	4/26	10/15	20/20
	55.7% (43–68%)	15% (6–34%)	67% (41–85%)	100%
Negative predictive value	52/63	35/38	15/17	2/8
	82.5% (71–90%)	92% (79–97%)	88% (65–96%)	25% (7–60%)
Accuracy	86/136	39/72	25/35	22/29
	63.2% (55–71%)	54.1% (43–65%)	71.4% (55–84%)	75.8% (58–88%)
Positive likelihood ratio	0.739/0.422	0.57/0.462	0.83/0.348	0.69/<0.01
	1.75	1.23	2.39	>69
Negative likelihood ratio	0.261/0.578	0.43/0.538	0.17/0.652	0.31/1
	0.45	0.8	0.26	0.31

(95% CI are given in brackets)