Use of T-SPOT.*TB* in latent tuberculosis infection diagnosis in general and immunosuppressed populations

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SUMMARY

Immunosuppressed patients have a nine-fold greater risk of developing active tuberculosis (TB) disease given the latent TB infection than the general population. Few data are available on the predictivity of T-SPOT.*TB* in immunosuppressed patients.
We had a T-SPOT.*TB* determination and a TST from 197 immunosuppressed haematological patients and 324 community contacts of infectious TB cases. In the general population, TST was positive in 275 (84.9%), T-SPOT.*TB* in 167 (51.5%) ($p<0.0001$). In immunosuppressed patients, TST was positive in 34 (17.3%), T-SPOT.*TB* in 70 (35.5%). T-SPOT.*TB* is not influenced by immunosuppression and even an indeterminate result may yield useful information on patient's anergy.

KEY WORDS: Tuberculosis, IGRA test, Immunosuppression, LTBI

The number of people living under iatrogenic immunosuppressive conditions for onco-haematological diseases, transplants, anti-TNFα treatment and steroid therapy is constantly increasing. This population has a nine-fold greater risk of developing active tuberculosis (TB) disease given the latent TB infection (LTBI) than the general population (Malone *et al.,* 2004). Mantoux test (TST) is used for the screening of infected patients, but the test has shown a particularly low sensitivity in this category of patients due to their anergy (Cordonnier *et al.,* 2004). A test based on the enumeration of effector T cells specific for *M.tuberculosis* is becoming widespread for the diagnosis of LTBI, the T-SPOT.*TB*™ (Oxford Immunotec, Abingdon, UK). This test has proved to be more sensitive and specific in diagnosing active TB, and more related to intensity of exposure for detecting LTBI in contact tracing investigation than TST (Pai *et al.,* 2006). Some studies on HIV-positive patients revealed no difference in the performance of this test compared to the general population (Chapman *et al.,* 2002), and preliminary data are available on the use of this test in immunosuppressed haematological patients nosocomially exposed to active tuberculosis cases (Piana *et al.,* 2006).
Aim of this study is the evaluation of the T-SPOT.*TB* in the management of immunosuppressed patients at high suspicion of LTBI.
We enrolled 197 immunosuppressed haematological patients, contacts of two infectious TB cases and 324 community contacts of infectious TB cases as a control population. Both groups had a T-SPOT.*TB* determination and a TST performed during the same visit.
Contacts were interviewed to identify their pre-

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vious history of TB, vaccination with BCG and the extent of their contact with the index case.
TST was performed according to international standards: 5 tuberculin units (0.1 ml) of PPD were injected intradermal and the induration's diameter was read after 48-72 hours. Reactions were considered positive when the diameter was ≥5 mm, according to the Italian guidelines (Italian Ministry of Health, 1998).
T-SPOT.*TB* was performed according to the manufacturer's instructions and spots counted with the automatic reader ELISPOT (AID-GmbH, Dusseldorf, Germany), and visually checked. The presence of ≥20 spot forming cells (SFC) in the positive control well, demonstrating T-cell function, validates the assay results. An indeterminate result was defined as high background prevented interpretation or when <20 SFC were detected in the positive control wells (Chee *et al.*, 2007).
We used the logistic regression with PROC LOGISTIC in SAS/STAT version 9.1.3 (Cary, NC), with Windows 2000 Professional for the comparison of the results obtained by TST and T-SPOT.*TB*. Stepwise regression was used to select up to 4 statistically significant terms to be in the model. The level of statistical significance was $p<0.05$.
Data analysis showed that in community contacts TST was positive in 275 (84.9%), while T-SPOT.*TB* in 167 (51.5%) with a highly significant statistical difference ($p<0.0001$). In immunosuppressed patients, TST was positive in 34 (17.3%), T-SPOT.*TB* in 70 (35.5%). As a true gold-standard for the diagnosis of LTBI is not currently available, we tried to understand which of the two tests was less influenced by immunosuppression and more likely to detect LTBI, estimating the risk factors with a multivariate analysis for a positive result (Porsa *et al.*, 2006) (Table 1).
The TST was 10 times more affected than T-SPOT.*TB* by immunosuppression (T-SPOT.*TB*: OR: 0.323, $p<0.0001$, 95%CI: 0.172-0.587; TST: OR: 0.038, $p<0.0001$, 95%CI: 0.016-0.081).
We noticed that both tests were related to the age of patients, with elderly patients being more likely to be positive, and to previous history of treated TB disease, although T-SPOT.*TB* was less strongly so. Only T-SPOT.*TB* was significantly related to birth in a TB endemic country. TST showed a significant correlation to prior BCG

TABLE 1 - *Logistic regressions analysis for all contacts.*

		*T-SPOT.*TB			*TST*		
Factor	*N*	*Odds Ratio*	*P value*	*95% CI*	*Odds Ratio*	*P value*	*95% CI*
Immunosuppressed	197	0.323	p<0.0001	[0.172, 0.587]	0.038	p<0.0001	[0.016, 0.081]
Male sex	280	1.045	0.8923	[0.706, 1.548]	1.807	0.0413	[1.022, 3.226]
Elderly (60+ years)	155	4.509	p<0.0001	[2.503, 8.441]	3.173	0.0028	[1.424, 7.728]
Birth in a TB high endemic country*	186	1.897	0.0114	[1.147, 3.155]	0.302	0.0095	[0.116, 0.765]
Previous history of TB disease†	17	4.266	0.0295	[1.127, 20.522]	31.971	0.0016	[2.901, >1000]
BCG vaccinated	193	1.074	0.9295	[0.563, 2.024]	13.659	p<0.0001	[6.024, 34.257]
Family contact	156	0.725	0.2937	[0.407, 1.277]	0.551	0.1629	[0.224, 1.238]

*Defined as any country with TB case notification rates >40/100,000; †Previous history of TB as recorded by the investigator.

vaccination, whereas the T-SPOT.*TB* test did not (Table 1).

We supplemented the above regression analysis with a separate analysis to examine the factors explaining the discordances between the two tests. These results are shown in table 2. The negative effect of immunosuppression on the TST was supported by the fact that discordant TST +ve, T-SPOT –ve results were far less likely to appear in these patients compared to when the tests agreed, while discordant T-SPOT +ve, TST –ve results were also far more likely to appear. We also found an association of BCG vaccination with the discordant TST +ve, T-SPOT –ves, while T-SPOT +ve, TST –ves were underrepresented amongst those who were BCG vaccinated. The discordance TST+ve, T-SPOT –ve fell significantly in people born in a high TB incidence country. The same discordance decreased considerably in the elderly (60+ years of age).

Among community contacts, T-SPOT.*TB* was indeterminate (less than 20 spot in the positive control well) in 9 cases (2.8%), while amongst immunosuppressed patients 12 (6.0%), with a non-statistically-significant difference. All immunosuppressed patients with an indeterminate result were TST-negative.

Our findings highlight that TST is 10-fold more affected by immunosuppression than T-SPOT.*TB*, and confirm that T-SPOT.*TB* is a better marker for LTBI, being more related than TST to the well known risk factors for TB infection. In fact, a positive T-SPOT.*TB* result is associated with the age of patients, and it is known that LTBI is more frequent in the elderly population as a result of exposure when TB rates were higher than currently (Moro *et al.,* 1999). Interestingly, only T-SPOT.*TB* is significantly related to birth in a TB endemic country, another important marker of TB exposure.

TABLE 2 - *Logistic regression analysis for disagreements.*

	T-SPOT.TB +ve, TST –ve				*TST +ve, T-SPOT.TB –ve*			
agree	*Compared to cases where the tests agree*				*Compared to cases where the tests*			
Factor	*N*	*Odds Ratio*	*P value*	*95% CI*	*N*	*Odds Ratio*	*P value*	*95% CI*
Immunosuppressed	162	7.709	p<0.0001	[2.650, 30.716]	134	0.293	0.0011	[0.129, 0.635]
Male sex	188	0.820	0.6722	[0.399, 1.675]	234	1.266	0.3882	[0.770, 2.085]
Child (0-12 years)	12	1.146	1.0000	[0, 9.822]	18	3.475	0.1128	[0.774, 14.992]
Elderly (60+ years)	128	1.874	0.1326	[0.848, 4.324]	103	0.374	0.0214	[0.145, 0.880]
Birth in a TB high endemic country*	119	0.883	1.0000	[0.017, 9.433]	174	0.182	p<0.0001	[0.075, 0.408]
Previous history of TB disease†	9	0.426	0.5033	[0, 3.548]	10	0.530	0.9540	[0.011, 4.381]
BCG vaccinated	107	0.118	0.0259	[0, 0.804]	184	5.864	p<0.0001	[2.669, 13.715]
Family contact	97	0.624	1.0000	[0.011, 8.175]	148	1.369	0.3516	[0.743, 2.548]

*Defined as any country with TB case notification rates >40/100,000; †Previous history of TB as recorded by the investigator.

Moreover, we find out that both tests are related to previous history of treated TB disease, although T-SPOT.*TB* was less strongly so; this can be partially explained by the possible negativization of specific effector T-cells after an effective treatment (Ewer *et al.,* 2006), while this is not the case for TST ("once positive, always positive") (Menzies, 1999). As expected, and as previously reported, (Lalvani *et al.,* 2001; Farhat *et al.,* 2006) TST shows a significant correlation to prior BCG vaccination, whereas the T-SPOT.*TB* test does not.

Another interesting result is that the estimated LTBI prevalence varies markedly among immunosuppressed and non-immunosuppressed individuals when TST results are considered (17.3% and 84.9%, respectively). Otherwise, if T-SPOT.*TB* results are taken into account, the apparent prevalence seems more stable (35.5% and 51.5%, for immunosuppressed and non-immunosuppressed, respectively). The slight difference of T-SPOT.*TB* positivity seen among immunosuppressed and general population might be due to a lesser degree of exposure (the former being nosocomial contacts and the latter close family contacts). These findings further corroborate the hypothesis that the T-SPOT.*TB* might overcome the problem of immunosuppression.

A major concern of TST in immunosuppressed patients is the poor sensitivity due to the typical anergy of this population. A negative TST result might be due either to lack of LTBI, and to anergy itself and there is no way, for the clinician, to distinguish among these two conditions.

In the T-SPOT.*TB* assay the presence of an internal positive control allows the result to be validated and guarantees the effective vitality of the lymphocytes used to carry on the determination of the interferon-gamma release assay.

In our study, the indeterminate rate was not statistically significant different between the healthy community contacts (2.8%) and the immunosuppressed patients (6.0%); although, as previously observed (Piana *et al.,* 2006), the rate of indeterminates shows a trend with the immunosuppression state.

It should be noted that in an immunosuppressed patient, even an indeterminate result may be useful to the clinician, as it will distinguish between a simply negative result (such as the skin test one) from anergy, detected, in this test, by the lack of mitogen response (Pai *et al.,* 2005). Interestingly, all immunosuppressed patients with an indeterminate result are TST-negative.

In conclusion, in this study, the largest one available to date on immunosuppressed haematological patients, the T-SPOT.*TB* test proved to be a reliable tool for contact tracing investigation in immunosuppressed patients.

Oxford Immunotec kindly provided the reagents to perform this study. This work was partially supported by a grant from the Italian Institute of Health to DMC n RF04/126. We thank Dr. R. Centis for the revision of statistics. We are grateful to all the patients who participated in this study and to the health care workers of the Villa Marelli Institute.

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