ERJ Express. Published on March 29, 2006 as doi: 10.1183/09031936.06.00110205

USE OF A T-CELL BASED TEST FOR DETECTION OF TB INFECTION AMONG IMMUNOCOMPROMISED PATIENTS

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Keywords: contact tracing, haematology, Interferon-gamma, T-SPOT.*TB*, Tuberculosis.

Short title: T-cell based test in LTBI in immunosuppressed

Body text word count: 2666

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We compared the performance of T-SPOT.*TB*™, a T-cell based test, versus TST to diagnose LTBI in 138 immunosuppressed haematology patients who had been nosocomially exposed to a case of smear-positive tuberculosis.

Overall, 44.2% of the contacts were positive by T-SPOT.*TB*, 17.4% by TST (concordance 67.8%, κ=0.34, $p<0.0001$). The apparent prevalence of infection fell from 25.9% to 14.5% with the TST with increasing immunosuppression, although this difference was not significant ($p=0.12$). In contrast, the apparent prevalence of infection with the T-SPOT.*TB* test was unaffected at 44.6% and 44.3% respectively. The T-SPOT.*TB* test had an overall indeterminate rate of 4.3% and this was also unaffected by the level of immunosuppression.

Our study suggests that T-SPOT.*TB* maintains sensitivity and performance in immunocompromised patients identifying a large number of truly-infected patients anergic to TST.

Abstract word count: 130

Introduction.

In January 2005, a male patient with multiple myeloma, attending the Haematology Chemotherapy Unit of Niguarda Hospital in Milan, was diagnosed with a smear-positive pulmonary TB. There was considerable concern over the exposure of other patients attending the same Chemotherapy Unit, as the index case had attended the Haematology Department for about 3 months, with at least 55 visits within this period.

As the risk of progression from latent to active tuberculosis (TB) amongst an immunosuppressed haematology patient group would be higher than that of the general population [1;2], it was very important to correctly identify those who had become infected. Unfortunately the sensitivity of the current method of determining infection, the Tuberculin Skin Test (TST), is known to be low [3;4]. We therefore wanted to examine whether the new γ-interferon-based tests for tuberculosis infection might give useful additional information, as currently the performance of these tests in immunosuppressed haematology patients is unknown [5].

Of the two commercial tests currently available, we chose the T-SPOT.*TB* assay (Oxford Immunotec, UK), which enumerates individual activated Tuberculosis-specific T-cells using ELISPOT methodology. We considered this test more useful than QuantiFERON-TB Gold In Tube [6] as, at that time, only T-SPOT.TB had an internal positive control and the lymphocyte numbers and function are checked as part of the assay [7]. These two features were very important in this setting because we had to work with lymphocytes from people with underlying hematological diseases and who were under chemotherapy, so we needed to know if a negative result was a true negative or it was due to a lack of function of T-cells. Moreover, the counting of T-cells, and thus the ability to correct for low lymphocyte counts, would give the best chance of getting a valid test result.

All the patients with possible nosocomial contact with the infectious patients were identified and underwent contact tracing. Contact tracing included clinical examination, Mantoux test (TST), T-SPOT.*TB* test and chest-radiography.

Materials and methods.

Patient population. All 138 patients (66 men, 72 women, mean age: 61 years, range:18-93, 136 Italians, 2 foreign-born) identified as nosocomial contacts of the index case were recalled for screening. The Ethical Committee approved the study and patients gave their informed consent. 4 patients had anamnesis of previously-treated active TB disease and one had previously undergone isoniazid chemoprophylaxis for LTBI. All were HIV-negative.

The haematological diseases were: non-Hodgkin lymphoma (46; 33%), multiple myeloma (41; 30%), chronic lymphocytic leukaemia (20; 14%), bone marrow dysplasia (11; 8%), Hodgkin lymphoma (10; 7%), acute myeloid leukaemia (3; 2%), acute lymphocytic leukaemia (1; 0.7%) and chronic myeloid leukaemia (1; 0.7%). 5 (4%) patients were treated for severe anaemia.

Mantoux testing was performed in January, soon after the identification of the infectious case (>3 months after first exposure), T-SPOT.*TB* testing was performed 15-30 days later.

TST screening. The TST was performed according to international standards (5TU of PPD BiocineTest-PPD, Chiron, Italy) and indurations ≥5 mm were considered positive, according to the Italian guidelines (MoH. Art.115.D.lgs.31/3/1998). Nurses performing the TST were experienced in administering and reading the test, but were not blinded to the patient's history.

T-SPOT.*TB* test. T-SPOT.*TB* (Oxford Immunotec, UK) was performed according to the manufacturer's instructions. Briefly, 8 ml of blood were drawn into a BD Vacutainer® CPT™ tube and centrifuged to separate blood components. Lymphocytes were washed twice, the cell concentration adjusted and 250,000 cells seeded in each of 4 wells of the assay plate. The cells were stimulated (37°C 5%CO_2) for 16-20 hours with media (nil control), phytohaemoagglutinin (mitogen positive control) or the specific antigens (ESAT-6 and CFP-10 in separate wells). The IFN-γ released by the single cells was quantified by ELISPOT technology (4). Results are expressed as the

number of Spot Forming Cells (SFC) A positive antigen-specific result is defined as a well containing at least six SFCs more than the negative control.

The presence of a satisfactory reaction (>20 SFCs) to the mitogen positive control demonstrates T cell function and also validates the assay result. An “indeterminate” result was reported when high background levels prevented interpretation or when less than 20 SFCs were detected in the positive control wells.

Statistical analysis. Statistical analysis was performed using SAS® v8.2.

Results.

At the time of screening no signs of active TB in the contacts were detected by clinical examination and/or chest-radiography. One year later, no patient has developed active TB.

In 16 people no TST result was obtained either due to contraindications or non-returns for reading (11,6%).

Among the 138 patients tested by T-SPOT.*TB,* we were not able to collect sufficient T-cells in 3 cases (2.1%) and we obtained an indeterminate result in 6 cases (4,3%).

Valid T-SPOT.*TB* results were therefore available in 129 patients and TST results in 122.

Overall, 24 subjects (17.4%) were positive by TST and 61 (44.2%) were positive by T-SPOT.*TB*. T-SPOT.*TB* was positive in 34 TST-negative patients and the TST was positive in 3 T-SPOT.*TB* negative patients (Table 1). Concordance between the two tests (in the 115 patients for whom both test results were available) was 67.8% (κ=0.34, $p<0.0001$).

BCG-vaccination data was obtained for 84 patients (60.9%), only 2 were BCG-vaccinated. This is consistent with the fact that BCG-vaccination has never been extensively used in Italy. In both BCG-vaccinated patients TST was positive and T-SPOT.*TB* was negative.

Details about the duration of contact were very difficult to collect as the only calculable data in most cases was the number of visits to the haematology unit coincident with the index case. Furthermore, it was not possible to classify the contacts by their closeness to the index case as the physical location of the patients during their visits is not routinely recorded. However, in 53 people with up to three visits coincident with the infectious case, T-SPOT.*TB* was positive in 20 (37.7%) subjects. Interestingly, this rate increased to 66% in the 6 patients with nine or more coincident visits with the infectious case. The difference between the two groups, however, did not reach statistical significance due to the small number of patients. The Mantoux test showed no

relationship to this one available measure of exposure, being negative in all the patients in both the above groups.

Results of white blood cell (WBC) counts were available for all patients. 70 patients (51.9%) had a pathological WBC count (<4.3 or >10.8x10^3 WBC/mm^3). Among these we had 2 (2.8%) indeterminate T-SPOT.*TB* results due to lack of lymphocyte function. In the 65 patients with a normal count there were 4 indeterminate results (6.2%) showing that the extent of immunosuppression did not affect the T-SPOT.*TB* test's performance (no statistical significance). The T-SPOT.*TB* test was positive in 44.6% of those with normal WBC count and in 44.3% in those with a pathological WBC count. In contrast, the level of positivity by the TST fell from 25.9% to 14.5% between the same groups, although this fall did not quite reach statistical significance (p=0.09 χ^2 test).

Stratifying our population according to their age, we found an increase in the rate of positive T-SPOT.*TB* tests with the age of the patients. In particular, in people who were less than 60 years old, 26.4% of patients were positive, while this rate increased to 55.3% in people aged more than 60 years (OR=3.17, 95%CI=1.48-6.79, p=0.0033). No such relationship was found with the TST (OR=1.54, 95%CI=0.58-4.1, p=0.4816).

Discussion.

By screening 138 haematological patients who have been in contact with an infectious TB case we found a significantly higher rate of T-SPOT.*TB* test compared to TST screening. T-SPOT.*TB* was positive in 44.2% of the contacts versus 17.4% for the TST. It was important for us to determine whether the higher apparent prevalence of infection found with T-SPOT.*TB* is due to the TST being falsely negative due to anergy, or due to the T-SPOT.*TB* test being falsely positive in a number of patients.

Our data, whilst clearly not definitive, support the former view that T-SPOT.*TB* was correctly identifying infected patients anergic to the TST. Firstly, the results demonstrate that the T-SPOT.*TB* test result was not affected by the WBC counts of the patients; whereas the TST results were affected by increasing immunosuppression. The response to the positive control mitogen in the T-SPOT.*TB* assay provided a control against anergy (notwithstanding the fact that the indeterminate rate was only 4.3% overall, and actually lower in those with weaker immune systems). Secondly, the view that the T-SPOT.*TB* test gives false-positive results compared to the TST is not supported by the fact that in routine contact tracing of non-immunosuppressed close household contacts at the same institution, we find the reverse of what was found here, with the T-SPOT.*TB* test actually identifying less patients than the TST (54.5% vs. 84.8%) (F. Piana personal communication, 26th ESM congress, Istanbul, Turkey, 25-29 June 2005). The clear differentiator with this population was the widespread immunosuppression of all patients. Lastly, we know that in the Italian population, TST positivity correlates with age, presumably as a result of exposure when TB rates were higher than today [8]). The fact that the T-SPOT.*TB* test, and not the TST, was related to the age of the subjects further suggests that the T-SPOT.*TB* test was more closely reporting the true infection status of the patients than was the TST.

Our tentative conclusions are supported by the substantial body of literature demonstrating T-SPOT.*TB* is more closely correlated to exposure [9-13], more sensitive than the TST [7;14-17] and not prone to cross-reaction to BCG and most non-tuberculous mycobacteria [10;18-19].

Based on the above considerations and additional clinical parameters including the risk of side effects, only the 61 T-SPOT.*TB* positive subjects were considered for chemoprophylaxis for LTBI (due to present or past exposure); 8 patients older than 75 years were not treated (due to the high risk of side effects) as well as the 4 patients with previous history of TB and the patient with history of chemoprophylaxis. The 3 TST-positive, T-SPOT.*TB* negative patients were not treated. This decision was taken based on the fact that 2 of them were BCG-vaccinated, while the third was a 18-year old girl with a Mantoux test of 11 mm exposed 5 times, but for very short time to the index case, as reported by the patient. We considered that it was more likely that these patients were false-positive to the TST rather than falsely negative to the T-SPOT.*TB* test. False-negative T-SPOT.*TB* results can be excluded as the positive control demonstrated the presence of functional T-cells, even in the severely immunosuppressed patients.

None of the screened contacts has, after one year, developed active TB.

Immunosuppressed groups are arguably the most important target for screening and treatment of LTBI [20] because of their high risk of progression to active TB [2]. The poor performance of the TST in these groups greatly hinders this effort [14;16]. For this reason there is great interest surrounding the use of new blood tests in the diagnosis of tuberculosis infection in immunosuppressed people [21] and whether they can be more effective than TST.

Recently, Ferrara et al [22] reported that the QuantiFERON-TB Gold assay was significantly adversely affected by immunosuppression, giving a high proportion of indeterminate results. We report here the application of T-SPOT.*TB* for the LTBI screening of 138 haematology patients. In these immunosuppressed individuals, the indeterminate rate of T-SPOT.*TB* was only 4,3% and we

believe that the T-SPOT.*TB* test demonstrated greater sensitivity than TST, enabling us to identify 34 individuals candidates for chemoprophylaxis.

To our knowledge, this is the first study demonstrating the utility of new blood tests in immunosuppressed non-HIV positive patients. It suggests that the T-SPOT.*TB* test maintains sensitivity and performance in immunosuppressed patients (a finding recently confirmed in HIV patients [23]) and can identify infected patients anergic to TST. Further investigation is required to prove whether our observations are corroborated by larger-scale studies.

Acknowledgments.

T-SPOT.*TB* kits were provided by Oxford Immunotec, Oxford, UK.

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	T-SPOT.*TB* +ve	**T-SPOT.*TB* –ve**	**T-SPOT.*TB* indeterminate**	**Insufficient T-cells**	Total	
TST+ve	**21**	**3**[†]	**0**	**0**	24	17.4%
TST–ve	**34**	**57**	**5**	**2**	98	71.0%
No result	**6**	**8**	**1**	**1**	16	11.6%
Total	61	68	6	3	138	
	44.2%	49.3%	4.3%	2.2%		

Table 1:- Comparison of TST to T-SPOT.*TB* results in entire patient group.

[†] 2 BCG-vaccinated