

Steroid therapy does not affect the rate of indeterminate results with the T-SPOT.TB interferon gamma release assay

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Abstract

Background: The underlying causes of indeterminate (invalid) interferon-gamma release assays are not clear and predictors of indeterminate results do not exist. To date the effect of steroid concomitant therapy on the rate of indeterminate tests is unknown.

Methods: We performed a retrospective analysis of the results of 1339 tests, including clinical data from patient records, of which 914 were suitable for complete data analysis.

Results: 130 (15.2 %) tests were positive and 719 (83.8 %) were negative, 9 tests (1 %) were borderline positive (according to manufacturer's definition). Indeterminate results (56 (6.1 %) arose from failure of the negative control in 24 (42.9 %) and failure of the positive control in 32 (57.1 %) tests. Of 914 patients, 196 were taking cortisone at the time of testing; among these patients 180 (91.8 %) returned a valid result and 16 (8.2 %) an indeterminate result compared to 40 indeterminate tests of 718 (5.6 %) results from patients not on corticosteroid therapy at the time of testing ($p = 0.180$), (relative risk [RR] 1.46, [95 % CI 0.84 to 2.56]). No significant difference in the rate of indeterminate results was found with immunosuppressive diseases or medications other than with HIV; among HIV-positive patients, 11 (11.7 %) had an indeterminate test result, compared to 45 (5.5 %) in HIV-negative individuals ($p = 0.017$, relative risk [RR] 2.13, [95 % CI 1.14 to 3.98]).

Conclusions: steroid therapy does not have an effect whereas HIV positivity has a negative effect on the number of indeterminate results in the T-Spot.TB test.

Background

Tuberculosis (TB) remains one of the most lethal diseases worldwide. According to the WHO, one-third of the world's population has been infected with *Mycobacterium tuberculosis* and has latent TB (LTBI). Although immunocompetent people with LTBI only have a 10 % lifetime risk of developing active TB, immunocompromised persons, such as those with HIV infection, malnutrition or diabetes mellitus have a much higher risk of developing active TB.[1] People taking immunosuppressive medication have an increased risk for primary active TB infection and reactivation of LTBI.[1] Therefore most guidelines recommend that patients should be submitted to screening for LTBI before starting immunosuppressive therapy with medications such as anti-tumour necrosis factor (TNF) therapy. Screening protocols prescribe LTBI detection with an interferon (IFN)- γ release assay (IGRA). A number of studies have confirmed that IGRAs have a higher specificity and sensitivity than the tuberculin skin test (TST). [2] In addition IGRA tests are not influenced by prior immunisation with BCG or exposure to non-tuberculous mycobacteria (with a few exceptions) as is the tuberculin skin test. In Europe, two commercial IGRA-tests are available: The QuantiFERON-TB Gold In-Tube (QFT-GIT) (Qiagen, Hilden, Germany) and the T-SPOT.TB (TSPOT) test kit (Oxford Immunotec Ltd, Abingdon, Oxfordshire, United Kingdom). Both tests utilize an in vitro detection of IFN γ released by memory T-cells after stimulation with *M. tuberculosis*-specific recombinant antigens. The two antigens; early secreted antigenic target 6 (ESAT-6) and culture filtrate

protein 10 (CFP-10), used are encoded by genes located on the region of difference 1 (RD 1) of the *M. tuberculosis* genome. The QuantiFERON test utilizes a third additional antigen, TB-7.7 derived from the RD 2 region. These antigens are not encoded on most mycobacteria other than *M. tuberculosis*-complex, *M. marinum*, *M. kansasii*, *M. szulgai*, and *M. flavescens* [3] and therefore there is less cross reactivity and fewer false positive results than with the tuberculin skin test. The results of the TSPOT are derived from a defined number of spots, i. e. T-cells, above a cut-off derived from the number of spots detected in unstimulated cells from the same patient. A positive control consisting of mitogen stimulation of the patient's cells is run for each test. This test allows for a degree of standardisation by defining the PBMC count used in the assay. A specific T-cell deficiency is, however not compensated for. Indeterminate results may arise due to a high background response in the unstimulated cells or a lower than expected response to mitogen-stimulated cells. There are a number of variables that may impact both tests as both require viable T-cells for stimulation. A summary of these factors is given in a review by Pai et al. [4]

Since IGRAs were introduced in 2005 many studies have investigated the performance and utility of both QFT-GIT and TSPOT in different settings and patient groups. [5] Studies comparing the performance of the tuberculin skin test and QFT-GIT in regard to the influence of immunosuppressive therapy showed that prednisolone affects the performance of both the QFT-GIT and TST. [6] There has been one report from Japan, in which the effect of corticosteroid therapy on the validity of TSPOT and QFT-GIT among children receiving steroids was investigated. This study found no correlation between steroid therapy and indeterminate results for TSPOT compared to 9.9 % indeterminate results for QFT-GIT. [7] A significant indication for LTBI testing arises from the need to treat with TNF antagonists, and a proportion of these patients will receive various other immunosuppressive therapies with or without steroids.

Our primary goal was to determine if steroid therapy influenced the validity of the T-Spot. *TB* IGRA and secondarily whether we could identify other immune modulating conditions or medications that may have a similar influence.

Methods

Between Jan. 2014 and Dec. 2015 a total of 1339 samples were tested for LTBI with the T-SPOT. *TB* assay at the University Hospital of Düsseldorf. Blood samples were collected and sent to the microbiology laboratory where the TSPOT was performed according to the manufacturer's instructions.

TSPOT uses an enzyme linked immunospot method to enumerate the patient's TB-specific activated effector T-cells when incubated with the two recombinant TB-specific proteins ESAT-6 and CFP-10 each in separate wells. To perform the assay, a standardised number of peripheral blood mononuclear cells is used. A positive control is included to confirm functionality of the mononuclear cells using phytohaemagglutinin (PHA); as well as a nil control to identify any non-specific T-cell activation. TSPOT test results are recorded as positive if the wells A or B (containing ESAT-6 and CFP-10 antigens) have 8 spots more than the nil control. A result is considered borderline if the highest spot number of well A or B is 5, 6 or 7 spots more than the nil control. A negative result is a spot count, minus the nil control of less

than 5 in both wells. In this study an indeterminate test was defined as a test, in which either the positive control or the negative control was not in the expected range. A number of reasons for a non-responsive positive control can be postulated including a relative reduction in the number of T-cells (in the test the total mononuclear cells are used, which include B-cells and monocytes), non-vital cells or unspecified T-cell anergy. A non-specific stimulation of T-cells camouflages a true positive result and may be caused by a number of patient and non-patient related conditions.

After approval of the ethics committee of the University of Düsseldorf, we retrospectively reviewed the medical records of patients whose samples were sent for TSPOT analysis and extracted data concerning the medical history for all patients.

The medical records were surveyed in view of possible influences regarding the T-cell response, such as HIV status and immunosuppressive medication, in particular corticosteroids. Other factors indicating a dysfunction of the immune system were also characterized and included in the analysis; presence of chronic inflammatory bowel disease (CIBD) and psoriasis vulgaris were pooled and reviewed for deviations from the control group. As the numbers of patients with other immunosuppressive diseases were very small no further analyses were conducted for these subgroups. Immunosuppressive treatment included cytostatic drugs (azathioprine, methotrexate, colchicine, 5-aminosalicylic acid, cyclophosphamide or mycophenolic acid), calcineurin inhibitors (cyclosporine, tacrolimus, sirolimus or everolimus), TNF-inhibitors (infliximab, adalimumab, etanercept), biopharmaceutical agents (rituximab, anakinra, alemtuzumab, sekukinumab, ustekinumab, tocilizumab) and other immunomodulating agents (dimethylfumarat, fingolimod).

Data analysis was performed using the SPSS software package version 24.0 (IBM Corp. Released 2016. IBM SPSS Statistics for Macintosh, Version 24.0. Armonk, NY: IBM Corp.)

To determine the correlation between concomitant cortisone therapy and the results of the interferon gamma release assay, the groups' results were statistically analysed using chi-squared test and Cramers V.

Results

Out of the 1339 tests, 914 were suitable for data analysis. 425 tests were excluded for the following reasons: For 201 samples no or insufficient clinical data was available, 61 tests were repeated within a time range of 6 weeks and 163 tests were not performed due to an insufficient PBMC count.

The age range of the tested population was between 0 and 91 years, the median age was 46 years. 507 (55.5%) tested patients were male. Only 15 patients with solid organ transplants were included and 17 patients with rheumatoid arthritis.

Of the tests evaluated 858 (93.9%) were valid and 56 (6.1%) indeterminate.

Among the valid test results, 719 (83.8%) were negative and 130 (15.2%) positive, 9 tests (1%) were borderline positive (according to manufacturer definition). Indeterminate results arose from failure of the negative control in 24 (42.9%) and failure of the positive control in 32 (57.1%) cases.

Out of 914 patients, 196 were taking cortisone at the time of testing; among these patients 180 (91.8%) returned a valid result and 16 (8.2%) an indeterminate result. This compares to 40 indeterminate tests of 718 (5.6%) results from patients not on corticosteroid therapy at the time of testing ($p = 0.180$), (relative risk [RR] 1.46, 95% confidence interval [95% CI] 0.84 to 2.56). These results are shown in Table 1. As can be seen by the frequencies in Table 1, there is no significant relationship between concomitant corticosteroid therapy and an indeterminate test result.

Table 1
Correlation between concomitant corticosteroid therapy and result validity

	No steroid therapy	Steroid therapy	p
	N (%)	N (%)	
Number of patients	718 (78.6)	196 (21.4)	
Valid result	678 (94.4)	180 (91.8)	
Indeterminate result	40 (5.6)	16 (8.2) *	0.180
* $\chi^2 (1, N = 914) = 1,799, p = 0.180$			

In terms of other immunosuppressive states, among HIV positive patients, 83 (81.3%) had valid test results, whereas in HIV negative individuals the number of valid test results was 775 (94.5%). There was a significant correlation between HIV status and result validity ($p = 0.017$) (relative risk [RR] 2.13, 95% confidence interval [95% CI] 1.14 to 3.98) as shown in Table 2.

Table 2
Correlation between disease or therapy and result validity

	Result valid N, (%)	Result indeterminate N, (%)	p
HIV positive	83 (88.3)	11 (11.7) ^a	0.017
HIV negative	775 (94.5)	45 (5.5)	
Immunosuppressive Medication	164 (94.8)	9 (5.2) ^b	0.573
no immunosuppressive medication	694 (93.7)	47 (6.3)	
CIBD	52 (94.5)	3 (5.5) ^c	0.830
no CIBD	806 (93.8)	53 (6.2)	
Psoriasis vulgaris	118 (95.9)	5 (4.1) ^d	0.305
no Psoriasis vulgaris	740 (93.6)	51 (6.4)	
CIBD, chronic inflammatory bowel disease			

173 patients were undergoing immunosuppressive treatment at the time of testing, of these patients 9 (5.2%) had indeterminate test results, compared to 47 (6.3%) patients not on immunosuppressive therapy (relative risk [RR] 0.82, 95% confidence interval [95% CI] 0.41 to 1.64). The chi square test reveals no significant association between current immunosuppressive medication and result validity ($p = 0.573$).

In a subgroup of patients with CIBD, 52 out of 55 patients had valid results (94.5%) ($p = 0.830$), (relative risk [RR] 0.88, 95% confidence interval [95% CI] 0.29 to 2.74) and in the subgroup of patients suffering from psoriasis vulgaris, the rate of indeterminate results was even lower (5 out of 123 (4.1%), $p = 0.305$), (relative risk [RR] 0.63, 95% confidence interval [95% CI] 0.26 to 1.55).

All in all, significant differences in clinical characteristics among the patients with indeterminate results could only be identified in the HIV sub-group.

Discussion

Santin et al. compared 18 studies to estimate the diagnostic performance of TSPOT and estimated a pooled rate of indeterminate results that was 5.9 % in a total of 2239 individuals. However, the number of indeterminate tests in low and intermediate burden countries was only 3.5 %. [8] Our rate of indeterminate results was 6.1 % out of 914 tests and thus higher than comparators for a low burden country. Lee et al. found a similar rate of indeterminate TSPOT (8.7 %) in their study population of patients with suspected extra-pulmonary tuberculosis and no correlation between indeterminate result and immunosuppression, however they did not analyse the different immunosuppressive medications. [9]

In a number of studies, indeterminate test results have been found more frequently in patients with malignant diseases, patients receiving immunosuppressive treatment and patients with consumptive (under nutrition) conditions for both T.Spot-TB and QFT . [10]

HIV and indeterminate test results

Several studies have evaluated the performance of both TSPOT and QFT-GIT in HIV positive individuals. The sensitivity of TSPOT in HIV positive individuals has been found to be lower in comparison to HIV negative individuals. [8, 11]

In our study, out of 88 HIV infected individuals, 11 (11.7 %) had indeterminate results, whereas among the HIV uninfected individuals there were only 5.5 % of indeterminate results. This outcome is consistent with the findings of other reports, as the number of indeterminate test results both in QFT-GIT and TSPOT has been known to be higher in HIV-positive than in HIV-negative individuals. Of all of the immunomodulating factors, HIV-positivity was the only one that reached statistical significance on TSPOT result validity.

CD4+ and indeterminate test results

The sensitivity of TSPOT has been equally high in patients with normal CD4+ T-cell count as in patients with low CD4+ T-cell count in two studies. [12, 13] Also, it could not be confirmed that the level of circulating CD4+ T-cells was in any way related to the number of indeterminate test results. [11, 14] According to these studies, the level of CD4+ T-cell depletion in HIV-positive individuals does not interfere with the functionality of TSPOT. However, various studies have confirmed that QFT-GIT and TST are both influenced by the number of circulating CD4+ T cells, more accurately, that the QFT-GIT result inversely correlated with CD4+ lymphocyte count. [14, 15]

This finding may indicate that the diagnostic performance of TSPOT may be superior to QFT-GIT in patients that are HIV positive and have a low CD4+ T-cell count. In our study, we did not statistically analyse the influence of the CD4+ T-cell count on the performance of TSPOT as most of the HIV positive patients were undergoing antiretroviral treatment and a current CD4+ T-cell count was not available for most of our patients. It would thus be reasonable to assume that the majority of patients on ART would have an adequate CD4+ count, however this remains speculative.

Immunosuppressive medication and indeterminate test results

Previous studies assessing the effect of immunosuppressive treatment on IGRA performance have been inconsistent. While some studies detected an effect on IGRA performance, especially on QFT-GIT performance, others, similar to our analysis, found no effect for TSPOT. [6, 16]

In 2017, Edwards et al. conducted a study that used an ex vivo model to determine the influence of corticosteroids and infliximab on the performance of QFT-GIT assays. [17] Both corticosteroids and infliximab were added after the blood was taken from the patient, the results of these assays were compared with the test results of patients' blood without added drugs. Both drugs impaired the QFT-GIT performance significantly. Unfortunately, there is to date no such study for TSPOT.

Cortisone and indeterminate test results

Corticosteroids have been known to influence and suppress the cytokine and chemokine production of T-cells, including IFN-g. [18, 19] This effect explains why the performance of QFT-GIT in patients receiving corticosteroids is impaired compared to the performance of patients not taking any corticosteroids. A significant increase in indeterminate tests due to reduced IFN-g release in the control after mitogen stimulation (PHA) was reported by Latorre et al. in patients receiving steroids. [20] In a QuantiFERON Gold in-tube assay they were able to show that this effect was dose related with a sharp decrease in IFN-g release at doses greater than 20 mg/day.

Belard et al. demonstrated that oral prednisolone suppresses both QFT-GIT and TST performance and was associated with a greater risk of indeterminate QFT-GIT results (adjusted odds ratio, 16.1) whereas long-acting corticosteroids and other immunosuppressants do not have a similar effect. [6] This effect was dose related with doses of > 10 mg prednisolone per day associated with a 27 % risk of an indeterminate result. A recent study reported a significant risk of indeterminate results in patients receiving steroids using the QuantiFERON Gold in-tube assay but were not able to demonstrate a dose-related effect. [21] Another study conducted by Arias-Guillén in 2014 underlined these findings; all patients with indeterminate test results in QFT-GIT were also receiving corticosteroid treatment. [22] In this study T-cell sub-populations were analysed by flow cytometry and the authors were able to demonstrate that in 9 of 12 indeterminate TSPOT assays the T-cell sub-population counts for CD3⁺, CD4⁺ and CD8⁺ cells were normal. None of these studies found any statistically significant evidence that prednisolone or corticosteroids in general influence the functionality of TSPOT. While in our study the number of indeterminate test results among patients taking corticosteroids was higher than in the control group, the statistical analysis did not support the hypothesis that corticosteroids impair TSPOT validity.

CIBD, Psoriasis vulgaris and indeterminate test results

Neither CIBD nor Psoriasis vulgaris were associated with indeterminate test results. Interestingly, patients with psoriasis vulgaris had a lower rate of indeterminate test results than patients who do not have psoriasis vulgaris.

There are several limitations to our study. We were unable to distinguish between different groups of immunosuppressive agents and doses and also did not account for duration of treatment or disease activity. All of these factors could have an effect on indeterminate results. In addition, the sub-group analysis only contained relatively small numbers of patients, which may impact on the ability to detect significant differences. HIV was associated with a higher risk of indeterminate result and it would be logical to assume that the CD4⁺ cell count would influence this observation; we were unable to determine with accuracy the current cell count as most patients were on a stable treatment regime. That the CD4⁺ cell count was likely to be high is however speculative. Other determinants for an indeterminate result, such as hypoalbuminaemia or anaemia [23] were not measured as this study was primarily aimed at the

effect of steroid use on TSPOT performance. Furthermore, the study was performed retrospectively and thus not all datasets could be included as the medical records were not always complete.

In conclusion, the study demonstrated that steroid therapy did not influence the validity

of the T.Spot-TB IGRA and secondarily that, of the conditions investigated in this study, only HIV was associated with an increased rate of indeterminate tests.

Declarations

Ethics approval and consent to participate:

This retrospective study was approved by the Ethics Committee of the Medical Faculty, Heinrich-Heine University, Düsseldorf, study number 5266, 24.11.2015. Due to the retrospective and anonymous nature of the study informed consent from patients was not deemed necessary by the ethics committee.

Consent to publish:

Not Applicable

Availability of data and materials:

The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare no conflict of interest.

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Authors' Contributions:

Anna-Teresa Schulz: designed the study and conducted the primary data access, performed the data analysis and statistical evaluation and wrote the manuscript

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