

Quantiferon TB Gold and tuberculin skin tests for the detection of latent tuberculosis infection in patients treated with tumour necrosis factor alpha blocking agents

M. Klein^{1,2}, K. Jarošová^{1,2}, Š. Forejtová^{1,2}, R. Bečvář^{1,2}, L. Šedová^{1,2}, K. Pavelka^{1,2}, G. Šimková¹, R. Svobodová^{1,2}, K. Hviščová¹, H. Mann^{1,2}, I. Půtová¹, J. Vencovský^{1,2}

¹Institute of Rheumatology, and ²Charles University, Dept. of Rheumatology, 1st Medical Faculty, Prague, Czech Republic.

Abstract

Objectives

The risk of activation of latent tuberculosis infection (LTBI) is increased in patients treated with anti-TNF- α drugs. Tuberculin skin test (TST) and Quantiferon-TB Gold test (QFT) are used to detect LTBI before and during anti-TNF- α treatment. We describe here a relation of these tests at various timepoints and also longitudinal QFT data.

Methods

Study group consisted of 305 patients with several rheumatic inflammatory diseases treated and/or scheduled for anti-TNF- α drugs. The QFT was performed in 303 patients during therapy and in 177 patients also during screening. The TST was used in 284 patients. Both tests simultaneously were utilised in 360 instances.

Results

Twenty-two patients were QFT positive; 3.9% before and 5.9% during anti-TNF- α treatment. Two patients who became QFT positive developed active tuberculosis. The TST was positive in 42% and 38% of patients before and during treatment, respectively. There was poor agreement between the two tests. Patients on glucocorticoids had a negative TST more frequently. The IFN- γ response to mycobacterial antigens significantly increased after application of tuberculin, but never reached the positive threshold. There was a significant increase in mitogen-induced IFN- γ production after initiation of anti-TNF- α therapy.

Conclusion

Poor correlation between the QFT and TST renders the TST non-specific for LTBI. QFT is more specific to detect LTBI and conversion to a positive result may predict active TB. An increase in IFN- γ production in response to mycobacterial antigens is seen when the TST is performed before the QFT. Mitogen-induced IFN- γ production increases after initiation of anti-TNF- α therapy.

Key words

TNF inhibition, tuberculosis, arthritis, IFN- γ

Martin Klein, MD
 Kateřina Jarošová, MD
 Šárka Forejtová, MD, PhD
 Radim Bečvář, MD
 Liliana Šedová, MD
 Karel Pavelka, MD, PhD
 Gabriela Šimková, MD
 Radka Svobodová, MD
 Katarína Hviščová, MD
 Heřman Mann, MD
 Ivana Půtová, MD
 Jiří Vencovský, MD, PhD

Please address correspondence to:

Jiří Vencovský, MD, PhD,
 Institute of Rheumatology,
 Na Slupi 4,
 12850 Praha 2, Czech Republic.
 E-mail: venc@revma.cz

Received on March 15, 2012; accepted in revised form on April 19, 2012.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2013.

Introduction

Tumour necrosis factor alpha (TNF- α) blocking agents are routinely used to treat several rheumatic diseases, with good benefits for the patients. An increased risk of tuberculosis infection has been reported in patients treated with anti-TNF- α drugs, particularly with monoclonal antibodies (1-3). It usually manifests as a reactivation of latent tuberculosis infection (LTBI) in the first year of treatment (4); although newly acquired disease also occurs during the treatment period (5). Tuberculosis represents a major health complication and leads to the administration of potentially toxic therapy and to the interruption or discontinuation of anti-TNF- α treatment. It has been shown that testing for LTBI before the initiation of anti-TNF- α treatment significantly decreases the risk of developing active tuberculosis (TB) infection and this testing has become routine (6, 7). Screening includes a careful medical history and chest x-ray, as well as the tuberculin skin test (TST). There are several factors that limit the usefulness of the TST for accurate LTBI prediction. In many countries, the Bacillus Calmette-Guérin (BCG) vaccination has been compulsory; consequently, positive TST may be attributed to reaction to antigens contained in the original vaccine. On the other hand, disease itself due to impaired T cell function, or immunosuppressive treatment may diminish the reactivity, leading to false negative results (8-10). Interferon gamma release assays (IGRA) have recently been introduced into daily clinical practice (11-13). These assays are believed to be more specific and can be used repeatedly to check for changes during treatment (14). The impact of repeated use of these assays on the identification of LTBI before anti-TNF- α therapy and on detecting active tuberculosis during treatment have not been fully described. In this paper, we compared the TST with the QuantiFERON assay. Both tests were performed at the same time before treatment with anti-TNF- α agents and the performance of both tests was assessed during the longitudinal follow-up of patients on treatment. Furthermore, we examined the effect

of the TST on subsequent interferon release assays and estimated the ability of lymphocytes to produce interferon gamma (IFN- γ) in response to mitogen stimulation at different time points before and during anti-TNF- α treatment.

Materials and methods

Patients

Data were collected during routine care in the Institute of Rheumatology from June 2006 to November 2009 from patients screened before and/or during anti-TNF- α treatment. They were consecutive patients and all with clinical data made available by treating physician were included. The screening guidelines for LTBI during the respective period of time, imposed primarily by pulmonologists – Section for Tuberculosis of the Czech Society of Pneumology and Phthisiology required the simultaneous performance of TST and interferon release assay. These guidelines also required frequent verification of both assays during treatment. QuantiFERON assays were typically performed before treatment and 3, 6, and 12 months after treatment began, and then yearly during treatment; while the TST was typically performed before treatment and yearly. If both tests were performed, they were done simultaneously the same day or within a difference of maximally one week. The tests were performed more often if required, usually after consultation with a TB specialist.

The studied group consisted of 305 patients with rheumatoid arthritis (RA, n=117), ankylosing spondylitis (AS, n=110), and psoriatic arthritis (PsA, n=6). There was also a group of patients with polyarticular juvenile idiopathic arthritis who all reached adult age at the time of inclusion (JIA, n=72). Patients were treated with infliximab (n=150), adalimumab (n=79), and etanercept (n=77). All the patients signed informed consent and the institutional ethics committee approved the study.

Interferon release assay and tuberculin skin test

The QuantiFERON-TB Gold (in-tube method) (Cellestis, Chadstone, Australia) (QFT) was used to measure IFN- γ release from effector T cells.

Funding: This work was supported by the Czech Ministry of Health (Institutional support no. 00023728).

Competing interests: none declared.

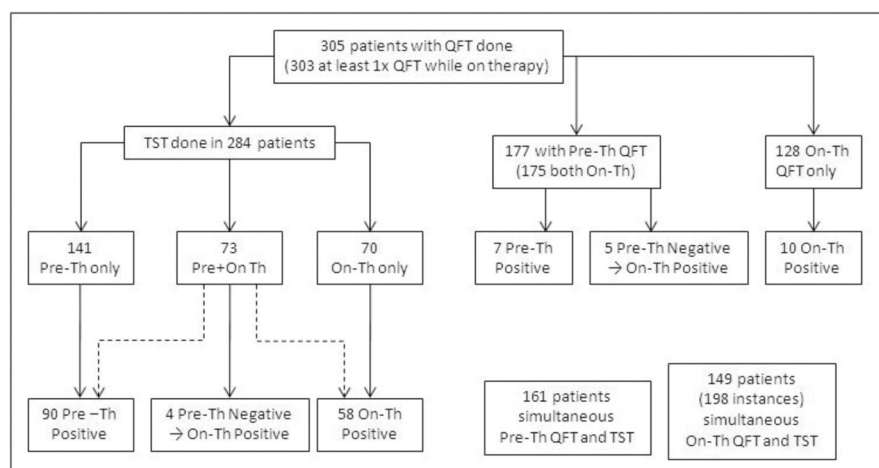


Fig. 1. Distribution of patients with Quantiferon (QFT) and tuberculin skin test (TST). Pre-Th – before anti-TNF therapy initiation; On-Th – on anti-TNF therapy.

Table I. Characteristics and therapeutic data of the study population.

Patients, n	305
Gender, males/females, n	140/165
Age, years [§]	44.18±14.77
Diagnosis [*]	RA=117 (38.4%); AS=110 (36.1%); PsA=6 (1.9%); JIA=72 (23.6%);
Anti-TNF α drug [*]	Infliximab=149 (48.9%); adalimumab=79 (25.9%); etanercept=77 (25.2%)
Concomitant therapy	
Glucocorticoids	RA 67.6%; AS 11.8%; PsA 83.3%; JIA 45.8%
Methotrexate	RA 63.2%; AS 14.5%; PsA 33.3%; JIA 58.3%
Leflunomide	RA 14.5%; AS 1.8%; PsA 16.6%; JIA 12.5%
Sulfasalazine	RA 5.9%; AS 25.4%; PsA 33.3%; JIA 12.5%
No DMARDs/GC therapy	RA 11.1%; AS 65.4%; PsA 0%; JIA 18.1%

[§]Data are expressed as mean value $\pm$ SD.

^{*}Data are expressed as absolute number, n (%).

RA: rheumatoid arthritis; AS: ankylosing spondylitis; JIA: juvenile idiopathic arthritis; PsA: psoriatic arthritis; DMARDs: disease-modifying anti-rheumatic drugs; GC: glucocorticoids. Concomitant therapy in some patients consisted of a combination of DMARDs.

The test was performed in 177 of patients before the start of anti-TNF- α treatment (Fig. 1). These patients were prospectively followed with further assessments at least once after 3, 6, 12, and 24 months. In selected patients, the QFT was also performed 2 weeks after the first infliximab infusion. Another 128 patients were assessed only during the anti-TNF- α treatment with no pre-treatment QFT results available. These patients began receiving anti-TNF- α treatment before QFT testing became mandatory. The test was repeated regularly in most patients; a single result was available only in nine patients. The QFT was performed according to the manufacturer's instructions. Briefly, whole blood was cultured overnight at 37°C and spontaneous (S), mitogen stimulated (phytohemagglutinin) and mycobacterium tuberculosis (MT) spe-

cific antigen (ESAT-6, CFP-10 and TB 7.7[p4]) stimulated INF- γ production were measured in plasma by ELISA. The test is considered positive when the difference between MT stimulated production and spontaneous production of INF- γ (MT-S) exceeds 0.34 IU/ml and is greater than 25% of spontaneous production at the same time. The result is considered indeterminate when spontaneous production of INF- γ exceeds 8.0 IU/ml, when there is a low mitogen induced INF- γ production (<0.5 IU/ml) along with MT-S production that is lower than 0.35 IU/ml, or when MT-S exceeds 0.34 IU/ml, but is lower than 25% of the spontaneous production. Tuberculin skin tests were performed in 284 patients (Fig. 1). The test was carried out by trained hospital staff before and/or during anti-TNF- α treatment by intradermal application of two T.U. in

0.1 ml, which equals 0.04 μ g of tuberculin PPD RT 23 SSI (Statens Serum Institut, Copenhagen, Denmark), into the skin of the forearm. The induration was measured after 48–72 hours. A diameter of 5 mm and more was considered positive. The study was performed in a BCG vaccinated population due to mandatory vaccination in the Czech Republic.

Statistics

Demographics, clinical characteristics, and results are presented as descriptive statistics. The continuous not-normally distributed variables were analysed by Wilcoxon signed rank tests, Anova (Kruskal-Wallis test); correlation was computed using Spearman correlation coefficient; categorical data were analysed by χ^2 test and Fisher's exact test. The concordance of Quantiferon and TST was assessed using percentage agreement and κ coefficient. We used SPSS (SPSS, Chicago, Illinois), GraphPad Prism 5 (GraphPad Software, La Jolla, California), and QuickCalcs online calculators (graphpad.com) for statistical analysis.

Results

Overall Quantiferon and tuberculin skin test results

Basic characteristics of patients are shown in table (Table I). QFT was positive in seven (3.9%) of the 177 individuals in whom the test was performed before anti-TNF- α initiation. Ten patients (5.6%) had indeterminate results (due to non-reactivity to mitogen stimulation) and the QFT was negative in the remaining patients. Of the 303 patients that were studied at least once during anti-TNF- α treatment, 18 (5.9%) tested positive with the QFT (including three patients positive at the pre-treatment screening). Only four patients (1.3%) had indeterminate test results, including one patient who also had an indeterminate result before treatment.

The TST was performed in 73 patients both before and during treatment, in 141 patients only at pre-treatment screening, and in 70 patients only during anti-TNF- α therapy. Ninety (42%) patients who were examined before treatment had positive TSTs. When the

TST was performed during treatment, the test was positive at least once in 58 (38%) patients. In 73 patients with test results before and during treatment, four patients who initially tested negative tested positive by TST during the course of therapy (three patients after one year and one after three years). None of these patients tested positive in QFT. Since also clinical investigation was negative, which included new chest X-ray, isoniazid was not given after a consultation with a TB specialist.

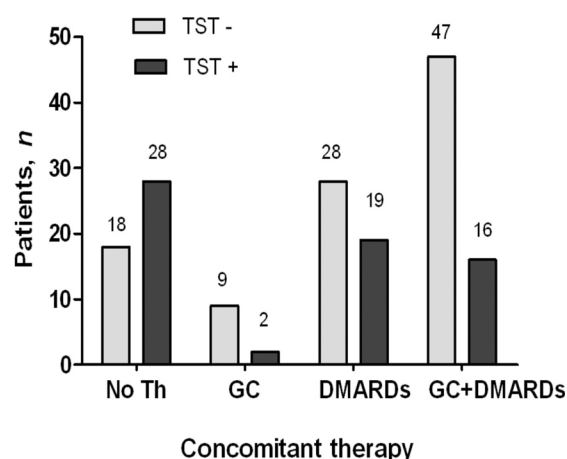
Effect of concomitant treatment on the tuberculin skin test and interferon gamma release assay before anti-TNF- α therapy

Most patients were treated with glucocorticoids (GC) and/or disease modifying antirheumatic drugs (DMARDs) at the time of screening for anti-TNF- α treatment (Table I). We compared the effects of concomitant treatment on the TST and QFT at screening in 167 and 177 patients, respectively. Patients who were on GC and/or a combination of GC with DMARDs, but not patients on DMARDs alone, had a significantly lower frequency of positive TST results, compared to patients on no treatment (Fig. 2). There was no effect of concomitant treatment on median mitogen stimulated IFN- γ production (no treatment, 17.7 [95% CI 13.1 to 20.3; n=47]; GC, 16.3 [95% CI 7.4 to 17.5; n=12]; DMARDs, 13.0 [95% CI 10.4 to 18.2; n=50]; GC+DMARDs, 12.4 [95% CI 10.5 to 14.9; n=68]). All ten patients (5.6%) who had indeterminate results were on combination of GC and DMARDs. Concomitant therapy did not have any effects on spontaneous production, which was very low in all patients (data not shown).

Characteristics of QFT positive patients

QFT was positive in a total of 22 patients. Seven patients who tested positive at the pre-treatment screening received six months of isoniazid (INH) prophylaxis; four of these patients tested negative within six months, and six of them were negative at the first annual check-up. All seven patients were treated with anti-TNF- α agents and none developed TB.

Fig. 2. Tuberculin skin test results in relation to concomitant therapy in patients before anti-TNF- α treatment. Patients receiving GC treatment either alone ($p=0.0172$) or in combination with DMARDs ($p=0.0003$), or both groups together (GC and GC+DMARDs) ($p<0.0001$) had positive TSTs significantly less frequently than patients not receiving concomitant therapy. No significant difference was found between patients not receiving therapy and those receiving DMARDs treatment. GC: glucocorticoids; DMARDs: disease-modifying anti-rheumatic drugs.



In five patients, the screening QFT was negative but the test became positive during anti-TNF- α treatment. Two of these patients developed active TB. The first was a 33-year old male with ankylosing spondylitis with negative pre-treatment QFT. The TST was 4 mm, there were no signs of TB on chest X-ray, and his history was negative. There was no need for isoniazid prophylaxis. The QFT was re-checked after two weeks, after the patient received only one infliximab infusion. This repeated QFT was positive and the patient rapidly developed pulmonary TB. He was successfully treated with a combination of anti-tuberculosis drugs. The second patient was a 38-year old male with ankylosing spondylitis, treated with adalimumab, who was also QFT negative at screening. His TST was 13 mm. There were no signs of TB on high resolution computer tomography scan of his chest (HRCT). Since this patient had a hepatopathy, adalimumab was started without INH prophylaxis after consultation with a TB specialist. The QFT was checked after three months and after one year of treatment and both results were negative. However, at 18 months, the patient's QFT was positive. The TST was repeated and was positive at 14 mm. HRCT showed tuberculous cavity, pleural effusion, and TB dissemination in the lungs. Adalimumab was discontinued and the patient was successfully treated with a combination of antituberculosis drugs. The other 3 positive patients received isoniazid treatment for 6 months, resumed anti-TNF treatment and tested negative in QFT after 3–12 months.

In the remaining 10 positive patients, the pre-treatment QFT was not available. One patient was first tested with the QFT after two years of anti-TNF- α treatment. Initial QFT at 1 year was negative, but the patient tested positive one year later. The first available QFT during anti-TNF- α treatment was positive in nine patients (duration of treatment: 1 year – 1 patient; 2 years – 2 patients; 3 years – 4 patients, and 4 years – 1 patient), three of whom later tested negative 12 (two patients) and 24 (one patient) months later. All 10 patients received INH for six months and none developed TB. All these patients continued anti-TNF- α treatment with interruptions of varying lengths.

Comparison between Quantiferon and TST results

In 161 patients, the pre-treatment QFT and TST were performed at the same time (one patient was tested twice); in 149 patients, both tests were simultaneously administered 198 times while receiving anti-TNF- α therapy. The QFT was positive in 12.1% of the patients with positive TSTs and in 2.2% of patients with negative TSTs, when performed simultaneously. A good proportion (34.6%) of Quantiferon negative tests were TST positive and 31.3% of QFT+ tests were TST negative at the same time (Fig. 3). After excluding indeterminate results, there was a poor agreement between the two tests (66% concordance; $\kappa=0.121$). Patients with a positive QFT had significantly larger mean indurations in the TST (10.7 mm [95%CI 7.3–14.2] vs. 3.8 mm [95% CI

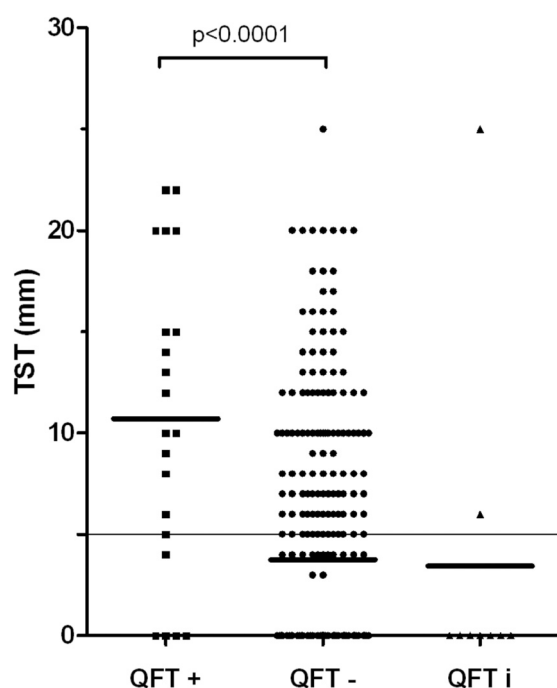


Fig. 3. Comparison of Quantiferon (QFT) and tuberculin skin test (TST) results. The induration diameter in TSTs is compared with the results from the QFT when both tests performed simultaneously. Horizontal line at 5 mm is the arbitrary positive value for the TST. Thick horizontal lines mark the mean diameters of TST inductions. QFT±/i Quantiferon positive/negative/indeterminate; TST± TST positive/negative.

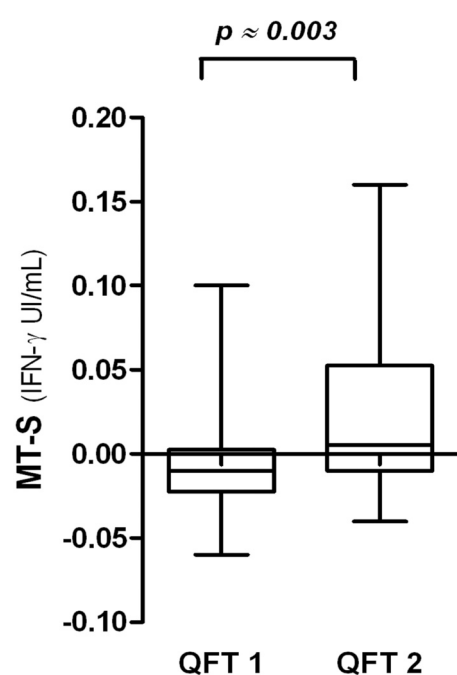


Fig. 4. Mycobacterium tuberculosis antigen stimulated IFN- γ production before and after application of tuberculin during the skin test (median interval 2.0 weeks; 0.5–5.0). There was a significant increase in IFN- γ production after the TST, but there was no conversion to a positive result.

3.2–4.4], $p < 0.0001$). When absolute levels of MT stimulated minus spontaneous IFN- γ production (MT-S) were compared in IU/ml to the diameter of the TST result in millimetres in each patient, there was a weak but significant correlation (Spearman correlation coefficient $r = 0.1374$, $p = 0.009$, $n = 357$).

TST – QFT interaction

The potential sensitisation to tuberculous antigens due to the exposure to

tuberculin during skin testing and the effect on the QFT performance were investigated in 22 patients scheduled for treatment with infliximab. These patients were first screened with the Quantiferon assay (QFT1) and tuberculin skin test, which was applied on the same day or a short time after the QFT1. Blood for the next Quantiferon test (QFT2) was taken before administration of the first infliximab infusion, a median of 2 weeks (minimum

0.5, maximum 5.0 weeks) after the TST. Median IFN- γ levels (measured as MT-S) detected in the QFT2 significantly increased after application of tuberculin (from -0.01 [95%CI -0.02 – 0.01] IU/ml in QFT1 to 0.005 [95%CI 0.00 – 0.04] IU/ml in QFT2; $p < 0.003$ [Fig. 4]); however, none of these QFTs was positive. There was no change in spontaneous IFN- γ production.

IFN- γ release before and during anti-TNF- α treatment in response to mitogen stimulation

Stimulation with mitogen reflects the ability of peripheral blood mononuclear cells to produce IFN- γ . In the QFT, phytohaemagglutinin (PHA) stimulation is instrumental as an internal positive control. We assessed PHA stimulated IFN- γ production in 177 individuals with pre-treatment QFT results available and compared it with PHA stimulated IFN- γ production in patients receiving anti-TNF- α therapy after two weeks ($n = 64$) and 3 ($n = 75$), 6 ($n = 27$), 12 ($n = 116$), and 24 ($n = 33$) months of treatment. There were significant increases in median PHA stimulated IFN- γ production after 3 months (15.0 [95%CI 12.8 – 18.4] vs. 18.9 [95%CI 17.3 – 21.0] IU/ml) as well as after 6 months (13.6 [95%CI 10.3 – 17.3] vs. 23.9 [95%CI 20.7 – 25.3] IU/ml), 12 months (13.1 [95%CI 11.7 – 15.4] vs. 23.4 [95%CI 20.8 – 23.8] IU/ml), and 24 months (5.5 [95%CI 5.9 – 13.1] vs. 23.8 [95%CI 20.1 – 28.0] IU/ml) (Fig. 5).

Discussion

The tuberculin skin test and, more recently, Quantiferon assay have been used for detection of latent tuberculosis in patients considered for anti-TNF- α treatment. In this study, we addressed several questions regarding these tests. We found a high number of patients with positive TSTs both before and during anti-TNF- α therapy. Treatment with glucocorticoids and/or DMARDs compromises sensitivity of TST (9) and despite this fact we still detected positivity in around 40% of patients. The tests in these patients are most likely positive due to compulsory BCG vaccination (15) in the Czech republic,

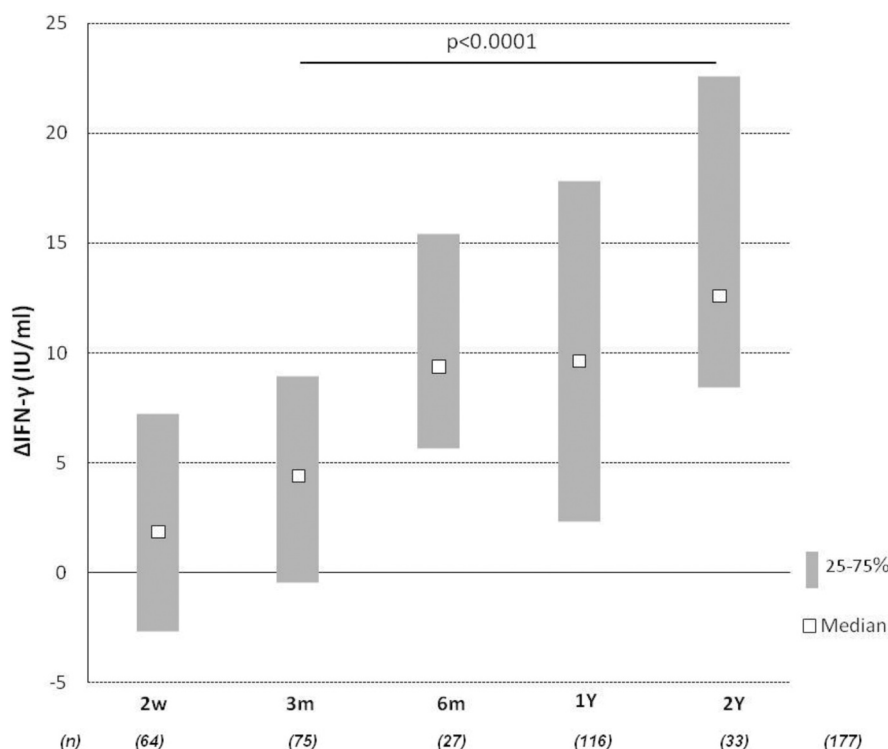


Fig. 5. Patients on anti-TNF- α treatment at 2 weeks and 3, 6, 12, and 24 months. The graph shows the median changes from before therapy in phytohemagglutinin (PHA) induced IFN- γ production (IU/ml) at various time points. The median PHA stimulated IFN- γ production significantly increased starting from month 3, in comparison with production at pre-treatment, when treated with TNF- α neutralising agents.

which is a country with a low incidence of tuberculosis (6.8 per 100,000 in 2009) (16). Apart from BCG vaccination, we also cannot rule out a contribution of cross-reaction with non-tuberculous mycobacteria (17). Therefore, we confirm that the TST is not a specific for latent TB and a positive result should not be used as the sole indicator for isoniazid treatment, since too many patients would be exposed to a potentially toxic therapy unnecessarily.

Substantially fewer patients showed positivity in the QFT than the TST. The QFT is not affected by previous BCG vaccination and any positive finding should be interpreted as specific for latent or active tuberculosis. A change in a patient's test result to positive during anti-TNF- α treatment may indicate the development of active disease, as documented in the two of 5 such cases described here. In the first case, TB developed extremely rapidly, a few weeks after the start of infliximab, and we detected the positive change by QFT after just one infusion. The assay was repeated so early because this patient

was a member of a substudy investigating the potential effect of anti-TNF- α on the ability to produce IFN- γ . In the second case, a positive QFT occurred after long-term treatment with adalimumab and correctly predicted active TB; whereas, the TST remained the same, although positive. These observations underscore the good sensitivity and predictive capacity of the assay.

There is no gold standard to determine LTBI. However, none of the patients who were QFT positive at pre-treatment or who tested positive during anti-TNF- α treatment and were treated with isoniazid developed tuberculosis. Besides, there were not any cases of TB in patients who were TST positive and QFT negative. This finding suggests that preventive treatment should be given only to those with a positive QFT, which would reduce exposure to isoniazid. A question is the length of the isoniazid treatment that varies in different countries, usually from 6 to 9 months. Generally, it seems that longer duration is optimal (18). Guidelines in our country recommend 6-month du-

ration of isoniazid therapy, which, in the limited number of our patients, appeared to be sufficient, because none of our treated patients developed TB.

An interesting observation is the reversion of positive QFT into negativity in 12 out of 20 patients who were treated with isoniazid and had the test rechecked after six to twelve months. This was previously noted in 25% of Japanese patients who were in contact with TB, tested positive for QFT and were treated with isoniazid for 6 months (19). Most studies, however, describe remaining positivity in QFT after isoniazid treatment in a majority of patients (20). Patients who completed therapy showed a significant decrease of IFN- γ response to mycobacterial antigens but did not revert into negativity (21). It has been suggested that the immune response may be dependent on pathogen replication and antigenic load (21), which would point to a possibility that it was both low in our patients. What is the significance of test reversion and whether it can be used to measure treatment success is currently unknown (22).

Intradermal injection of tuberculin may theoretically lead to sensitisation to TB antigens and interfere with the QFT assay. We have tested this possibility in a group of patients who had a TST performed between two QFTs, with a short interval inbetween, before any anti-TNF- α treatment. There was a small, but significant increase in the level of IFN- γ production, which suggests the QFT should be done first, before the TST, if both tests are planned. In our cohort, we did not see a conversion from a negative to positive result in the QFT.

One explanation for activation of tuberculosis in patients treated with TNF- α neutralising agents includes their possible effect on the ability to produce IFN- γ . Therefore, our finding of a clear increase in mitogen induced IFN- γ production in patients on anti-TNF- α therapy was somewhat unexpected. Several investigators reported suppressive effects of anti-TNF- α agents on IFN- γ production (23-28); yet, these were mainly *in vitro* studies. NK cells have been described as one of the major

sources of IFN- γ and, recently, a significant increase in the proportion of IFN- γ producing NK cells was detected after 4 months of treatment with adalimumab (29). Similarly, infliximab treatment induced a significant increase in the number of cells secreting IFN- γ in patients with Behçet's disease (30). Our results show that at least the phytohaemagglutinin response of lymphocytes is not suppressed, but rather increased after anti-TNF- α treatment.

In summary, we found the Quantiferon assay was less frequently positive in patients before and during anti-TNF- α treatment than the tuberculin skin test. The fact that only two patients developed tuberculosis and clinical symptoms in both of them were preceded by a conversion to a positive Quantiferon test seems to indicate that this test is more specific. Use of the Quantiferon test may prevent unnecessary administration of potentially toxic isoniazid. Administration of tuberculin mildly sensitises patients to TB antigens and increases the response in the Quantiferon assay. If both tests are to be performed, the Quantiferon should be done first.

References

- DIXON WG, HYRICH KL, WATSON KD *et al.*: Drug-specific risk of tuberculosis in patients with rheumatoid arthritis treated with anti-TNF therapy: results from the British Society for Rheumatology Biologics Register (BSRBR). *Ann Rheum Dis* 2010; 69: 522-8.
- TUBACH F, SALMON D, RAVAUD P *et al.*: Risk of tuberculosis is higher with anti-tumor necrosis factor monoclonal antibody therapy than with soluble tumor necrosis factor receptor therapy: The three-year prospective French Research Axed on Tolerance of Biotherapies registry. *Arthritis Rheum* 2009; 60: 1884-94.
- WOLFE F, MICHAUD K, ANDERSON J, URBANSKY K: Tuberculosis infection in patients with rheumatoid arthritis and the effect of infliximab therapy. *Arthritis Rheum* 2004; 50: 372-9.
- KEANE J, GERSHON S, WISE RP *et al.*: Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med* 2001; 345: 1098-104.
- CAGATAY T, AYDIN M, SUNMEZ S *et al.*: Follow-up results of 702 patients receiving tumor necrosis factor-alpha antagonists and evaluation of risk of tuberculosis. *Rheumatol Int* 2010; 30: 1459-638.
- GÓMEZ-REINO JJ, CARMONA L, ANGEL DESCALZO M; BIOBADASER GROUP: Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk: a multicenter active-surveillance report. *Arthritis Rheum* 2003; 48: 2122-7.
- GÓMEZ-REINO JJ, CARMONA L, ANGEL DESCALZO M *et al.*: Risk of tuberculosis in patients treated with tumor necrosis factor antagonists due to incomplete prevention of reactivation of latent infection. *Arthritis Rheum* 2007; 57: 756-61.
- VAN BESOUW NM, VAN DER MAST BJ, VAN DE WETERING J, RISCHEN-VOS J, WEIMAR W: Tapering immunosuppressive therapy significantly improves *in vivo* cutaneous delayed type hypersensitivity responses. *Transpl Immunol* 2008; 19: 229-34.
- SOBORG B, RUHWALD M, HETLAND ML *et al.*: Comparison of screening procedures for Mycobacterium tuberculosis infection among patients with inflammatory diseases. *J Rheumatol* 2009; 36: 1876-84.
- PONCE DE LEÓN D, ACEVEDO-VÁSQUEZ E, SÁNCHEZ-TORRES A *et al.*: Attenuated response to purified protein derivative in patients with rheumatoid arthritis: study in a population with a high prevalence of tuberculosis. *Ann Rheum Dis* 2005; 64: 1360-1.
- BOCCHINO M, MATARESE A, BELLOFIORE B *et al.*: Performance of two commercial blood IFN-gamma release assays for the detection of Mycobacterium tuberculosis infection in patient candidates for anti-TNF-alpha treatment. *Eur J Clin Microbiol Infect Dis* 2008; 27: 907-13.
- SHOVMAN O, ANOUK M, VINNITSKY N *et al.*: QuantiFERON-TB Gold in the identification of latent tuberculosis infection in rheumatoid arthritis: a pilot study. *Int J Tuberc Lung Dis* 2009; 13: 1427-32.
- LALVANI A, MILLINGTON KA: Screening for tuberculosis infection prior to initiation of anti-TNF therapy. *Autoimmun Rev* 2008; 8: 147-52.
- PARK JH, SEO GY, LEE JS, KIM TH, YOO DH: Positive conversion of tuberculin skin test and performance of interferon release assay to detect hidden tuberculosis infection during anti-tumor necrosis factor agent trial. *J Rheumatol* 2009; 36: 2158-63.
- TISSOT F, ZANETTI G, FRANCIOLI P, ZELLWEGER JP, ZYSSET F: Influence of bacille Calmette-Guérin vaccination on size of tuberculin skin test reaction: to what size? *Clin Infect Dis* 2005; 40: 211-7.
- NOVAKOVA Z: Incidence of tuberculosis in the Czech Republic in 2009. Fast Information 2010 38/10. uzis.cz. accessed 21 Jul 2010. <http://www.uzis.cz/en/fast-information/incidence-tuberculosis-czech-republic-2009>.
- LATORRE I, DE SOUZA-GALVÃO M, RUIZ-MANZANO J *et al.*: Evaluating the non-tuberculous mycobacteria effect in the tuberculosis infection diagnosis. *Eur Respir J* 2010; 35: 338-42.
- COMSTOCK GW: How much isoniazid is needed for prevention of tuberculosis among immunocompetent adults? *Int J Tuberc Lung Dis* 1999; 3: 847-50.
- HIGUCHI K, HARADA N, MORI T: Interferon-gamma responses after isoniazid chemotherapy for latent tuberculosis. *Respirology* 2008; 13: 468-72.
- DYRHOL-RIISE AM, GRAN G, WENTZEL-LARSEN T, BLOMBERG B, HAANSHUUS CG, MØRKVE O: Diagnosis and follow-up of treatment of latent tuberculosis; the utility of the QuantiFERON-TB Gold In-tube assay in outpatients from a tuberculosis low-endemic country. *BMC Infect Dis* 2010; 10: 57.
- GOLETTI D, PARRACINO MP, BUTERA O *et al.*: Isoniazid prophylaxis differently modulates T-cell responses to RD1-epitopes in contacts recently exposed to Mycobacterium tuberculosis: a pilot study. *Respir Res* 2007; 8: 5.
- ESMAIL H, BARRY CE 3RD, WILKINSON RJ: Understanding latent tuberculosis: the key to improved diagnostic and novel treatment strategies. *Drug Discov Today* 2012; doi:10.1016/j.drudis.2011.12.013.
- AMITAL H, BARAK V, WINKLER RE, RUBINOW A: Impact of treatment with infliximab on serum cytokine profile of patients with rheumatoid and psoriatic arthritis. *Ann N Y Acad Sci* 2007; 1110: 649-60.
- GIARDINA AR, ACCARDO-PALUMBO A, CICCIA F *et al.*: Blocking TNF *in vitro* with infliximab determines the inhibition of expansion and interferon gamma production of Vgamma9/Vdelta2 T lymphocytes from patients with active rheumatoid arthritis. A role in the susceptibility to tuberculosis? *Reumatismo* 2009; 61: 21-6.
- AGNHOLT J, DAHLERUP JF, KALTOFT K: The effect of etanercept and infliximab on the production of tumour necrosis factor alpha, interferon-gamma and GM-CSF in *in vivo* activated intestinal T lymphocyte cultures. *Cytokine* 2003; 23: 76-85.
- HAMDI H, MARIETTE X, GODOT V *et al.*: Inhibition of anti-tuberculosis T-lymphocyte function with tumour necrosis factor antagonists. *Arthritis Res Ther* 2006; 8: R114.
- POPA C, BARRERA P, JOOSTEN LA *et al.*: Cytokine production from stimulated whole blood cultures in rheumatoid arthritis patients treated with various TNF blocking agents. *Eur Cytokine Netw* 2009; 20: 88-93.
- SALIU OY, SOFER C, STEIN DS, SCHWANDER SK, WALLIS RS: Tumor-necrosis-factor blockers: differential effects on mycobacterial immunity. *J Infect Dis* 2006; 194: 486-92.
- ARAVENA O, PESCE B, SOTO L *et al.*: Anti-TNF therapy in patients with rheumatoid arthritis decreases Th1 and Th17 cell populations and expands IFN- γ -producing NK cell and regulatory T cell subsets. *Immunobiology* 2011; 216: 1256-63.
- MISUMI M, HAGIWARA E, TAKENO M *et al.*: Cytokine production profile in patients with Behçet's disease treated with infliximab. *Cytokine* 2003; 24: 210-8.