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Do results of the T-SPOT.*TB* interferon-γ release assay change after treatment of tuberculosis?

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Summary
Background: Interferon-gamma (IFN-γ) production by lymphocytes exposed to antigens specific of *Mycobacterium tuberculosis* has been shown to correlate with antigen load and disease activity.
Aim of study: To determine whether treatment of tuberculosis (TB) led to a decrease and/or a reversion of results of a IFN-γ release assay (T-SPOT.*TB*, Oxford Immunotec, UK) and thus if T-SPOT.*TB* could be used to monitor response to treatment.
Methods: Qualitative and quantitative analysis (SFUs: spot-forming units) of T-SPOT.*TB*® in HIV-negative patients with TB, during initial 2 weeks of treatment (T0), at end of treatment (TE) and 6 months later (TE + 6).
Results: Mean SFU (SD) was 75 (58; $n = 62$) at T0, 46 (55; $n = 55$) at TE, and 33 (46; $n = 41$) at TE + 6; positive rate was 98%, 93% and 98%, respectively. SFUs (paired samples, $n = 36$) decreased significantly between T0 and TE; 2 reversions occurred between T0 and TE (6%), but none between TE and TE + 6. Of 6 patients (17%) with an increase in SFUs between T0 and TE, 5 had a favourable outcome at TE and TE + 6.
Conclusion: Decrease in SFUs under treatment suggests a relationship with antigen load; however, persisting high SFUs were not predictive of unfavourable outcome and test reversion was rare.
This trial was registered at www.clinicaltrials.gov (NCT00595907).

Introduction

Over the past 5 years, two assays (T-SPOT.*TB*, Oxford Immunotec, UK and QuantiFERON-GOLD in tube®, Cellestis,

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Australia) measuring the production of interferon-gamma (IFN-γ) by peripheral blood lymphocytes exposed to antigens highly specific of *Mycobacterium tuberculosis* (ESAT-6 and CFP-10), have become commercially available.[1] In patients with tuberculosis (TB), these tests, referred to as IGRA (IFN-γ release assays) have been shown to be at least as sensitive as the tuberculin skin test (TST) for the detection of infection by *M. tuberculosis* in immunocompetent individuals, and much more specific. Their major contribution is that they do not yield false positive results in subjects with previous BCG vaccination, or infection by most MOTT.[2] IGRA have been recently included in several national guidelines for the detection of latent tuberculosis infection (LTBI) either as a replacement of the TST, or as a complement in a ''two-step procedure'' (i.e., confirming positive TST results with an IGRA).[3–5]

Quantitative assessment of IFN-γ production by sensitized lymphocytes in response to ESAT-6 and/or CFP-10 has been shown to correlate with disease activity although these results are still debated. Monitoring of spot-forming units (SFUs) in ELISPOT response to antigens specific of *M. tuberculosis* (ESAT-6 and CFP-10) could thus theoretically be useful for assessing response to treatment. This study aimed therefore to determine, in immuno-competent subjects treated for TB: (1) whether reversion of T-SPOT.*TB* assay and/or a significant decrease in SFUs could be documented after treatment; (2) whether an increase in SFUs was associated with an unfavorable clinical outcome; (3) how results of the T-SPOT.*TB* evolved 6 months after treatment completion.

Patients and methods

Between October 1, 2004 and June 30, 2006, all subjects either treated for culture-proven TB or having completed their treatment within the past 6 months, and followed by the Division of Pulmonary Diseases (Geneva University Hospital) were prospectively invited to participate in this study after informed consent. Exclusion criteria were age under 18 years, history of prior active tuberculosis, and HIV infection.

The study protocol was accepted by the ethics committee on clinical research of Geneva University Hospital. All subjects included provided written informed consent. The trial was registered at www.clinicaltrials.gov (NCT00595907).

Blood samples were taken within 2 weeks of initiation of treatment (T0), at treatment completion (TE), and 6 months after treatment completion (TE + 6) (Fig. 1).

Peripheral venous blood samples (8 ml) were processed by our laboratory within 3 h of sampling for determination of *M. tuberculosis* specific IFN-γ secreting T cells using the T-SPOT.*TB* IGRA (Oxford Immunotec, Oxford, UK) according to the previously reported manufacturer's recommendations.[6] Tests were considered as indeterminate if SFUs in the positive control were <20, or if SFUs in the negative well were >10. Results were scored as positive if SFUs in either ESAT-6 or CFP-10 wells were >6 spots above SFUs of the negative control. SFU values are reported as [highest SFU value of ESAT-6 or CFP-10 antigen − SFU value of negative control]. We also individually scored SFUs for ESAT-6 and CFP-10 according to the same procedure ([SFU value of either antigen − SFU value of negative control]).

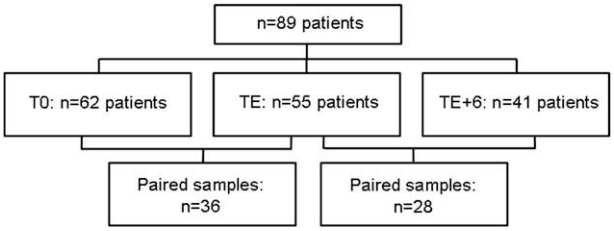

Figure 1 Study structure and number of samples analysed. T0: blood samples within 2 weeks of beginning of treatment; TE: blood samples at the end of treatment; TE + 6: blood samples 6 months after end of the treatment.

Statistical analysis

Data are reported as mean ± standard deviation (SD) unless specified otherwise.

Paired observations (SFUs, T0—TE, TE—TE + 6) were compared by Wilcoxon signed rank test for results of T-SPOT.*TB*, ESAT-6 and CFP-10 wells.

Unpaired observations were compared by Mann—Whitney rank-sum test. Level of significance was set at $p < 0.05$.

Results

Inclusion criteria were met by 89 HIV-negative patients (43 male, 46 female, aged 37 ± 15 years, 90% foreign born). TB was pulmonary in 70 subjects (79% of total, 86% smear positive), extra-thoracic in 34 (38% of total), and both in 15 (17%). All patients had *M. tuberculosis* strains susceptible to major tuberculostatic drugs, and were treated according to international ATS/CDC guidelines. None received corticosteroids or any other immunosuppressive treatment. Compliance to treatment was monitored either by DOT, or through monthly urinary testing for isoniazid, and attendance to clinical visits, and was optimal for all patients studied.

T-SPOT.*TB* was performed at T0 in 62 subjects, at TE in 55, and at TE + 6 in 41 (Fig. 1). One T-SPOT.*TB* result was indeterminate (1.1%).

Fig. 2 shows box plots for all subjects included; T-SPOT.*TB* was positive in 62 of 63 samples at T0 (98%), 51 of 55 samples at TE (93%), and 40 of 41 samples at TE + 6 (98%). SFU counts were significantly lower at TE than at T0 ($p < 0.001$), but did not differ significantly between TE and TE + 6.

Paired samples between T0 and TE were available for 36 subjects (1 indeterminate; Fig. 3). Average SFU counts decreased from 101 ± 47 to 55 ± 60 ($p = 0.0002$). SFU counts increased in 6 cases, 5 of whom had an unremarkable clinical resolution of their TB. One older patient with disseminated TB relapsed shortly after a 12-month treatment, and subsequently died of pneumonia. Two patients reverted their T-SPOT.*TB* between T0 and TE.

Between TE and TE + 6, paired samples ($n = 28$) showed a non-significant decrease in SFUs (67 ± 60 vs. 54 ± 54, $p = 0.36$), and no test reversion. Twelve subjects slightly increased their SFU counts (Fig. 4); none relapsed during study period.

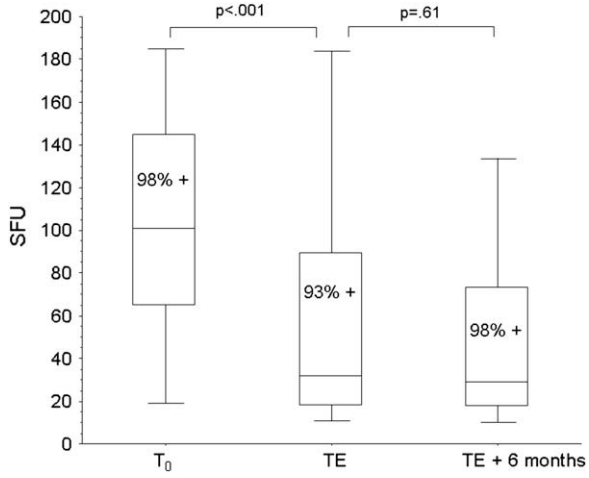

Figure 2 Box plot of spot-forming unit (SFU) counts of subjects at the beginning of treatment for culture-proven tuberculosis (T0; $n = 62$), at the end of treatment (TE; $n = 55$), and 6 months after end of treatment (TE + 6; $n = 41$). The box shows median value, 25th and 75th percentiles; bars show 5th and 95th percentiles. p Values indicate results of Mann–Whitney rank-sum tests. Percentages in box plot indicate rate of positive T-SPOT.*TB* according to manufacturer's recommendations.

For 20 patients (Fig. 5), samples were available at T0, TE and TE + 6; SFU counts decreased significantly between T0 and TE (111 ± 60 vs. 60 ± 57, $p = 0.01$), and non-significantly between TE and TE + 6 (60 ± 57 vs. 39 ± 29, $p = 0.10$); among these patients, non-reverted.

SFU counts for both ESAT-6 or CFP-10 antigens decreased significantly between T0 and TE, but not between TE and TE + 6 (data not shown).

Discussion

Data reported show that (1) the T-SPOT.*TB* was positive in 98% of subjects with TB during the first 2 weeks of treatment (which is in the upper range of previously reported sensitivity of the T-SPOT.*TB*)[2] and remained positive in most patients 6 months after treatment completion; (2) SFU counts decreased significantly between T0 and TE, but test reversion was rare; 6 months after treatment completion, SFU changes were non-significant, and no further test reversion occurred; (3) a small number of subjects increased their SFU counts between T0 and TE; in most cases, however, this was not associated with an adverse outcome.

Our results confirm previous observations suggesting a relationship between bacterial load and number of γ-IFN producing antigen-specific effector T cells enumerated by the T-SPOT.*TB* assay. The decrease in bacterial load resulting from efficient antituberculous therapy would explain why the number of ESAT-6 and CFP-10 specific

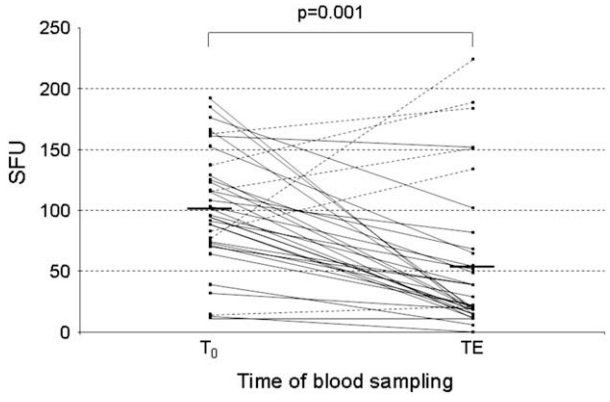

Figure 3 Individual results for patients with paired samples ($n = 36$) between beginning of treatment (T0) and end of treatment (TE); horizontal bars indicate mean values; p value given for Wilcoxon signed rank tests. Dotted lines indicate subjects ($n = 6$) with an increase in SFU between T0 and TE.

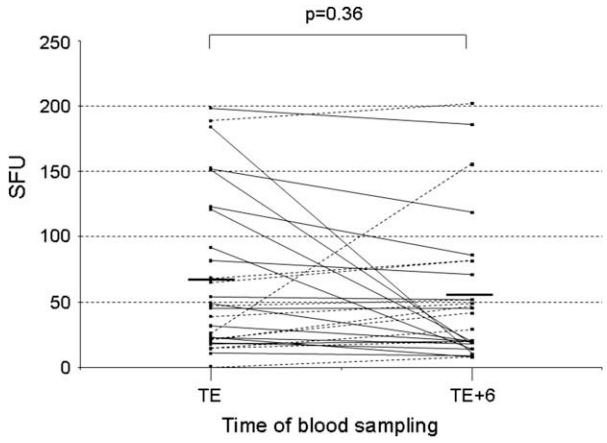

Figure 4 Individual results for patients with paired samples ($n = 28$) between end of treatment (TE) and 6 months later (TE + 6); horizontal bars indicate mean values; p value given for Wilcoxon signed rank tests. Dotted lines indicate subjects ($n = 12$) with an increase in SFU between TE and TE + 6.

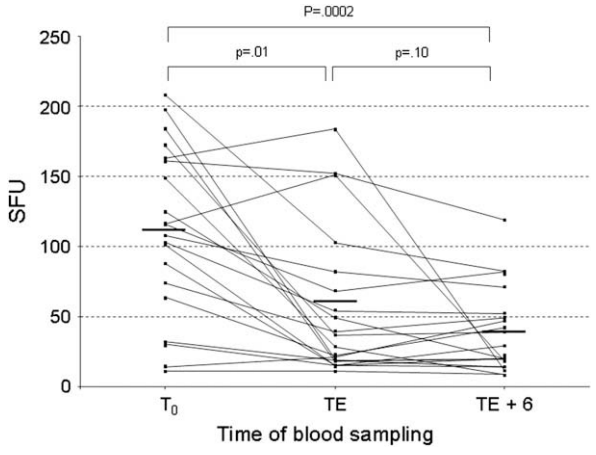

Figure 5 Individual results for patients with paired samples ($n = 20$) available at beginning of treatment (T0), end of treatment (TE) and 6 months later (TE + 6); horizontal bars indicate mean values; p value given for Wilcoxon signed rank tests.

T cells decreases during treatment.[7] Quantitative assessment of T-SPOT.*TB* results (SFU counts) could thus in theory reflect bacterial burden and be used to monitor response to TB treatment.[7] Indeed, SFU counts have been reported as significantly higher in subjects with TB than in subjects with LTBI and no evidence of active disease.[6] One case report documented a progressive increase in ELISPOT response to ESAT-6 and CFP-10 prior to diagnosis of TB, followed by a decrease in SFUs under treatment.[8] ELISPOT assays using either ESAT-6 alone,[9,10] or ESAT-6 and CFP-10[11] have shown, in adults with TB, a decrease in SFUs under treatment. A slightly different time course has been reported in children with TB, with an initial increase in response to ESAT-6 during the first month of treatment, followed by a progressive decrease in response to both ESAT-6 and CFP-10.[12]

One study documented a delayed drop in SFUs in late responders to treatment in patients treated for TB: in these patients, *M. tuberculosis* could still be cultured from either sputum, blood or pleural fluid.[9] The authors suggested that a delayed drop in SFUs could be an indicator of adverse prognosis and poor response to treatment. In the present study, however, among 6 patients who increased their SFU counts between T0 and TE (Fig. 3), 5 had a normal response to treatment; none had persistent culture positive samples after 2 months; one elderly patient relapsed early after treatment completion and died subsequently of pneumonia. Among 12 patients who increased their SFU counts during the 6 months following completion, none relapsed (Fig. 4).

Reversion of IGRA results has been shown in two studies: among 82 HIV-negative cases of TB successfully treated in the Gambia, 44 (55%) were ELISPOT negative 12 months after diagnosis.[13] In Cape Town, 17 of 21 (81%) HIV-negative patients who had successfully completed TB treatment were IGRA negative.[14] Our data do not confirm these high reversion rates: 5.5% of patients reverted their T-SPOT.*TB* between T0 and TE, and none during the following 6 months. Furthermore, patients sampled at TE and 6 months later (Fig. 1) had, respectively, 93% and 98% of positive T-SPOT.*TB* results.

Two limitations of this study must be mentioned: the first is the relatively small number of cases, which is related to the epidemiology of tuberculosis in Switzerland, a low incidence country with an annual incidence of 8/100,000 inhabitants; the second is the 25% rate of ''lost to follow-up'' subjects between TE and TE + 6: 90% of our cases were foreign born, many of whom were either asylum seekers or illegal migrants; although compliance was excellent during treatment, this is a very mobile population for whom long term follow-up studies are difficult to perform.

In summary, number of γ-IFN producing antigen-specific effector T cells decreased significantly in most TB cases between diagnosis and treatment completion, thus confirming several reports showing a quantitative relationship between disease activity (or antigen load) and IGRA results; test reversion was, however, exceptional; furthermore, most subjects with an increase in SFUs between T0 and TE had an uneventful clinical improvement. These results challenge the idea that IGRA can be used to monitor treatment efficacy or risk of relapse.

Conflict of interest statement

There are no conflicts of interest regarding this study for Valerie Bosshard.
There are no conflicts of interest regarding this study for Pascale Roux-Lombard.
There are no conflicts of interest regarding this study for Thomas Perneger.
There are no conflicts of interest regarding this study for Marie Metzger.
There are no conflicts of interest regarding this study for Regis Vivien.
There are no conflicts of interest regarding this study for Thierry Rochat.
There are no conflicts of interest regarding this study for Jean-Paul Janssens.

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