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CLINICAL REPORT

Evaluation of an IFN-gamma Assay in the Diagnosis of Latent Tuberculosis in Patients with Psoriasis in a Highly Endemic Setting

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Screening for latent tuberculosis infection is mandatory before starting anti-tumour necrosis factor treatments, but its diagnosis still poses a challenge. While studies performed in developed countries have demonstrated superior performance of T-cell based interferon-γ release assay (IGRA) compared with the tuberculin skin test, there is a debate about whether this holds true in tuberculosis endemic areas. The performance of an IGRA kit (T-SPOT.*TB*) was evaluated in 33 moderate-to-severe untreated psoriasis patients and, as controls, 30 patients with common dermatological diseases at a tuberculosis highly endemic setting. The frequency of positive tuberculin skin test responses and induration size in controls were higher than in psoriasis patients (53% vs. 18% and 9.3 ± 1.4 vs. 2.6 ± 0.7 mm, respectively, $p < 0.001$). In contrast, the frequency of positive response and mean number of spots elicited with the T-SPOT.*TB* test were not significantly different between patients and controls (47% vs. 43% and 14.7 ± 3.2 vs. 20.5 ± 3.1 spots/well, respectively). The two tests presented good agreement in the control, but not the psoriasis group (κ values of 0.625 and 0.375, respectively). Thus, in a highly tuberculosis-endemic setting the T-SPOT.*TB* test was superior to the tuberculin skin test in diagnosing latent tuberculosis infection in psoriasis, probably because the immune dysregulation of psoriasis shows a lower interference in the *in vitro* test. *Key words: psoriasis; TNF; tuberculin skin test; latent tuberculosis infection; interferon-γ release assay; ELISPOT.*

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Anti-tumour necrosis factor (anti-TNF) treatments may reactivate latent tuberculosis infection (LTBI). Thus, screening for LTBI is mandatory, and preventive treatment should be given to all patients with evidence of this infection before starting any anti-TNF therapy, even if it does not offer complete protection (1, 2). Given that the tuberculin skin test (TST) is not an ideal screening test for identification of cases of LTBI in patients with psoriasis (3), alternative tests have been developed. It has been suggested that the standardized antigen-specific (ESAT-6 and CFP-10) T-cell interferon-γ release assay (IGRA) may be a useful surrogate marker of LTBI, since it is more sensitive and specific than the TST, specially in non-endemic areas, where it has been shown to avoid cross-reaction with BCG vaccination and other mycobacterial infections (4, 5). However, some controversy still persists in its performance regarding TST in endemic areas (6). The results of studies carried out in these areas are contradictory to those of non-endemic areas. A study of household contacts of TB cases in Gambia showed the TST to be more sensitive than the IGRA, although at the cost of some specificity (7). In a study of Indian healthcare workers, the IGRA and TST demonstrated good agreement and comparable performances (8). Studies with healthy adults from a rural community in South Africa demonstrated poor agreement between TST and IGRAs of different generations and found a high proportion of individuals (30–56%) with indurations >15 mm who tested negative on the IGRAs (9). Besides this, no studies compared the TST with a standardized IGRA kit for the diagnosis of LTBI in psoriasis patients living in a highly endemic area. The aim of this study was to evaluate the performance of such a T-cell-based IGRA in the diagnosis of LTBI, comparing it with TST in patients with psoriasis in a highly TB endemic city (128.7/100,000 persons year in 2005), Recife, in the northeast of Brazil.

MATERIALS AND METHODS

Patients and controls

A cross-sectional study was carried out to evaluate the diagnostic test. Thirty-three patients with psoriasis (psoriasis group) and 30 patients with acne, seborrhoeic dermatitis, or other common dermatological diseases (control group), who were followed at the General Dermatology Out-patients Department of Santa Casa de Misericórdia, Recife, Brazil, from February to November 2009, were recruited by non-probabilistic sampling, according to convenience. The exclusion criteria were: age

under 18 years; any other disease that compromised immunological competence or posed an increased risk to TB; treatments with immunosuppressive drugs; active tuberculosis infection; BCG vaccination within the past 15 years; and pregnancy. The study was approved by the Ethics Committee for Research on Human Beings of Federal University of Pernambuco (number 229/2008) and informed consent was obtained from all subjects before inclusion in the study. Blood for the IGRA was collected just before the administration of 0.1 ml (2 tuberculin units) of purified protein derivative (PPD) RT-23 (Statens Serum Institut, Copenhagen, Denmark). The diameter of the induration was read after 72 h, and responses were considered to be positive at > 5 mm for psoriasis patients and > 10 mm for controls, based on the III Brazilian Consensus on TB, which takes into account the immunological background of the patients and the indication of anti-TNF treatment. All patients also had a chest X-ray.

*T-SPOT.*TB *test*

The T-SPOT®.*TB* test (Oxford Immunotec, Abingdon, UK), hereafter called TB-spot test, is a variant of the enzyme-linked assay technique for enumerating effector T cells (ELISPOT) that secrete IFN-γ upon overnight stimulation with peptide pools of two specific antigens from *Mycobacterium tuberculosis*; ESAT-6 and CFP-10 (4, 5). IFN-γ responses to these pools were performed according to manufacturer's instructions using peripheral blood mononuclear cells (250,000 cells/well) obtained through Ficoll-Hypaque gradient as in previous studies (10). The test was considered positive according to the following criteria: (*i*) in cases in which the negative control presented from 0 to 5 spots: spot count with either ESAT-6 or CFP-10 minus negative control spot count was ≥6; (*ii*) in cases in which the negative control presented more than five spots: spot count with either ESAT-6 or CFP-10 ≥ twice the negative control. The assay was considered valid when the positive control well gave > 20 spots.

Statistical analysis

Comparison between results of TST and TB-spot was done using agreement and κ statistics. The level of agreement was determined by Cohen's κ, with a κ value > 0.8, representing an excellent agreement, 0.7–0.8 very good, 0.5–0.69 good, and < 0.5 poor.

RESULTS

Patients and controls characteristics

All patients and controls included in the study had valid TB-spot tests and TST readings. Patients psoriasis severity was either moderate ($n = 15$, Psoriasis Area Severity Index (PASI) of 8–12) or severe ($n = 18$, PASI > 12). None received previous systemic treatment to psoriasis. Table I shows the main clinical and demographic characteristics. All patients had been BCG vaccinated during the first month of life and none had findings suggestive of previous or current TB on chest X-ray. The patients and controls' groups studied presented a similar low education and socio-economical level.

TB-spot test and TST performance

There was a significantly higher prevalence of TST reactors in the control group than in the patient group (53 vs. 18%, $p < 0.001$) (Fig. 1). This high prevalence in the control group is equivalent to that reported for a healthy adult population in Brazil (51%) (11). In agreement with this, the mean TST induration in the control group was significantly higher than that in the psoriasis group (9.3 ± 1.4 vs. 2.6 ± 0.7 mm, $p < 0.001$).

In contrast, similar prevalence of positive TB-spot test was observed in patients (43%) and controls (47%) ($p > 0.05$, Fig. 1). When the mean number of spots elicited with either ESAT-6 or CFP-10 was analysed there was no significant difference between patients and controls (ESAT-6: 3.2 ± 1.0 vs. 5.7 ± 1.8 spots/well, $p > 0.05$, respectively; CFP-10 3.6 ± 1.4 vs. 4.5 ± 1.8 spots/well, $p > 0.05$, respectively). When only the positive ESAT-6 and CFP-10 responses were considered together the number of spots produced by patients and controls' cells were also comparable (14.7 ± 3.2 vs. 20.5 ± 3.1 spots/

Table I. *Characteristics of patients and controls with no abnormalities in chest X-rays*

	Psoriasis ($n = 33$)	Controls ($n = 30$)
Age distribution, years, %		
>50	24.2	3.3
30–50	54.5	63.3
20–29	21.3	33.3
Male, %	57.6	33.3
Psoriasis score, median and range	16 (8–24)	NA
Income (as a multiple of the minimum wage), %		
>2	2 (6.7)	1 (3)
>1–2	1 (3)	1 (3)
≤1	27 (90)	28 (94)
Years of education, %		
≥12	6.0	13.3
4–11	81.8	80.0
<4	12.1	6.6
Agglomeration per room, %		
1–3 persons	51.5	53.3
>3 persons	48.5	46.7

NA: not applicable.

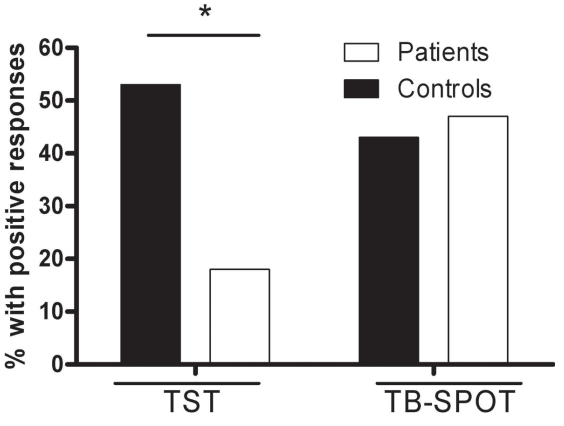

Fig. 1. Frequencies of positive responses to tuberculin skin test (TST) and T-SPOT.*TB* test in psoriasis patients and controls. *$p < 0.001$, Fisher's exact test.

well, $p > 0.05$). Moreover, the number of spots elicited in the positive control wells was comparable between the two groups (patients, 184 ± 32; controls, 229 ± 40 spots/well, $p > 0.05$). Neither TST induration nor TB-spot counts presented a significant correlation with the PASI score (not shown). Also, the frequency of positive and negative TST and TB-spot responses were comparable between moderate and severe psoriasis patients.

Agreement performance between TST and TB-spot

The two tests presented a good agreement in the control group, with a concordance of 80% and a κ value of 0.624. In contrast, in the patients group agreement was poor, with 66% of concordance and a κ value of 0.375 (Table II).

DISCUSSION

While studies performed in developed countries have shown superior sensitivity and specificity of the currently available IGRA kits (QuantiFERON-TB Gold, Cellestis, Melbourne, Australia and T SPOT-*TB* test, Oxford Immunotech) for the diagnosis of latent TBI as compared with TST (12, 13), there still is a debate on whether this holds true in TB endemic areas (6). While TST and the QuantiFERON-TB Gold performed comparably in a study of healthcare workers in India (8), in Korea the latter was a better indicator of LTBI in a BCG-vaccinated population with variable degree of TB exposure than was the TST (14). More recent studies in Gambia have shown an excellent performance of the TST for the diagnosis of LTBI from recent exposure and that both tests may be required to better diagnose LTBI in case contacts, thus not favouring the simple replacement of the TST by an ELISPOT assay (15, 16).

This issue is further complicated when populations with immune disturbances are addressed; for example, patients with immuno-mediated inflammatory diseases (IMID). We have previously shown that patients with severe psoriasis have depressed *M. tuberculosis*-specific immune responses, *in vivo* (TST) and *in vitro* (lymphocyte proliferative responses and IFN-γ production); however, the *in vivo* immune responses were more intensely affected, since those individuals who were non-reactors to the TST still exhibited normal responses to an in-house ELISPOT assay with different *M. tuberculosis* antigens (10). Several studies have investigated the cell-mediated reactivity status of psoriasis patients. Tsiouri et al. (17) described exacerbated TST responses, while other *in vitro* and/or *in vivo* studies found either depressed or normal reactivity (18–21). The reasons for such discrepancies are unclear, but may be related to differences in severity of the psoriasis, immunosuppressive treatment, and immunological tests employed. On the other hand, patients with rheumatoid arthritis (RA) have also been shown to present decreased TST reactivity: the proportion of TST reactors was at least two-fold higher in healthy adults than in RA patients in endemic countries, such as Peru and Brazil, or non-endemic countries, such as Japan (22–24). This was also shown for patients with inflammatory bowel diseases (25).

Table II. *Agreement between the T-SPOT.*TB *test and tuberculin skin test (TST) in psoriasis and control groups*

Group	T-SPOT.*TB*$^+$	T-SPOT.*TB*$^-$	Total	Agreement (%)	κ
Control				80.0	0.624
TST+	12	4	16		
TST–	2	12	14		
Total	14	16			
Psoriasis				66.6	0.375
TST+	3	3	6		
TST–	8	19	27		
Total	11	22			

These findings may explain the markedly superior performance of the IGRA for the diagnosis of LTBI in IMID patients in both endemic and non-endemic settings (23, 24, 26, 27). Few studies have addressed this issue in psoriasis patients. Laffitte et al. studied the frequency of LTBI in moderate to severe psoriasis patients in Switzerland, and demonstrated the TB-spot could favourably replace the TST in this setting (28). An earlier study of 11 psoriasis patients at an apparently low risk for TB also suggested the utility of this assay (29). Our study is first to suggest that the IGRA is superior to TST in a TB high-risk psoriasis population. Despite the limited number of patients studied, the frequency of LTBI in psoriasis patients was found to be significantly higher using the TB-spot than the TST, and then comparable with that of the control, non-immunosuppressed group, as well as to that observed in a survey of a healthy adult population in Brazil (12). This is reinforced by the observation that, in the control, but not in the patient group, both tests presented good agreement. While both the frequency of TST reactors and the size of the TST induration were diminished in the patient group, the number of spots elicited with either ESAT-6 and/or CFP-10 was comparable between the two groups. This supports the idea that the immune imbalance of the IMID decreases the TST response more than the IGRA reactivity. The immunological mechanisms underlying this observation remain to be determined.

In conclusion, our data suggest that, in highly TB endemic settings, the IGRAs are superior to the TST, probably because the immune dysregulation of psoriasis shows a lower interference in the *in vitro* test. Further studies with larger cohorts are warranted to confirm these results.

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The authors declare no conflicts of interest.

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