Rheumatology 2008;47;212–218 doi:10.1093/rheumatology/kem351

Evaluation of latent tuberculosis infection in patients with inflammatory arthropathies before treatment with TNF-α blocking drugs using a novel flow-cytometric interferon-γ release assay

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Objective. To compare the efficacy of the conventional skin test and a novel flow cytometric whole blood assay in the diagnosis of latent tuberculosis infection (LTBI) in patients with rheumatological diseases evaluated for treatment with TNF-α-blocking agents.
Methods. Prospective study of 97 consecutively enrolled patients, who were assessed for the presence of LTBI through clinical history, Mendel–Mantoux skin testing and chest X-ray. In addition, T-cell reactivity towards tuberculin (PPD, purified protein derivative) and the *Mycobacterium tuberculosis*-specific proteins ESAT-6 and CFP-10 was determined *ex vivo* using a flow cytometric whole blood assay.
Results. After standard screening, 15% of patients receiving TNF-α-blocking therapy were pretreated with isoniazide (INH), another 5% of patients did not receive TNF-α-blocking therapy because of LTBI. PPD-reactivity in the skin was observed in 14% of patients compared with 39% with the whole blood test. Analysis of the *M. tuberculosis*-specific response to ESAT-6 and CFP-10 revealed positive results in 16% of patients. Using a decision tree incorporating history, chest X-ray and either skin-test or ESAT-6/CFP-10 results, 18 or 22% of the patients, respectively, were classified as latently infected with *M. tuberculosis*. Four patients treated with INH because of a positive skin reaction did not show reactivity to ESAT-6/CFP-10 in the whole blood assays. Another six patients not pretreated with INH because of negative skin tests would have received INH, had the results of the whole blood assay been taken into account.
Conclusion. The Mendel–Mantoux skin test has a low sensitivity and specificity for the diagnosis of LTBI in this cohort of patients, potentially resulting in both over- and under-treatment with prophylactic INH when compared with the flow cytometric analysis of whole blood T-cell reactivity to proteins specific to *M. tuberculosis*. Use of T-cell based *in vitro* tests may help to refine diagnostic testing for LTBI.

Key words: Latent tuberculosis, Tuberculin, TNF-α-blocking drugs.

Introduction

The introduction of drugs blocking TNF-α has widely changed rheumatological practice since an increasing number of patients with rheumatoid arthritis, spondyloarthropathies and psoriatic arthritis experience significant clinical benefit from this treatment. One of the major obstacles for TNF-α-blocking therapy is the increasing incidence of active tuberculosis [1, 2]. In the United States, up to 50 cases per 100 000 patient-years have been reported, corresponding to an ~8-fold increase in risk [3]. Thus, screening for the presence of latent tuberculosis infection (LTBI) has become mandatory prior to initiation of TNF-α-blocking therapy [4, 5], which has efficiently decreased the risk of reactivation of tuberculosis in this group of patients [6, 7].

In general, screening for LTBI consists of an evaluation of the medical history of patients having tuberculosis in combination with a chest X-ray and intracutaneous skin testing using tuberculin (PPD, purified protein derivative) as Mendel–Mantoux test [4, 5]. The clinical decision for treating rheumatic disease with TNF-α-blocking drugs is based on the results of this screening procedure in that either the presence of a history of tuberculosis, a positive PPD skin test, or a chest X-ray showing changes suspicious of LTBI result in prophylactic treatment with isoniazide (INH) prior to TNF-α-blocking therapy, or the use of alternative disease-modifying anti-rheumatic drug regimens.

The screening procedure is hampered by the uncertainty of medical history and the difficulty of finding specific radiological signs in a population with low disease prevalence. While the PPD skin test is well established and standardized, it suffers from several shortcomings: (i) it is inconvenient in an outpatient setting, as the patient has to return after 48–72 h for the determination of the test result; (ii) in the presence of chronic immunosuppression including inflammatory arthritis, the test result may be falsely negative [8–10]; (iii) in patients vaccinated against tuberculosis with bacillus Calmette–Guérin (BCG) as well as in the frequent situation of subclinical infection with non-tuberculous mycobacteria, the skin test may lead to false positive results [11–13].

Recently, *in vitro* tests using whole blood or isolated peripheral blood mononuclear cells have been evaluated for the diagnosis of latent infection with *Mycobacterium tuberculosis*. Two commercially available interferon-γ-release assays, Quantiferon-TB Gold and T-Spot-B, appear to have an increased precision for the diagnosis of active infection after exposure to tuberculosis [14]. These tests may also increase the sensitivity of diagnosing LTBI in the setting of rheumatoid arthritis [15]. We have recently developed an alternative whole blood test, a quantitative flow cytometric assay [16, 17], measuring antigen-activated T cells after stimulation with tuberculin PPD and two proteins considered specific for *M. tuberculosis*, early secretory antigenic target 6 (ESAT-6) and 10 kDa culture filtrate protein (CFP-10), which are also used in the commercial assays mentioned above [18–20]. We have previously performed a comparative evaluation of the flow cytometric test and the ELISPOT assay. As expected from the similarity in the assay principle (intracellular accumulation vs local secretion of cytokines), the results of both tests showed a strong correlation [17]. The flow cytometric assay may be performed within one working day directly from whole blood (<1 ml) and has demonstrated an increased sensitivity and specificity compared with the PPD skin-testing in the diagnosis of

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Submitted 30 April 2007; revised version accepted 30 November 2007.

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LTBI in immunocompromised patients due to chronic haemodialysis or to immunosuppressive drug treatment after renal transplantation [16, 17]. This study was carried out to characterize the clinical usefulness of this test in a prospective study of patients with inflammatory rheumatic diseases evaluated for treatment with a TNF-α-blocking agent.

Patients and methods

Between May and December 2006, 97 consecutive patients with inflammatory rheumatic disease referred to the rheumatology ward from our outpatient department or from private practice rheumatologists for an evaluation of treatment with a TNF-α-blocking agent were included in this study. The study protocol was approved by the local ethics committee and all patients gave their informed consent. We followed the guidelines of the German Society for Rheumatology (DGRh) and the European consensus statement for the use of biologic treatments [4, 5] with regard to the clinical decisions for the indication and contraindication for a TNF-α-blocking treatment. Patients were asked for a history of tuberculosis or vaccination against tuberculosis. In case of a positive history, this information was verified with information from medical records available to the hospital or the primary care physician. History of vaccination was not verified further. The presence of LTBI was assessed with the Mendel–Mantoux skin test using an intracutaneous injection of 5 U of tuberculin PPD (Biocine-Test PPD Lyophil, Chiron, Siena, Italy) in the volar surface of the forearm. The resulting induration after 48–72 h was measured in millimetres and recorded. An induration of $\geq$5 mm was considered positive according to the national guidelines [4, 5]. In addition, chest X-rays were performed and analysed by an experienced radiologist for the presence of typical signs of tuberculosis infection, i.e. lymph node calcifications, cavernae or multiple nodules in the upper lobes of the lung. From each patient, we also obtained 4.7 ml of whole blood in lithium heparin in addition to the blood tests performed routinely to assess disease activity and comorbidity. This heparinized whole blood was sent to the laboratory in Dewar containers containing water cooled to 4°C for *in vitro* testing of T-cell reactivity and processed within the next 24 h. In addition, eight immunocompetent individuals (47 ± 23 yrs of age) in the absence of any rheumatic disease or an indication for TNF-α-blocking therapy were analysed as typical examples of BCG vaccinated and non-vaccinated subjects without any risk for *M. tuberculosis* infection, of subjects with known history of tuberculosis and of patients with active tuberculosis ($n = 2$ for each group). Results of these individuals are shown in Fig. 1A.

The heparinized whole blood samples were analysed for the presence of specific T-cell reactivity against PPD, ESAT-6 or CFP-10 using the quantitative flow cytometric whole blood assay described previously [16, 17]. In brief, 222 IU/ml PPD (Tuberkulin-GT-1000; Chiron Behring, Marburg, Germany), 10 µg/ml recombinant ESAT-6 or 10 µg/ml recombinant CFP-10 (Lionex, Braunschweig, Germany) were used as stimuli in the presence of 1 µg/ml anti-CD28 and anti-CD49d (clones L293 and 9F10; BD, Heidelberg, Germany), respectively. As negative controls, cells were stimulated with diluent (Chiron Behring). The blood was incubated in polypropylene tubes at 37°C at 6% CO_2 for a total of 6 h. During this time, effector T-lymphocytes are specifically stimulated, resulting in the up-regulation of CD69 and the production of cytokines. During the last 4 h, 10 µg/ml of brefeldin A (Sigma, Deisenhofen, Germany) was added to block extracellular transport of cytokines. Thereafter, the blood was treated with 2 mM EDTA for 15 min. Subsequently, erythrocytes were lysed and leucocytes fixed for 10 min using Becton Dickinson lysing solution according to the manufacturer's instruction. Cells were washed once with FACS buffer (PBS, 5% filtered FCS, 0.5% BSA, 0.07% NaN_3). Staining was done using anti-CD4 (clone SK3), anti-interferon-γ (clone 4SB3), and anti-CD69 (clone L78; all antibodies from BD). At least 15 000 CD4 T cells were analysed on a FACSCalibur (BD) using the Cellquest-Pro 4.0.2 software. The percentage of specific T cells was calculated by subtracting the frequency obtained by the control stimulation. The lower limit of detection of the flow cytometric assay is 0.05% as previously established [21].

In this study, the results from *in vitro* testing did not affect final decision making. If treatment with TNF-α-blocking agents was considered in the presence of LTBI, patients were pretreated with INH at a dose of 5 mg/kg of body weight daily and pyridoxine for 4 weeks. This treatment was continued for another 8 months parallel to the TNF-α-blocking therapy.

Statistical analysis was performed using GraphPad Prism 4.01. Correlation between skin test induration and PPD-specific T-cell frequencies was calculated according to Spearman. Other tests were used as indicated.

Results

Typical examples of the results obtained with the flow cytometric assay used in immunocompetent individuals are shown in Fig. 1A. Whole blood was incubated with PPD, the *M. tuberculosis*-specific antigens ESAT-6 and CFP-10, before antigen-specific activation and cytokine induction was analysed. The frequency of specific CD4 T cells was calculated as the percentage of CD69 and interferon-γ-positive CD4 T cells. Among individuals without any known tuberculosis contact, T-cell reactivity towards all antigens was below detection limit in BCG non-immunized individuals, whereas BCG-vaccinated individuals showed a response towards PPD in the absence of ESAT-6 or CFP-10 reactivity (Fig. 1A, upper panel). In contrast, individuals with either known history of tuberculosis or active tuberculosis prior to therapy (Fig. 1A, lower panel) were characterized by T-cell reactivity towards both PPD as well as ESAT-6 and/or CFP-10. In general, the diluent failed to induce any specific cytokine production. Thus, the flow cytometric whole blood assay appeared suitable for the specific detection of CD4 T cells towards *M. tuberculosis* in immunocompetent individuals. A similar response pattern was identified in patients with inflammatory arthropathies (Fig. 1B) so that the flow cytometric test may also be extended for the use in patients on immunosuppressive drug therapy.

A total of 97 patients with inflammatory arthropathies were assessed for medical indications and contraindications to TNF-α-blocking treatment. The clinical characteristics including the underlying rheumatological diagnosis are shown in Table 1. The eight patients summarized under the heading of 'other' rheumatic diseases were classified as undifferentiated spondyloarthropathy in four cases, as well as enteropathic spondylitis, juvenile arthritis, dermatomyositis and Sharp syndrome in one case each.

Every patient was asked whether he/she was aware of a tuberculosis infection in the past or a vaccination with BCG (Table 2). Four patients remembered a treated infection and this information was verified using their medical records. Five patients reported a childhood vaccination against tuberculosis. Skin testing could be performed in every patient. A total of 13 patients showed an induration of at least 5 mm in the PPD skin test. No induration (0 mm) was seen in 69 patients. Among those with a documented history of infection, two patients showed a positive reaction of $>$5 mm and two patients showed no reaction. Both patients with positive reactions also reacted in the whole blood tests. One of the two patients with negative skin test showed T-cell reactivity against CFP-10 and ESAT-6 in the whole blood assay. An additional patient with ankylosing spondylitis was found to have active tuberculosis after a recent exposure by positive culture of *M. tuberculosis* from bronchoalveolar lavage fluid. Among patients reporting vaccination, one showed a positive reaction in the skin test and four patients did not. Three of these last four patients had never received immunomodulatory drugs. Another patient without a history of tuberculosis or vaccination and

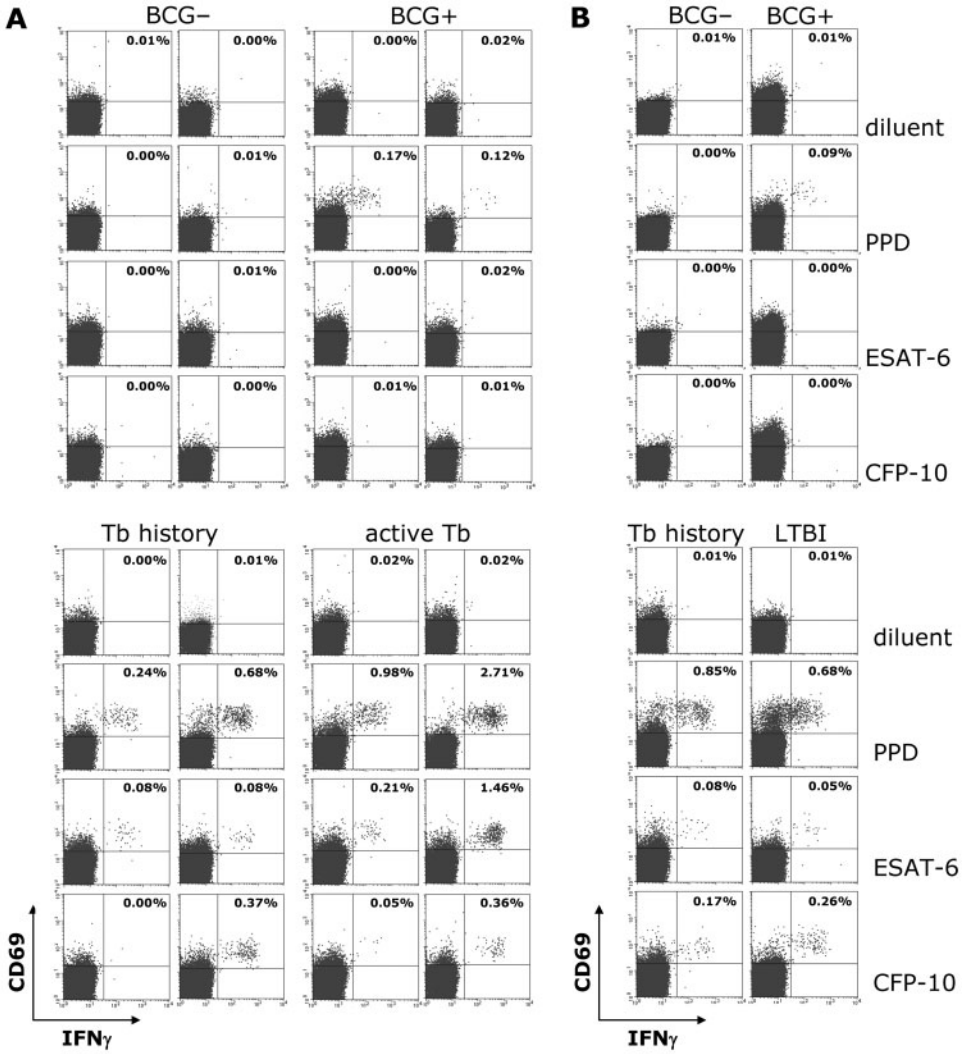

FIG. 1. Quantitation of specific CD4 T-cells reacting against *M. tuberculosis proteins* using flow cytometry in immunocompetent individuals and in patients with inflammatory arthropathies. (**A**) Dotplots of two immunocompetent individuals for each of the following groups are shown: non-BCG vaccinated individuals without known history of tuberculosis contact, BCG-vaccinated individuals without known history of tuberculosis contact, individuals with known history of treated tuberculosis and patients with active tuberculosis (bone and lung tuberculosis, respectively). Antigens used for stimulation are indicated. (**B**) Similar response patterns are found in patients with inflammatory arthropathies.

TABLE 1. Patient characteristics

Diagnosis	*n*	Age (yrs) (M ± S.D.)	Sex (% female)	Disease duration (yrs) (M ± S.D.)	Number of previous DMARDs (M ± S.D.)	Prednisolone dose in mg (M ± S.D.)
Rheumatoid arthritis	48	60 ± 10	73	9 ± 9	2.2 ± 1.4	13 ± 10
Ankylosing spondylitis	23	46 ± 12	35	7 ± 9	0.2 ± 0.5	1 ± 4
Psoriatic arthritis	18	54 ± 12	61	2 ± 3	0.7 ± 0.8	3 ± 5
Other	8	45 ± 19	38	7 ± 14	1.5 ± 1.5	16 ± 16
Total	97	54 ± 13	59	7 ± 9	1.4 ± 1,4	8 ± 10

Patients evaluated for treatment with TNF-α antagonists between May and December 2006. M, mean.

normal computed tomography of the chest developed a skin necrosis measuring 2 cm in diameter. The use of PPD as a common stimulus in the skin test and in the whole blood assay allowed for a direct comparison of results from skin-testing and from the whole blood assay. As compared with 13 patients with positive skin tests, T-cell reactivity against PPD as assessed by the whole blood assay was observed in 39 patients (Table 2). This indicates an increased sensitivity of the flow cytometric assay (see also Table 4). Moreover, these data suggest that exposure to PPD through infection or vaccination is more common than expected from history or Mendel–Mantoux testing. In the group of patients with rheumatoid arthritis and psoriatic arthritis, ~10% showed a positive skin reaction and 20% showed T-cell reactivity against *M. tuberculosis*-specific proteins *in vitro* (Table 2). Interestingly, only one patient in the group with spondylitis ankylosans showed significant *in vitro* T-cell reactivity, whereas the proportion of patients with positive skin test was highest in this group (Table 2).

TABLE 2. Assessment of exposure to tuberculosis

Diagnosis	*n*	History of tuberculosis infection	History of vaccination	Suspicious chest X-ray	Positive skin test	Positive whole blood test against			
						PPD	ESAT-6 and/or CFP-10	ESAT-6	CFP-10
Rheumatoid arthritis	48	3	2	5	5	20	10	6	9
Ankylosing spondylitis	23	0	3	1	5	10	1	1	0
Psoriatic arthritis	18	1	0	1	3	8	5	4	5
Other	8	0	0	0	0	1	0	0	0
Total	97	4	5	7	13	39	16	11	14

The number of patients with a history of tuberculosis infection or BCG vaccination is indicated, as well as the number of patients showing reactivity against PPD in the skin test (Mendel–Mantoux, reaction of $\geq$5 mm) or in the whole blood tests analysing PPD, ESAT-6 and CFP-10 (cut-off 0.05% of CD4 T-cells positive). The difference between the results of the skin test and the whole blood assay for PPD is significant ($P < 0.001$ in the chi-square test).

TABLE 3. Treatment selection

Diagnosis	TNF-α *n* (%)	INH-treatment prior to TNF-α *n* (% of number treated with TNF-α)	Principal reason for withholding TNF-α blocking treatment (*n*)				
			LTBI	Malignant disease	Comorbidity	No medical indication	Other preference
Rheumatoid arthritis	28 (57)	4 (14)	3	1	5	8	3
Ankylosing spondylitis	17 (74)	3 (18)	1[a]			5	
Psoriatic arthritis	12 (67)	2 (17)	1			5	
Other	4 (50)	0			1	3	
Total	61 (60)	9 (15)	5	1	6	21	3

The number of patients started on TNF-α blocking drugs after screening for LTBI and clinical evaluation is shown as well as the reason for not choosing TNF-α-blocking drugs. Several patients with severe arthritis were found to have LTBI and were treated prophylactically with INH before starting TNF-α-blocking treatment, as indicated in the corresponding row. In other patients with LTBI and moderate to severe disease activity, the risk of INH and TNF-α-blocking drugs was deemed inappropriate in the clinical context. TNF-α-blocking agents were therefore withheld, as shown in the corresponding column. Other reasons for withholding TNF-α blocking agents are also listed. *n*, number of patients. [a]This patient was found to have active tuberculosis.

After evaluation, 60% of patients were treated with a TNF-α blocking agent. Among them, 15% received infliximab, 34% etanercept and the others adalimumab. The principal reasons for withholding TNF-α-blocking treatment are indicated in Table 3. In five patients, the risk associated with INH prophylaxis and TNF-α-blocking treatment in the context of LTBI was considered inappropriate with regard to the disease severity and an alternative treatment was chosen. Nine patients were treated prophylactically with INH before the treatment with TNF-α-blocking drugs was initiated (Table 3).

As compared with patients with positive skin tests ($n = 13$), more patients reacted in the whole blood test against PPD ($n = 39$) or against the antigens specific for *M. tuberculosis*, ESAT-6 and/or CFP-10 ($n = 16$, Table 2). The BCG-vaccination status frequently was not remembered or documented by patients. Thus, results were also analysed according to age group, as BCG vaccinations had been recommended in Western Germany between 1945 and 1975 [22]. No positive reactions were observed in four patients below the age of 31 yrs. The prevalence of positive skin reactivity was comparable among the younger (31–64 yrs) compared with the older population (>64 yrs), with 10 of 73 patients (14%) and 3 of 20 patients (15%) showing a reaction, respectively. A higher proportion of the non-vaccinated older population showed reactivity against PPD (11 of 20, 55%) as well as ESAT-6/CFP-10 (5 of 20, 25%) in the whole blood assay compared with the younger population (28 of 73, 38%, and 11 of 73, 15%, respectively), in line with an increasing risk of exposure to *M. tuberculosis* over a lifetime. Of the five patients reporting a vaccination against BCG, one was tested positive with the skin test and the whole blood assay against ESAT-6, three reacted only in the whole blood assay against PPD (not against the specific proteins CFP-10 and ESAT-6) and one remained negative in all tests. Of the seven patients with suspicious changes in chest X-ray, two patients reacted in both the skin and the whole blood assays, and the remaining patients were negative in both tests. One of these five patients had been inappropriately pretreated with etanercept before evaluation for LTBI.

When analysing internal concordance of the different tests used in this study, all but one patient who reacted in the skin test with PPD were also identified using PPD as a stimulus *in vitro* (92%, Table 4) and skin test induration showed a significant correlation with PPD-reactive T-cell frequencies ($P < 0.0001$, $r = 0.48$). Only one-third of patients with reactivity against PPD in the whole blood assay also reacted in skin testing, illustrating the inferior sensitivity of the skin test. Among patients with detectable T cells against PPD in the whole blood assay, 36% showed reactivity towards the *M. tuberculosis*-specific proteins ESAT-6/CFP-10, a group presumably representing truly latently infected individuals (an example being depicted in Fig. 1B). Among all patients with ESAT-6/CFP-10 reactivity, all except two also reacted against PPD *in vitro* (Table 4). As only 6/13 patients with positive skin test also showed a response against ESAT-6/CFP-10 (Table 4), more than half of all skin test reactivities seem to be due to BCG vaccination or infection with non-tuberculous mycobacteria. There were no obvious differences in the amount of steroids used, the number of current or previous disease-modifying drugs or the presence of comorbid conditions such as diabetes mellitus or renal insufficiency in patients divergent for the two test approaches.

TABLE 4. Comparison of test concordance

Concordance of test positive with (%)	PPD skin ($n = 13$)	PPD blood ($n = 39$)	ESAT-6/CFP-10 ($n = 16$)
PPD skin		12/39 (31)	6/16 (38)
PPD blood	12/13 (92)		14/16 (88)
ESAT-6/CFP-10	6/13 (46)	14/39 (36)	

The concordance of tests analysing PPD reactivity in the skin and in whole blood and of the whole blood tests against ESAT-6 and CFP-10 is shown. The number in parentheses refers to the percentage of the group analysed.

Finally, a classification tree for the diagnosis of LTBI in all patients evaluated for TNF-α-blocking treatment was constructed based on a history of tuberculosis, suspicious chest X-ray and either the skin test or the whole blood assay (Fig. 2). Five patients were considered positive due to the presence of a documented medical history or active infection (Fig. 2, step 1). Another 10 patients showed an induration of $\geq$5 mm in the PPD skin test (step 2, panel A). As expected, more patients were detected in the more

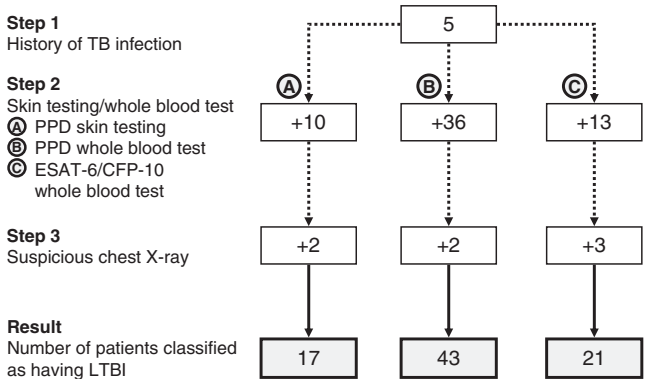

FIG. 2. Classification tree for LTBI. The number of patients with rheumatic diseases that were classified as having LTBI is shown following the indicated three-step diagnostic evaluation. Steps 1 and 3 comprise evaluation of TB history and suspicious chest X-ray, respectively. When comparing the PPD skin test (step 2, panel A) with the PPD whole blood test (step 2, panel B), the latter has an increased sensitivity in detecting exposure to PPD. The number identified when using T-cell reactivity against two proteins specific for *M. tuberculosis*, ESAT-6 and CFP-10 (step 2, panel C), was comparable with the PPD skin test, even though different patients were identified (see text).

sensitive whole blood PPD assay. In contrast, with the whole blood assay against the specific *M. tuberculosis* proteins ESAT-6/CFP-10, 13 additional patients showed T-cell reactivity (step 2, panel C). Depending on whether skin testing or ESAT-6/CFP-10 were considered for decision making, two or three further patients showed suspicious X-ray changes in the absence of a definite history or positive skin or ESAT-6/CFP-10 test (Fig. 2, step 3). In summary, comparing conventional screening with ESAT-6/CFP-10 testing, 17 *vs* 21 patients were identified as latently infected (18% and 22%, respectively). Among those patients, only 12 were targeted with both algorithms, whereas five or nine were exclusively identified by skin testing or ESAT-6/CFP-10 screening, respectively. It is tempting to speculate that the five individuals reacting in skin testing only are BCG-vaccinated without having LTBI, whereas the nine individuals with specific T-cell response towards ESAT-6/CFP-10 were identified due to increased sensitivity of *in vitro* testing. In contrast, screening exclusively based on whole blood PPD reactivity (Fig. 2, panel B) would identify significantly more patients due to increased sensitivity (as compared with skin PPD testing) but lower specificity compared with ESAT-6/CFP-10 screening.

Of the patients who were treated with TNF-α-blocking agents, four out of nine patients would not have been treated with INH if the ESAT-6/CFP-10 assay had been used instead of the skin test for the diagnosis of LTBI. On the other hand, 6 out of 52 patients would have been treated with INH if the positive ESAT-6/CFP-10 reactivity had been used for decision making instead of the skin test. In these cases, the evaluation with the conventional approach may be considered as falsely negative. Of the five patients in whom treatment with TNF-α-blocking drugs was withheld with the primary reason of LTBI (Table 3), the qualitative results for the skin test or the ESAT-6/CFP-10-based whole blood assay were not different. Notably, one patient developed an acute hepatitis after 3 weeks of INH prophylaxis.

Discussion

This is the first prospective study analysing different approaches for the detection of LTBI in rheumatological patients evaluated for the suitability for treatment with TNF-α-blocking drugs. Our data suggest that the flow cytometric analysis of T-cell reactivity towards specific mycobacterial proteins may increase the precision of the diagnosis of LTBI compared with Mendel–Mantoux skin testing. The results are in line with previous reports that the use of the skin test underestimates the prevalence of exposure to mycobacteria in immunocompromised patients [11, 16, 17, 23]. The need for adequate and precise screening tools is underscored by the observation that prophylactic treatment with INH was initiated in 15% of patients prior to treatment with TNF-α-blocking drugs, and 5% of patients did not receive a TNF-α-blocking agent due to the presence of latent or active tuberculosis using the conventional screening approach.

The analysis of the relative clinical value of each diagnostic test used in our study is hampered by the absence of a gold standard test to differentiate between truly latent tuberculosis, the presence of a vaccination response towards BCG, or an infection with non-tuberculous mycobacteria [14]. Three of five patients with documented past or current infection with tuberculosis were positive in skin testing in this study, as was one of five patients with self-reported BCG vaccination. However, the history for the presence of tuberculosis disease is imprecise, as is the history for vaccination. It is unlikely that only 6% of patients who were born in the time period in which BCG vaccination was generally recommended were vaccinated, as our history results appear to suggest. In contrast to other European countries, BCG vaccination was performed shortly after birth in Germany, which may explain the low recall rate observed. Thus, the conventional diagnostic approach recommended prior to treatment of rheumatic patients with TNF-α blockers [4, 5] appears to result in over-treatment with prophylactic INH due to the failure to identify mere vaccination responses. In addition, under-treatment may be common due to the low sensitivity of the skin test.

In theory, whole blood assays measuring T-cell activation towards PPD along with ESAT-6 and CFP-10 should both be more sensitive and more specific. Ideally, a positive whole blood test after stimulation with PPD would detect all patients with vaccination and most with other mycobacterial infections in addition to patients truly having LTBI. The latter could then be identified by using a second stimulation with ESAT-6 and CFP-10 [18–20]. Using these antigens, we indeed observed differences between the results of whole blood compared with conventional skin test screening that are compatible with the presence of over- and under-treatment with prophylactic INH. Of the six patients with documented tuberculosis, five exhibited reactive T-cells on stimulation with ESAT-6 and/or CFP-10. Four out of nine patients treated prophylactically with INH prior to TNF-α-blocking treatment would not have received INH, had the whole blood test been used for decision making instead of the skin test. In contrast, another 6 out of 52 patients not on prophylactic INH would have received INH before initialization of TNF-α-blocking treatment.

When analysing all patients irrespective of the final treatment decision using a screening algorithm consisting of medical history, chest X-ray and one of the assays analysed, the overall number of patients classified as having LTBI was similar using the specific ESAT-6/CFP-10 whole blood assay and the traditional Mendel–Mantoux skin test (Fig. 2, panels C and A, respectively). The poor specificity of the skin test is underscored by the poor correlation of positive results obtained with this compared with the other tests and by the high number of patients identified by the whole blood assay using the same antigen, PPD. Interestingly, while use of the whole blood assays will not reduce the number of patients needing INH prophylaxis in the setting of TNF-α-blocking treatment, our data suggest that the ESAT-6/CFP-10 test may help in a better and more specific selection of patients requiring prophylaxis. The importance of valid patient selection for prophylaxis is underscored by the observation that one of the nine patients receiving INH stopped treatment due to a toxic hepatitis. The whole blood assay is easier to handle in an outpatient setting [17], in which the need to return for reading the skin test may be too inconvenient for some patients. In addition, skin testing in patients taking steroids may be contraindicated due to dermal atrophy and suffusions. Moreover, the skin test is not without risk, as one of our patients developed a skin necrosis after intradermal injection of PPD.

Apart from the flow cytometric approach used in this study, two other *in vitro* tests, an ELISPOT- and an ELISA-based assay, have been established to quantify specific immunity towards *M. tuberculosis* in immunocompetent individuals [18, 24–26]. As all three assay systems rely on the same principle of inducing interferon-γ after stimulation with specific antigens, it is not surprising that we found a significant correlation between T-cell responses detected by flow cytometry and the results obtained by ELISPOT assay [17]. Thus, the flow cytometric approach is equally effective in detecting specific immunity towards *M. tuberculosis*. Both the flow cytometric and the ELISPOT assay have been evaluated as sensitive alternatives to skin testing in various conditions of immunodeficiency [16, 17, 27]. The flow cytometric test is well suited for clinical routine and similar in costs as compared with the commercially available *in vitro* assays. As a major advantage compared with the ELISPOT assay and Quantiferon test, it may be performed within one working day (~8 h) with very little hands-on time. It also allows for a simultaneous quantitation and characterization of cells with respect to T-cell subpopulations and phenotype [16, 17].

When interpreting the results of this study, several issues need to be considered. The Robert Koch Institute estimates the incidence of manifest tuberculosis in Germany as 7.3 per 100 000 inhabitants in 2005 [28]. While it is plausible that the number of cases with LTBI is higher than that of cases with tuberculosis disease, the number of patients classified as LTBI in our study is still surprisingly high, irrespective of whether the traditional screening approach recommended by expert committees [4, 5] or the novel blood test is used. This unexpectedly high prevalence may be a direct consequence of intensified screening programmes for latent tuberculosis infection in patients with inflammatory arthropathies and other cohorts of high-risk individuals and has been observed with the Quantiferon and ELISPOT assays as well [14]. In line with this evidence, we and others also found a high prevalence of *M. tuberculosis*-specific T-cell reactivity upon routine screening among haemodialysis patients, renal transplant recipients and health care workers [16, 17, 29]. As the T-cell assay mainly detects antigen-experienced effector T cells, this high prevalence of T-cell reactivity may in fact reflect the continuing presence of bacilli and/or mycobacterial antigens in the body. On the other hand, an immunological reaction to *M. tuberculosis* proteins may not necessarily prove the continuing presence of *M. tuberculosis* bacteria, even if the diagnostic test used was perfectly specific. In this case, one might speculate that a proportion of patients may have a persistent immune response without persisting infection in the sense of LTBI (i.e. after a transient contact with *M. tuberculosis*). Thus, the specificity of the lymphocyte stimulation tests for the diagnosis of LTBI could be lower than the specificity for the detection of a previous contact. This possibility has not been addressed for any of the existing assays. Together, this emphasizes that skin testing or *in vitro* assays to classify LTBI according to the presence of an immunological reaction to *M. tuberculosis* proteins [4, 5] should be accompanied by careful clinical examination to improve assessment of the individual risk of developing active tuberculosis. One approach to clarify the significance of these findings towards predicting the development of active tuberculosis could be a longitudinal outcome-centred study using different screening approaches as well as the integration of quantitative measures and phenotypical characteristics of specific T cells. However, due to the relatively low number of active tuberculosis infections, this study would have to include several centres that should ideally be located in countries with high prevalence of *M. tuberculosis* infection.

A potential technical issue deals with sample transport. All blood samples were transported overnight in cooled Dewar containers and analysed within 24 h after phlebotomy. Although this could have decreased the viability or reactivity of the T cells, we have previously shown that stimulation results of samples shipped under these conditions are identical to results of samples processed immediately (unpublished data). In addition, while concomitant immunosuppression could alter the results of both the whole blood as well as the skin tests, we have shown in long-term renal transplant recipients taking a combination of several immunosuppressive drugs that the whole blood assay is not adversely affected by immunosuppression, in contrast to the skin test [17].

In summary, this study prospectively describes the performance of different screening approaches for LTBI in a large number of rheumatic patients in a real-life situation. In this setting, the use of a whole blood T-cell stimulation assay using ESAT-6 and CFP-10 as specific stimuli may be more precise than conventional Mendel–Mantoux testing.

Rheumatology key messages

- Latent tuberculosis infection is frequently identified on screening prior to institution of TNF-α-blocking therapy.
- Whole blood T-cell assays may increase correct identification of patients.

Acknowledgements

We thank Dr Nicole Schröder, Dr Petra Saar, Dr Ingo Tarner and Dr Carola Rumbaur for including patients in the study and Rebecca Ruth for excellent technical assistance.

Funding: This study was supported by a grant from the Else Kröner Fresenius Foundation and from the Zentrale Forschungskommission der Universität des Saarlandes.

Disclosure statement: The authors have declared no conflicts of interest.

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