An interferon-gamma release assay test performs well in routine screening for tuberculosis

Allan Vestergaard Danielsen[1], Andreas Fløe[1], Troels Lillebaek[2], Hans Jürgen Hoffmann[1] & Ole Hilberg[1]

ABSTRACT

INTRODUCTION: A positive interferon-gamma release assay (IGRA) is regarded as proof of latent *Mycobacterium tuberculosis* infection. We conducted an evaluation of the IGRA test "T-SPOT.TB" to test its performance during clinical routine use by analysing the positivity rate and odds, effect of season and sensitivity.

MATERIAL AND METHODS: Data from T-SPOT.TB testing together with age and test indications (anti-tumour necrosis factor alpha (TNFα) candidate, contact investigation or suspicion of tuberculosis (TB)) were combined with mycobacteria culture results.

RESULTS: A total of 1,809 patients were tested. Conclusive results were achieved for 1,780 patients (98.4%). Among these, 4.6% of anti-TNFα candidates, 19.3% of contacts and 24.4% of TB suspects tested positive. Compared with anti-TNFα candidates, the odds for a positive result were significantly higher for contact investigations (odds ratio (OR), mean (95% confidence interval): 4.93 (3.11-7.81)) and TB suspects (OR: 6.83 (4.33-10.77)). Elevated odds of an inconclusive test were found during autumn and winter periods (OR: 2.53 (1.58-4.05)) and for patients > 75 years of age (OR: 2.66 (1.43-4.94)) and < 6 years of age (OR: 3.35 (1.58-7.09)). In all, 41 of 43 culture-verified M. tuberculosis infections tested positive with one false negative.

CONCLUSION: During routine testing, inconclusive tests were rare, but more frequent during autumn/winter periods and for patients < 6 and > 75 years of age. The T-SPOT.TB showed a high sensitivity in culture-verified TB, although false negative results did occur.

FUNDING: not relevant.

TRIAL REGISTRATION: not relevant.

Mycobacterium tuberculosis is a major cause of mortality and morbidity worldwide. Nine million new tuberculosis (TB) cases evolve every year, and an estimated one third of the world population are latently infected with *M. tuberculosis* (LTBI) which implies that there is a large reservoir for future TB disease [1]. Approximately 10% of all LTBI cases will progress to active TB [2].

Until recently, the only means of diagnosing LTBI was the tuberculin skin test (TST), which detects cell-mediated immunity through a delayed-type hypersensitivity reaction towards a purified protein derivative of tuberculin [3]. However, the TST can also react to bacille Calmette Guérin (BCG) vaccination and non-tuberculous mycobacteria (NTM), which constitutes a considerable diagnostic challenge [4]. These limitations have largely been overcome by the introduction of interferon-gamma release assays (IGRA) which quantify the ex vivo cellular immune response towards *M. tuberculosis*-specific antigens [5]. At present, the T-SPOT.TB assay (Oxford Immunotec, Abingdon, UK) and the QuantiFERON-TB Gold In-Tube (QFT-GIT) assay (Cellestis, Carnegie, Australia) are commercially available and widely used IGRAs. Both assays quantify the response towards the *M. tuberculosis*-complex specific proteins ESAT-6 (early secreted antigen target/6 kDa) and CFP-10 (culture filtrate protein/10 kDa). As LTBI is defined as an immunological reaction without evidence of TB disease, a positive IGRA in an otherwise asymptomatic person is regarded as a diagnosis of LTBI. Especially in the TB low-burden, high-income part of the world, IGRAs are recommended for contact investigations upon exposure to contagious TB patients [6] as well as for testing for latent *M. tuberculosis* infection prior to anti-tumour necrosis factor alpha (TNFα) therapy [7].

The performance of IGRAs has been studied and evaluated extensively throughout the past decade [4]. Whereas most studies focus on the performance of QFT-GIT, we wished to summarise and evaluate the test results of the T-SPOT.TB in the context of routine contact investigations prior to TNFα inhibitor therapy and in persons at risk of active TB. As age and season of the year have previously been reported to influence test results [8], we also evaluated these factors in our study. To our knowledge, only few evaluations of the performance of the T-SPOT.TB have been carried out in low-incidence settings [4, 8-13].

MATERIAL AND METHODS

We included all T-SPOT.TB results from the laboratory at the Department of Pulmonary Medicine, Aarhus University Hospital, from 1 January 2010 to 31 December 2011. Data on age, sex, date of testing and the clinical indication for testing (screening prior to anti-TNFα therapy, contact investigations or suspicion of TB) were collected for all patients. The test assays were performed and in-

ORIGINAL ARTICLE

1) Department of Respiratory Medicine and Allergology, Aarhus University Hospital
2) International Reference Laboratory of Mycobacteriology, Statens Serum Institut

Dan Med J 2014;61(6):A4856

terpreted by permanent staff members in accordance with the manufacturer's instructions. The laboratory is certified annually by the manufacturer to run the T. SPOT.TB assay. Results were reported as positive, negative or inconclusive. Data on concomitant culture-verified mycobacterial infections were obtained for all included patients. Culture-verified infections and T. SPOT.TB status were considered concomitant if they were performed within one year from the date of the T-SPOT.TB test.

Statistical analyses were performed using the χ^2-test. When appropriate, mean odds ratios (OR) with two-sided 95% confidence intervals (CIs) were calculated, and when needed, control for confounding was performed according to the Mantel-Haenzel method. For all results, a significance level of 0.05 was assumed.

RESULTS

A total of 2,018 T-SPOT.TB tests were performed in 1,809 patients; 185 patients were tested more than once. The crude results were 17.8% positive, 82.2% negative and 4.9% inconclusive tests (**Figure 1**).

Odds ratio for positive T-spot.TB

The indication for testing patients was reported in 1,631 patients (90.1%) as either LTBI screening prior to anti-TNFα therapy (31.7%), contact investigation (35.6%) or suspicion of TB (32.7%). When the T-SPOT.TB was performed for selective screening purposes prior to anti-TNFα therapy, 24 (4.6%) of 517 patients tested positive, whereas 112 (19.3%) of 581 patients tested due to contact investigation and 130 (24.4%) of 533 tested on suspicion of TB had a positive test result.

The odds for a positive T-SPOT.TB were significantly higher for patients tested on suspicion of TB than for anti-TNFα therapy candidates; OR: 6.83 (4.33-10.77), $p < 0.0001$). The same was true for patients tested in contact investigation compared with anti-TNFα therapy candidates; OR: 4.93 (3.11-7.81), $p < 0.0001$).

Patients tested on suspicion of TB had significantly higher odds for a positive result than patients tested in contact investigations; OR: 1.39 (1.04-1.85), $p < 0.0263$.

Inconclusive test results

The test date was recorded for 1,977 (97.9%) tests; 1,115 (53.6%) tests were performed during autumn and winter (September to February) and 862 (46.4%) were performed during spring and summer (March to August). Inconclusive test results occurred in 98 (4.9%); 72 tests (3.6%) done in autumn/winter and 26 tests (1.3%) done during spring/summer (**Figure 2**). The seasonal variation was significant (autumn/winter versus spring/summer (OR: 2.53 (1.58-4.05), $p < 0.0001$). A major contribution to the inconclusive results in wintertime was due to a high number of inconclusive results within one month (14 tests), all of which had an invalid negative control. The relation between inconclusive test results and the time of year remained significant when excluding these (OR: 1.78 (1.09-2.90), $p < 0.02$).

The initial test result was inconclusive for 30 (5.6%) of 533 patients with suspected TB, 23 (4.0%) of 581 tested in contact investigations and 23 (4.5%) of 517 anti-TNFα candidates. Test indication was unavailable for two patients with an initially inconclusive result. There was no statistically significant difference in the occurrence of inconclusive results according to test indication ($p > 0.4$). For patients above 75 years of age (n = 13), significantly more inconclusive tests were observed

FIGURE 1

Distribution of definitive results for tested patients.

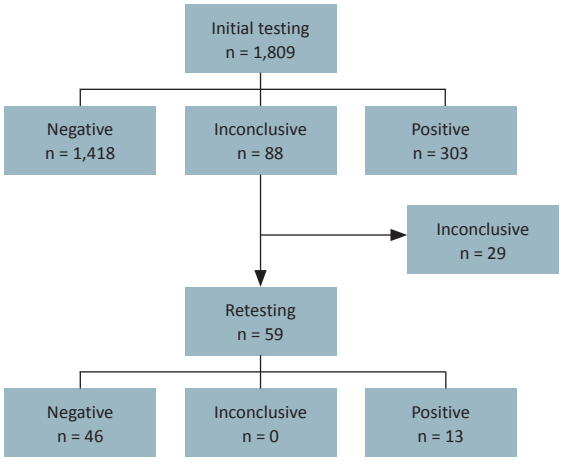
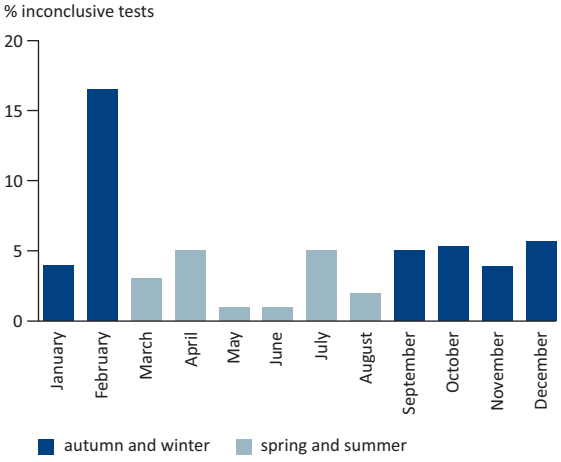


FIGURE 2

Distribution of inconclusive tests according to time of year.



than for patients aged between 6-75 years; OR: 2.66 (1.43-4.94), p < 0.0013). The same was true for patients < 6 years (n = 7) compared with patient aged between 6 and 75 years (OR: 2.89 (1.27-6.57), p < 0.019).

The impact of age on the odds for an inconclusive test became more evident when adjusting for the time of year; age < 6 years versus 6-75 years: OR: 3.35 (1.58-7.09), p < 0.0001 and age ≥ 75 years versus 6-75 years: OR: 3.39 (2.11-11.07), p < 0.0073.

A conclusive result was achieved in 49 (83.1%) of 59 retested patients. The median time passed between initial testing and retesting was seven days (range 1-117 days).

Dates, reason for retesting and final results were available for 53 (88.1%) retested patients (**Table 1**).

Verified mycobacterial infections

Sixty patients (3.3%) had a culture-verified, mycobacterial infection (**Table 2**). Of these, 43 were infected with *M. tuberculosis* and 41 had a concomitant positive T-SPOT.TB. Three of these patients were tested prior to anti-TNFα-therapy. Fifteen of 17 patients infected with NTM were tested on clinical suspicion of TB and two were tested prior to anti-TNFα therapy. One patient with culture-verified *M. tuberculosis* infection had an inconclusive T-SPOT.TB. Another patient tested in contact investigation had a false negative T-SPOT.TB, whereas *M. tuberculosis* was cultured from collected sputum, and the specimens for culture and T-SPOT.TB were collected the same day.

TABLE 1

Reason for retesting and test results after retesting. The values are n.

Time from initial inconclusive result to result of retest	Reason for retesting: defective positive control	defective negative control	controls not reported	Total
0-21 days				
Positive	0	3	3	6
Negative	5	14	16	35
Inconclusive	0	1	1	2
> 21 days				
Positive	0	2	0	2
Negative	3	1	2	6
Inconclusive	0	2	0	2
Total	8	23	22	53

TABLE 2

T-SPOT.TB status and mycobacterial species. The values are n.

Species	T-SPOT.TB status: negative	positive	inconclusive	Total
Mycobacterium tuberculosis complex				
M.tuberculosis[a]	1	41	1	43
Non-tuberculous mycobacteria				
M. marinum[a]	0	2	0	2
M. avium-intracellulare complex	7	0	1	8
M. gordonii[b]	3	1	0	4
M. celatum	1	0	0	1
M. xenopi	1	0	0	1
M. peregrinum	0	1	0	1
Total	13	45	2	60

CFP-10 = culture filtrate protein of 10 kDa; ESAT-6 = early secreted antigen target of 6 kDa.
a) Expresses T-SPOT.TB antigenic target proteins ESAT-6 and CFP-10.
b) May express ESAT-6.

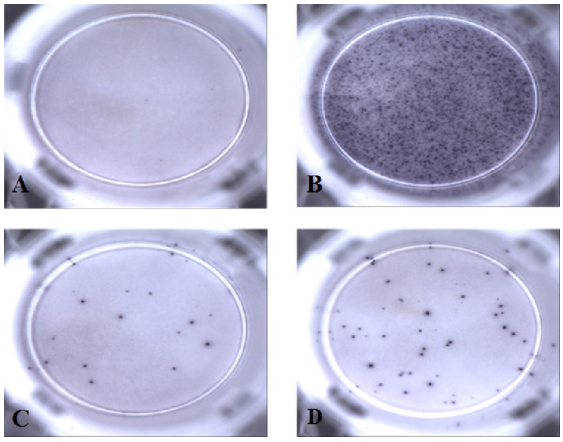
DISCUSSION

We have presented results of T-SPOT.TB testing performed under routine conditions during a two-year period at a single Danish laboratory affiliated with a hospital department responsible for TB treatment, contact investigations and LTBI screening.

Anti-TNFα therapy constitutes a major risk factor for reactivation of LTBI [14] as TNFα is pivotal for the integrity of granuloma formation, which plays a crucial role in containing surviving mycobacteria during latent infection [15]. Hence, screening with either TST or IGRA before initiating anti-TNFα therapy is highly recommended in order to offer preventive therapy in case of suspected LTBI [16]. Though anti-TNFα candidates are expected to be at no greater risk of exposure to *M. tuberculosis* than the background population in general, a higher risk of TB has previously been observed among patients with rheumatoid arthritis treated with prednisolone and disease-modifying anti-rheumatic drugs (DMARDs) [17]. This finding is probably due to co-morbidities as well as the immunosuppressive effects of this treatment. Prednisolone and DMARDs are known to possibly interfere with IGRAs and generally cause a higher incidence of inconclusive test results [9]. The overall prevalence of inconclusive test results (4.9%) was in accordance with the overall observed frequency among immunosuppressed individuals in a recent meta-analysis [4].

In the present study, the crude prevalence of positive test results among anti-TNFα candidates (4.6%) corresponded to the background prevalence observed in previous studies, although the reported prevalence spanned considerably (1.9-9.5%) [9, 10, 18]. We argue that anti-TNFα candidates can be regarded as a proxy for the background population in a low-incidence setting since this group of patients is at no greater risk of LTBI or exposure than the general population.

Among patients with suspected TB, 32.7% tested positive, which corresponds to previous findings in similar settings [8]. Likewise, 22.7% of those tested in contact investigations were positive, which is also in line with previous reports where the prevalence ranged from



T-Spot.TB reading. **A**. Negative control panel. **B**. Positive control panel. **C**. Panel coated with ESAT-6 antigens. **D**. Panel coated with CFP-10 antigens. The ESAT-6 panel (C) has 14 spots and the CFP-10 panel (D) has > 20 spots. The control panels (A, B) are valid. The test is positive for both antigens. CFP-10 = culture filtrate protein of 10 kDa; ESAT-6 = early secreted antigen target of 6 kDa.

11% to 34% among persons exposed to TB [8-11]. Compared to anti-TNFα candidates, who were regarded a proxy of the background population in this context, we found ORs of 6.83 and 4.93 for a positive test result in TB suspects and contact investigations, respectively. Previous studies have reported ORs of 0.7 and 1.99 when comparing background populations with contacts of TB patients [9, 10].

Many European centres depend on external authorities (public health departments etc.) to perform contact investigations. The responsibility of TB disease control in Denmark rests with the hospital departments in charge of diagnosing and treating TB. This structure may allow for a closer dialogue with both patient and contacts, yielding more precise contact investigations. On the other hand, a more selective screening procedure may be too narrow and might lead to testing of only individuals at obvious risk. This would yield a higher OR of a positive result because some patients at risk might be omitted. It has thus been documented that there is increased M. tuberculosis transmission in Denmark among socially marginalised persons, which underlines the need for a stronger focus on contact investigations and screening programmes [19].

Previous reports on T-SPOT.TB performance indicate a higher frequency of inconclusive test results during autumn and winter [8]. Inconclusive results can arise from an insufficient response to the mitogen-positive control, unspecific staining in the wells or unspecific production of interferon-gamma (IFNγ) by peripheral blood mononuclear cells. Furthermore, a compromised immune function due to immunosuppressive treatment or disease may lead to a weak mitogen response. A possible explanation for the increased occurrence of inconclusive results during the cold months is the higher frequency of benign viral infections leading to increased presence of IFNγ-immune cells in peripheral blood, causing an unspecific IGRA reaction. In addition, it has been proposed that failure to comply with suggested temperature limits during transportation during wintertime may interfere with test performance [8].

A higher frequency of inconclusive results has also been observed when testing patients < 5 years and > 75 years. Our findings are in accordance with previous reports from routine testing in low-incidence settings [8, 11].

We found a crude prevalence of inconclusive results of 4.9%, which is higher than in similar studies (0.3-3.6%) [8, 11]. Despite the relatively high frequency of initially inconclusive results, a conclusive result was achieved in 93.2% upon re-testing. The majority of the retests (81.1%) were performed within three weeks of initial testing. Local guidelines recommend that re-testing is postponed for at least three weeks following an inconclusive test because immunological activation due to trivial infections might theoretically interfere with the assay and give rise to more false positives and defective negative controls. However, we did not observe significantly more positive and inconclusive test results in connection with early retesting within three weeks.

A small subgroup of T-SPOT.TB-tested patients had a culture-verified mycobacteria infection. As noted, one patient with *M. tuberculosis* had a false negative T-SPOT.TB: A 66-year-old male with known exposure to TB. A tracheal suction sample from the patient was positive for acid-fast bacilli. In addition, radiological signs were strongly indicative of TB. Subsequently, PCR and culture confirmed *M. tuberculosis* infection. The patient was seriously ill at the time of diagnosis and the reactive T cells were probably at the site of infection rather than in circulation. This is a known shortcoming of IGRAs [4].

In four cases in which NTM infections were confirmed microbiologically, the T-SPOT.TB was positive (two patients infected with *M. marinum* and two infected with *M. peregrinum* and *M. gordonii,* respectively). *M. marinum* expresses the test target antigenic proteins ESAT-6 and CFP-10, and positive results were to be expected. *M. gordonii* has been reported to express ESAT-6 in some cases, which may very well lead to cross reactivity in T-SPOT.TB as well as in QFN-GIT [20]. The case of *M. peregrinum* may be considered as latent co-infection with *M. tuberculosis.*

CONCLUSION

The T-SPOT.TB performs well in routine screening prior to TNFα treatment as well as in contact investigation procedures. Furthermore, it is a useful diagnostic aid in low-incidence settings.

Test performance is influenced by the age of the subject and by the season of year, but mostly in terms of the occurrence of inconclusive results. In the majority of inconclusive cases, a subsequent test can provide a conclusive result.

One false negative result was recorded. Therefore, a negative T-SPOT.TB should never overrule the judgment of an experienced clinician if clinical findings or history are consistent or strongly suspicious of TB.

CORRESPONDENCE: *Allan Vestergaard Danielsen,* Lungemedicinsk Afdeling, Aarhus Universitetshospital, Nørrebrogade 44, 8000 Aarhus, Denmark.
E-mail: alladani@rm.dk

ACCEPTED: 28 March 2014

CONFLICTS OF INTEREST: none. Disclosure forms provided by the authors are available with the full text of this article at www.danmedj.dk.

LITERATURE

1. WHO. Global Tuberculosis Report 2013. Geneva: World Health Organization, 2013.
2. Chee CB, Sester M, Zhang W et al. Diagnosis and treatment of latent infection with mycobacterium tuberculosis. Respirology 2013;18:205-16.
3. Mack U, Migliori GB, Sester M et al. LTBI: latent tuberculosis infection or lasting immune responses to M. tuberculosis? A TBNET consensus statement. Eur Respir J 2009;33:956-73.
4. Diel R, Loddenkemper R, Nienhaus A. Predictive value of interferon-gamma release assays and tuberculin skin testing for progression from latent TB infection to disease state: a meta-analysis. Chest 2012;142:63-75
5. Pai M, Riley LW, Colford JM,Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. Lancet Infect Dis 2004;4:761-76.
6. Erkens CG, Kamphorst M, Abubakar I et al. Tuberculosis contact investigation in low prevalence countries: a European consensus. Eur Respir J 2010;36:925-49.
7. Vassilopoulos D, Tsikrika S, Hatzara C et al. Comparison of two gamma interferon release assays and tuberculin skin testing for tuberculosis screening in a cohort of patients with rheumatic diseases starting anti-tumor necrosis factor therapy. Clin Vaccine Immunol 2011;18:2102-8.
8. Beffa P, Zellweger A, Janssens JP et al. Indeterminate test results of T-SPOT. TB performed under routine field conditions. Eur Respir J 2008;31:842-6.
9. Mancuso JD, Tribble D, Mazurek GH et al. Impact of targeted testing for latent tuberculosis infection using commercially available diagnostics. Clin Infect Dis 2011;53:234-44.
10. Hinks TS, Varsani N, Godsiff DT et al. High background rates of positive tuberculosis-specific interferon-gamma release assays in a low prevalence region of UK: a surveillance study. BMC Infect Dis 2012;12:339.
11. 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;367:1328-34.
12. Ravn P. Screening for tuberculosis infection prior to medical therapy. Ugeskr Læger 2011;173:893-6.
13. Diel R, Loddenkemper R, Nienhaus A. Evidence-based comparison of commercial interferon-gamma release assays for detecting active TB: a metaanalysis. Chest 2010;137:952-68.
14. Ellerin T, Rubin RH, Weinblatt ME. Infections and anti-tumor necrosis factor alpha therapy. Arthritis Rheum 2003;48:3013-22.
15. Ehlers S. Tumor necrosis factor and its blockade in granulomatous infections: Differential modes of action of infliximab and etanercept? Clin Infect Dis 2005;41(Suppl 3):S199-S203.
16. Solovic I, Sester M, Gomez-Reino JJ et al. The risk of tuberculosis related to tumour necrosis factor antagonist therapies: a TBNET consensus statement. Eur Respir J 2010;36:1185-1206.
17. Winthrop KL, Iseman M. Bedfellows: mycobacteria and rheumatoid arthritis in the era of biologic therapy. Nat Rev Rheumatol 2013;9:524-31.
18. Bocchino M, Matarese A, Bellofiore B et al. Performance of two commercial blood IFN-gamma release assays for the detection of Mycobacterium tuberculosis infection in patient candidates for anti-TNF-alpha treatment. Eur J Clin Microbiol Infect Dis 2008;27:907-13.
19. Kamper-Jorgensen Z, Andersen AB, Kok-Jensen A et al. Clustered tuberculosis in a low-burden country: nationwide genotyping through 15 years. J Clin Microbiol 2012;50:2660-7.
20. Arend SM, de Haas P, Leyten E et al. ESAT-6 and CFP-10 in clinical versus environmental isolates of Mycobacterium kansasii. J Infect Dis 2005;191:1301-10.