Comparison of Screening Procedures for *Mycobacterium tuberculosis* Infection Among Patients with Inflammatory Diseases

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ABSTRACT. ***Objective.*** To test if *Mycobacterium tuberculosis* screening results differ among patients with inflammatory disease depending on whether the QuantiFeron TB-Gold test (QFT) or tuberculin skin test (TST) is used; and to evaluate if a possible difference is influenced by the presence of risk factors or immunosuppression.

Methods. The interferon-γ response to *in vitro* stimulation of *M. tuberculosis*-specific antigens was measured with QFT and results were compared with TST. Associations to bacillus Calmette-Guerin (BCG) vaccination, risk factors, and immunosuppression were analyzed for both tests.

Results. QFT and TST results were available for 294/302 and 241/302 patients, respectively; 234 had results from both tests. Twenty-one (7%) tested positive with QFT and 45 (19%) with TST. A positive QFT was associated with risk factors for *M. tuberculosis* infection: i.e., birth or upbringing in a TB-endemic area [risk ratio (RR) = 7.8, 95% CI 1.5–18.2, $p < 0.001$], previous TB treatment (RR 4.7, 95% CI 1.6–13.5, $p = 0.005$), and any latent TB infection risk factor (RR 4.7, 95% CI 2.1–11.0, $p = 0.0002$). Treatment with corticosteroids increased the risk for an inconclusive QFT result (RR 4.2, 95% CI 1.6–10.7, $p = 0.04$) and decreased the risk for a positive TST result (RR 0.4, 95% CI 0.1–1.0, $p = 0.04$). Agreement between the tests was low (kappa 0.2, 95% CI 0.02–0.3, $p = 0.002$).

Conclusion. The study documented a high degree of discordant positive QFT and TST results. A positive QFT was more closely associated with risk factors for *M. tuberculosis* infection than the TST. The use of corticosteroids affected test outcome by increasing the risk for an inconclusive QFT result and decreasing the risk for a positive TST result. (First Release Aug 1 2009; J Rheumatol 2009;36:1876–84; doi:10.3899/jrheum.081292)

Key Indexing Terms:
MYCOBACTERIUM TUBERCULOSIS TUBERCULOSIS SCREENING PROCEDURES
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Tumor necrosis factor-α (TNF-α) inhibitors are widely used for the treatment of patients with inflammatory diseases such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), psoriasis arthritis, and sarcoidosis. Roughly 400,000 patients have been treated worldwide with these drugs[1].

Patients with inflammatory diseases receiving TNF-α inhibitors have been shown to be at increased risk of developing severe disseminated tuberculosis (TB), some with fatal outcome[2-6]. The cytokine TNF-α is essential for host defence against mycobacterial infection by regulating macrophage activation, cell recruitment to the site of infection, and granuloma formation[7,8]. In patients with latent TB

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infection (LTBI), blocking the function of TNF-α may lead to disintegration of the granuloma and dissemination of *Mycobacterium tuberculosis* infection[6]. It is recommended that all patients who are candidates for TNF-α inhibitor treatment should be screened for *M. tuberculosis* infection prior to treatment[9,10]. Most current screening guidelines rely on the tuberculin skin test (TST) despite wide recognition that the TST has severe drawbacks; TST has poor specificity since it is unable to distinguish persons infected with *M. tuberculosis* from persons vaccinated with the *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) vaccine strain[11,12]. Exposure to environmental mycobacteria may lead to false-positive results[12], and the poor specificity can lead to unnecessary treatment with antituberculous antibiotics with the risk of drug toxicity[13-15]. In addition, sensitivity of the TST among patients with inflammatory diseases is potentially impaired due either to the inflammatory disorder itself or to the immunosuppressive treatment[16,17]. In some countries, interferon gamma release assays (IGRA) have been implemented as part of national TB screening programs[9,18-20]. IGRA tests are based on the release of interferon-γ (IFN-γ) in blood samples after stimulation *in vitro* with *M. tuberculosis*-specific antigens such as the 6 kDa early secreted antigenic target (ESAT-6) and the 10 kDa culture filtrate protein (CFP-10). These peptide antigens are highly *M. tuberculosis*-specific and are not present in BCG vaccine strains or the most widely disseminated species of nontuberculous mycobacteria[21].

The IGRA tests have been found to be highly specific in diagnosing active TB and LTBI[22,23]. Further, the structure of the IGRA tests, by including an unspecific lymphocyte-stimulating mitogen, allows evaluation of the T cell reactivity of the persons tested, which gives important information when handling immunocompromised patients. IGRA tests have to this point been approved for use in immunocompetent individuals > 17 years of age[24]. Knowledge about performance among immunocompromised patients is still limited, although new data are emerging[1,25-30].

The objectives of our study, in patients with inflammatory diseases who were candidates for TNF-α inhibitor treatment, were (1) to determine if results differed when screening for LTBI using the QuantiFeron TB assay (QFT) or the TST; and (2) to evaluate if a possible difference in test results is influenced by the presence of TB risk factors or immunosuppression due to immunosuppressive treatment or due to a low CD4 cell count.

MATERIALS AND METHODS

Study population. Inclusion criteria for study participation required patients with inflammatory diseases who were candidates for TNF-α inhibitor treatment and attending either The Heart Centre, Division of Lung Transplantation, Copenhagen, or one of 3 rheumatology departments in Copenhagen, Denmark. The patients were enrolled prospectively over a 2-year period from March 2005 to March 2007.

The exclusion criteria were age < 18 years, known pregnancy, hemoglobin < 6 mmol/l, or receiving TNF-α inhibitor treatment at study entry.

If the patients tested positive in either the TST or the QFT, the protocol strictly recommended 6-month prophylactic isoniazid treatment and clinical followup.

Patients gave their written informed consent and the study was approved by the ethical committee of Copenhagen and Frederiksberg Municipality (KF 01-258946).

Risk factors. A risk assessment questionnaire was completed in collaboration with the treating physician or a trained nurse. Along with demographic data and data on BCG vaccination status, the patients' TB history and risk factors for *M. tuberculosis* infection were collected, along with information on current immunosuppressive treatment.

Information on the following risk factors for *M. tuberculosis* infection were obtained: known contact to sputum-positive TB patient, birth or upbringing in a highly TB-endemic country (defined as TB incidence > 25:100,000), history of longterm residence in a highly TB-endemic country (defined as > 3 months), previous treatment for active TB, and chest radiographic findings consistent with previous, healed TB.

Based on information on current immunosuppressive treatment, patients were divided into 2 treatment groups: patients receiving disease modifying antirheumatic drugs (DMARD) or those receiving corticosteroid treatment. The CD4 cell count was measured in a randomly selected subgroup of patients and was used as a marker of immunosuppression. A value < 500×10^6/l was considered a low CD4 cell count. In the questionnaire, the attending physician was asked to evaluate if the patient had LTBI based upon the current Danish guidelines, which comprise patient's history, TST result, and chest radiography[31].

TST procedure. TST was applied the same day that blood was collected for the QFT. The TST was applied to the dorsal aspect of the forearm by the intradermal Mantoux method using 2 tuberculin units (0.1 ml) of purified protein derivative (PPD; RT-23, Statens Serum Institut, Copenhagen, Denmark). Seventy-two hours after inoculation the indurations were read by an experienced examiner and the maximum diameter of the induration was recorded in millimeters. All TST results are analyzed according to current Danish guidelines. The guideline applies for immunocompetent as well as immunocompromised samples and uses the limit of > 12 mm for BCG-vaccinated individuals and > 6 mm for those unvaccinated.

For comparison with US guidelines, we also compared the QFT/TST results using American Thoracic Society guidelines[32]. The US guidelines differentiate cutoff at > 5, > 10, or > 15 mm, depending on assessment of LTBI risk factors and groups at risk of progression of TB disease[32].

Quantiferon-TB Gold procedure. The QFT Gold is a whole-blood IFN-γ assay incorporating the TB-specific antigens ESAT-6 and CFP-10 to detect *M. tuberculosis* infection. In this study, all patients were tested with the QFT Gold second-generation test, performed as instructed by the manufacturer (Cellestis Ltd., Carnegie, Australia): 6 ml of whole blood was drawn from a cubital vein into a heparinized collection tube (Vacutainer™, Becton-Dickinson, Meylon, France). Within 6 hours of collection the samples were transferred to the National Reference Laboratory of Mycobacteriology at the Statens Serum Institut. As instructed by the manufacturer, samples were divided in 4 aliquots of 1 ml each, and stimulated in a 24-well culture plate (Nunc, Roskilde, Denmark) with ESAT-6 antigen, CFP-10 antigen, saline as negative control, or phytohemagglutinin (PHA) as positive control. After stimulation, samples were incubated at 37°C for 16–24 hours and the plasma was harvested. The amount of IFN-γ produced in the plasma was quantified in an ELISA.

The IFN-γ values obtained after antigen and mitogen stimulation were corrected for background activity by subtracting the IFN-γ value obtained in the negative control. An IFN-γ value ≥ 0.35 IU/ml obtained after antigen stimulation was considered positive and indicative of infection with *M. tuberculosis.* A test was indeterminate if the IFN-γ response in the mitogen well was < 0.50 IU/ml when corrected for background and with an antigen response < 0.35 IU/ml.

PPD in vitro. 1 ml of whole blood was stimulated with tuberculin PPD derived from the first-generation QFT assay for *in vitro* use (Cellestis). The PPD stock concentration was 20,800 IU/ml, with a final concentration of 2500 IU/ml in the test sample. The amount of IFN-γ produced in the plasma was quantified in an ELISA. The IFN-γ values obtained after antigen stimulation were corrected for background activity, by subtracting the IFN-γ value obtained in the negative control.

In order to increase specificity, an IFN-γ cutoff value for a positive PPD response was set at 1.5 IU/ml[33].

Statistical analysis. In the absence of a "gold standard" for diagnosis of LTBI one cannot directly determine the sensitivity and specificity of the QFT[34]; however, if the QFT is more specific and sensitive than the TST, it will be more closely associated with risk factors for *M. tuberculosis* infection[35], assuming that sensitivity and specificity for the TST and QFT tests are uncorrelated to sensitivity and specificity for the risk factors. The extent of association between test result and risk factors will therefore be used as a measure of misclassification induced by sensitivity and specificity.

Log-linear binominal regression models were used to analyze the association (expressed in risk ratios, RR) between QFT and TST and possible *M. tuberculosis* risk factors, immunosuppressive treatment, and BCG vaccination status. A similar approach was used to analyze PPD and inconclusive QFT results.

Correlations between *in vitro* PPD-induced IFN-γ production and CD4 cell count and correlations between PPD-induced IFN-γ production and PHA-induced IFN-γ production were presented using Spearman statistics.

Kappa statistics were used to determine the agreement between QFT and TST as well as between QFT and clinical diagnosis. The association between factors relevant to *M. tuberculosis* infection and QFT/TST concordance was evaluated by chi-square test. All reported p values are 2-sided and a p value < 0.05 was considered significant. All data were analyzed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

A total of 302 patients with inflammatory diseases were included in the study and a summary of their demographic data is shown in Table 1. All patients with a diagnosis fulfilled the international diagnostic criteria for their specific disease[36,37]. Overall, 62% of patients were female and the median age of enrolled patients was 49.8 years, ranging from a median age of 37.2 years for spondyloarthropathies to a median age of 56.9 years for patients with rheumatoid arthritis (Table 1).

A definite history of BCG vaccination was available for 200 participants (66%) and 152 (76%) of those 200 were BCG-vaccinated. In 12 (4%) of 302, the chest radiograph findings were consistent with previous, healed TB.

Twenty-two (9%) of 254 participants who had answered the questionnaire had a history of TB exposure due to known prior TB contact, 18 (8%) of 225 reported longterm residence in a TB-endemic area, and 7 (3%) of 255 had been previously treated for TB. Combining information on any of the LTBI risk factors (known contact to sputum-positive TB patient, birth or upbringing in a TB-prevalent country, history of longterm residence in a high-endemic country, previous treatment for active TB, chest radiographic findings consistent with previous healed TB), 69 (29%) of 264 of the patients had one or more LTBI risk factors.

CD4 cell count was obtained on a subgroup of 193 patients, with a median CD4 value of 907×10^6/l (95% CI $350–1700 \times 10^6$/l); 22 of 193 had a CD4 cell count below 500×10^6/l; and only one had a CD4 cell count below 200×10^6/l.

QFT and TST results. QFT results were obtained for 294 participants, of whom 21 (7%) had a positive result, 260 (88%) a negative, and 13 (5%) an indeterminate result. Information on QFT test was lacking for 8 participants.

TST results were available from 241 participants and unavailable from 61, as one participating rheumatology department did not apply the TST. According to Danish TST guidelines using the limit of ≥ 6 mm diameter for BCG-unvaccinated and ≥ 12 mm for BCG-vaccinated, 45 (19%) subjects presented a positive and 196 (81%) a negative TST result. Applying the US guidelines, with a differ-

Table 1. Demographic characteristics of 302 Danish patients with inflammatory diseases, screened for *M. tuberculosis* infection prior to tumor necrosis factor-α inhibitor treatment.

	Total, n = 302 (%)	Rheumatoid Arthritis, n = 153 (%)	Spondyloarthropathies, n = 40 (%)	Sarcoidosis, n = 51 (%)	Other*, n = 37 (%)	Unknown**, n = 21 (%)
Age, median yrs (25%–75% interpercentile range)	49.8 (36.7–60.2)	56.9 (45.3–65.4)	37.2 (29.5–46.8)	37.8 (31.8–48.7)	45.8 (31.9–60.2)	54.4 (38.1–62.9)
Sex						
Men	116 (38)	42 (28)	26 (65)	26 (51)	12 (32)	10 (48)
Women	186 (62)	111 (72)	14 (35)	25 (49)	25 (68)	11 (52)
Born in TB-endemic area***						
No	276 (91)	143 (93)	36 (90)	47 (92)	31 (84)	19 (90)
Yes	26 (9)	10 (7)	4 (10)	4 (8)	6 (16)	2 (10)
BCG vaccination						
No	48 (16)	19 (13)	10 (25)	6 (12)	9 (24)	4 (19)
Yes	152 (50)	89 (58)	17 (43)	13 (25)	20 (54)	13 (62)
Unknown†	102 (34)	45 (29)	13 (32)	32 (63)	8 (22)	4 (19)

* Including psoriatic arthritis, Morbus Still polymyositis, HLA-B27 arthritis, polyarthritis. ** Diagnoses were not further specified from the questionnaire. *** TB-endemic areas; defined by WHO as countries with a TB incidence > 25/100,000/year. † Where the questionnaire was incomplete, missing data were categorized as unknown.

entiated cutoff depending on risk factors and groups at risk for TB disease progression[18], 66 (28%) patients were TST-positive and 168 (72%) were TST-negative. The agreement of TST and QFT results is displayed in Table 2.

Test concordance using Danish guidelines was 81%, due to high concordance of TST- and QFT-negative test results; however, the agreement was poor (kappa = 0.2, 95% CI 0.04–0.3, p = 0.002). A TST result was available for 18 of 21 patients with a positive QFT: 9 (50%) of these were concordant-positive, whereas 9 (50%) were discordant QFT-positive/TST-negative. For the entire group of 45 who were TST-positive and for whom a corresponding QFT result was available, 9 (20%) subjects were concordant-positive, whereas 36 (80%) were discordant TST-positive/QFT-negative.

Due to the additional 21 TST-positive/QFT-negative subjects identified when applying US guidelines, the concordance dropped to 72%, with very poor agreement (kappa = –0.04, 95% CI –0.1 to 0.0, p = 0.05).

We analyzed QFT results by different cutoff levels (≥ 0.15 U/ml, ≥ 0.25 U/ml, ≥ 0.35 U/ml, ≥ 0.7 U/ml) to evaluate the effect of the cutoff level on the TST/QFT concordance. None of the chosen cutoff levels enhanced the TST/QFT concordance (data not shown).

Risk factors. The associations between factors relevant to *M. tuberculosis* infection and test reactivity in either a QFT or TST, stratified by Danish guidelines, are shown in Table 3.

A positive QFT result was significantly associated with previous TB treatment (RR 4.7, 95% CI 1.6–13.5, p = 0.005), birth or upbringing in a TB-endemic area (RR 7.8, 95% CI 1.5–18.2, p < 0.0001), and the combined variable, any LTBI risk factor (RR 4.7, 95% CI 2.1–11.0, p = 0.002).

A positive TST result was inversely associated to corticosteroid treatment (RR 0.4, 95% CI 0.1–1.0, p = 0.04). Neither a positive TST nor a positive QFT result was associated with BCG vaccination status.

The associations between test results and risk factors were further estimated among the subgroup of 234 patients with both test results available. The associations were found to be similar to the associations in Table 3 (data not shown).

The attending physicians assessed their patients' LTBI risk, based on current screening guidelines[31]: 249 responses were received and 31 (11%) patients were diagnosed with LTBI. Comparing the 249 patients in whom the attending physician's LTBI diagnosis as well as the QFT results were available, the attending physician identified 4 of 18 (22%) QFT-positive patients as LTBI. Agreement between the attending physicians' diagnoses and the QFT results was poor (kappa = 0.08, 95% CI –0.07 to 0.22).

Discordant TST and QFT results. The discordance of TST and QFT test results was analyzed according to the risk factors for *M. tuberculosis* listed in Table 3. None of the risk factors was associated with a discordant QFT/TST test result (Table 4).

Indeterminate results. QFT results were indeterminate in 13 of 294 patients (5%). Six of 13 (46%) of the indeterminate responders received DMARD, compared to 171 of 302 (57%) in the total study population. Five of 13 (38%) patients received corticosteroid treatment compared to 50 of 302 (17%) in the total study population. One patient received both DMARD and corticosteroids.

CD4 cell counts were available in 9 of the 13 patients, of whom 2 of 9 (22%) had a low CD4 cell count compared to 22 of 193 (11%) of the total study population. In a regression analysis, all factors relevant to *M. tuberculosis* infection (Table 3) were analyzed for an association to an indeterminate QFT result, and, adjusted for age and sex, only corticosteroid treatment was found to be significantly associated (RR 4.6, 95% CI 1.5–13.3, p = 0.008). There was no association between low CD4 cell count and indeterminate results, but high CD4 cell counts were found to correlate with PHA-induced levels of IFN-γ production (Spearman rho = 0.18, p = 0.02), indicating that a high CD4 count correlates to a high level of PHA-stimulated IFN-γ production (data not shown).

Association to in vitro PPD response. The IFN-γ production induced by PPD was measured *in vitro* on a randomly selected subgroup of 159 patients. There was a correlation between PPD response and CD4 cell count (Spearman rho =

Table 2. A comparative analysis of the tuberculin skin test and QuantiFeron test* (QFT) analyzed by the Danish and US tuberculin skin testing guidelines. Data in parentheses are percentages.

		Tuberculin Skin test				
		Danish Guidelines** Concordance 81% k = 0.2 (95% CI 0.04 to 0.3), p = 0.002			US Guidelines*** Concordance 72% k = –0.04 (95% CI –0.1 to 0.0), p = 0.05	
		Negative	Positive†		Negative	Positive
QuantiFeron test	Negative	180 (77)	36 (15)	Negative	159 (68)	57 (24)
	Positive	9 (4)	9 (4)	Positive	9 (4)	9 (4)

* Valid results of both tests were available for 234/302 patients. ** Tuberculin skin test cutoff ≥ 12 mm for Bacille Calmette-Guerin vaccinated and ≥ 6 mm for unvaccinated. *** Tuberculin skin test cutoff > 5, > 10, or > 15 mm, stratified by groups at risk and risk factors for *M. tuberculosis* infection[32]. † 18/45 of the TST-positives had a negative QFT and were unvaccinated or had unknown vaccination status. According to current guidelines all immunocompromised patients with either a positive TST or QFT result are eligible for 6 months of isoniazid treatment.

Table 3. The association between factors relevant to *M. tuberculosis* infection and test reactivity to either the QuantiFeron test or the tuberculin skin test.

	QuantiFeron Test				Tuberculin Skin Test			
	N	Test Positive, N (%)	RR (95% CI)*	p	N	Test Positive, N (%)	RR (95% CI)*	p
Total	294	21 (7)			241	45 (19)		
Diagnoses				0.4				0.08
Rheumatoid arthritis	151	13 (9)	1		141	28 (20)	1	
Sarcoidosis	50	1 (2)	0.3 (0.01–1.3)		8	1 (13)	0.7 (0.1–4.9)	
Spondyloarthropathies	39	2 (5)	0.6 (0.1–2.3)		39	11 (28)	1.6 (0.9–2.9)	
Other**	37	4 (11)	1.4 (0.4–3.5)		35	3 (9)	0.4 (0.1–1.4)	
Unknown***	17	1 (6)	—		18	2 (11)	—	
Born in a TB-endemic area†								
Yes	26	9 (35)	7.8 (1.5–18.2)	< 0.0001	18	6 (33)	1.7 (0.7–3.1)	0.2
No	268	12 (5)	1		223	39 (17)	1	
BCG vaccination								
Yes	148	9 (6)	0.8 (0.2–3.9)	0.7	140	23 (16)	0.9 (0.4–3.1)	0.9
No	45	4 (9)	1		42	5 (12)	1	
Unknown***	101	8 (8)	—		59	17 (29)	—	
Radiograph indicative of TB								
Yes	12	1 (8)	1.4 (0.1–5.7)	0.7	11	1 (9)	0.7 (0.04–2.6)	0.6
No	282	20 (7)	1		230	44 (19)	1	
Previous TB treatment								
Yes	7	2 (29)	4.7 (1.6–13.5)	0.005	6	2 (33)	2.7 (0.6–11.5)	0.5
No	240	16 (7)	1		231	42 (18)	1	
Unknown***	47	3 (6)	—		4	1 (25)	—	
History of TB contact								
Yes	22	1 (5)	0.3 (0.0–4.6)	0.6	20	5 (25)	1.4 (0.5–2.7)	0.4
No	224	17 (8)	1		216	40 (19)	1	
Unknown***	48	3 (6)	—		5	0	—	
Visit TB-endemic areas > 3 months								
Yes	17	1 (6)	1.0 (0.1–8.0)	0.9	17	5 (30)	1.5 (0.6–3.3)	0.4
No	202	14 (7)	1		206	37 (18)	1	
Unknown***	75	6 (8)	—		18	3 (17)	—	
Any LTBI risk factor††								
Yes	68	12 (18)	4.7 (2.1–11.0)	0.0002	58	16 (28)	1.5 (0.7–2.9)	0.3
No	226	9 (4)	1		183	29 (16)	1	
Corticosteroid treatment								
Yes	48	2 (4)	0.5 (0.1–1.6)	0.3	38	3 (8)	0.4 (0.1–1.0)	0.04
No	246	19 (8)	1		203	42 (21)		
DMARD treatment								
Yes	166	10 (6)	0.7 (0.3–1.7)	0.4	76	12 (16)	1.3 (0.7–2.3)	0.3
No	128	11 (9)	1		165	33 (20)	1	
CD4 count								
< 500	22	2 (9)	1 (0.2–3.2)	1.0	19	6 (32)	1.5 (0.7–3.3)	0.4
≥ 500	167	14 (8)	1		167	31 (19)	1	
Unknown***	105	5 (5)	—		55	8 (15)	—	

* The regression excluding the unknown numbers in the analysis. RR is adjusted for age and sex. ** Including psoriatic arthritis, Morbus Still, polymyositis, HLA-B27 arthritis, polyarthritis. *** Missing questionnaire data were categorized as unknown. † TB-endemic areas defined by WHO as countries with a TB incidence > 25/100,000/yr. †† Any LTBI risk factor is categorized as present, when at least one of the following risk factors is present: birth in TB-endemic area, visit to TB-endemic area > 3 months, prior TB contact, radiograph indicative of TB or prior TB treatment.

0.23, p = 0.01) and to PHA-induced levels of IFN-γ production (Spearman rho = 0.34, p < 0.0001), indicating that a high PPD response correlates to high CD4 cell counts as well as to high PHA-induced production of IFN-γ. PPD responses were tested in a regression analysis toward all factors relevant to *M. tuberculosis* infection listed in Table 3 as explanatory variables. When adjusted for age and sex, the PPD response was significantly associated only to the TST result (RR 2.7, 95% CI 1.1–6.6, p = 0.007) and to the diagnosis of sarcoidosis (RR 2.4, 95% CI 1.5–3.9, p = 0.006).

DISCUSSION

Patients with inflammatory diseases need sensitive and specific tests for detection of *M. tuberculosis* infection before

Table 4. Associations between factors relevant to *M. tuberculosis* infection and QuantiFeron and tuberculin skin test concordance.

	Total No. of Pair-wise Analyses, N	Concordant Test Results*, N (%)	Discordant Test Results*, N (%)	p**
BCG vaccination				
No	40	34 (85)	6 (15)	0.6
Yes	136	110 (81)	26 (19)	
Unknown***	58	—	—	
Corticosteroid treatment				
No	197	131 (76)	41 (24)	0.07
Yes	37	34 (92)	3 (8)	
DMARD treatment				
No	74	60 (81)	14 (19)	1.0
Yes	160	129 (81)	31 (19)	
CD4 count				
≥ 500	163	130 (80)	33 (20)	1.0
< 500	19	15 (79)	4 (21)	
Unknown***	52	—	—	
Visit TB-endemic area > 3 months				
Yes	16	10 (63)	6 (38)	0.09
No	201	166 (83)	35 (19)	
Unknown***	17	—	—	
History of TB contact				
Yes	20	13 (76)	4 (24)	1.0
No	209	169 (81)	40 (19)	
Unknown***	5	—	—	
Previous TB treatment				
Yes	6	5 (83)	1 (17)	1.0
No	224	182 (81)	42 (19)	
Unknown***	4	—	—	
Radiograph indicative of TB				
Yes	11	11 (100)	0 (0)	0.1
No	223	178 (80)	45 (20)	
Born in TB-endemic area				
Yes	18	13 (72)	5 (28)	0.4
No	216	176 (81)	40 (19)	
Any LTBI risk factor†				
Yes	57	43 (75)	14 (25)	0.3
No	177	146 (82)	31 (18)	
Diagnoses				
Rheumatoid arthritis	139	112 (81)	27 (19)	0.1
Sarcoidosis	8	8 (100)	0 (0)	
Spondyloarthropathies	38	27 (71)	11 (29)	
Other†	35	31 (89)	4 (11)	
Unknown***	14	—	—	

* Number of cases where both TST and QuantiFeron test results are either positive or negative or where the 2 test results differ (discordant). ** Chi-square test. *** Missing questionnaire data were categorized as unknown. † Any LTBI risk factor is categorized as present, when at least one of the following risk factors is present: birth in TB-endemic area, visit to TB-endemic area > 3 months, prior TB contact, radiograph indicative of TB or prior TB treatment. †† Including psoriatic arthritis, *M. Still*, polymyositis, HLA-B27 arthritis, polyarthritis.

initiation of TNF-α-inhibitor therapy, due to the high risk of reactivation of LTBI when on therapy. We report the comparison between the QFT and the TST for diagnosing LTBI among patients with inflammatory diseases who are candidates to receive TNF-α-inhibitor therapy. This study is the first to document an effect of corticosteroid treatment on QFT results, by increasing the amount of inconclusive test results.

The absence of a gold standard for LTBI makes it impossible to directly determine the sensitivity and specificity of new diagnostic tests[34]. We have used the strength of association between test results and LTBI risk factors as an indirect measure for the association between test result and LTBI. A positive TST can reflect prior BCG vaccination, exposure to nontuberculous mycobacteria, or exposure to mycobacteria belonging to the *M. tuberculosis* complex; whereas the specificity of the QFT is high, primarily reflecting exposure to mycobacteria belonging to the *M. tuberculosis* complex.

In our study, the overall prevalence of LTBI was 7% based on QFT results and 19% based on TST results. We observed that in patients with inflammatory diseases the QFT is more closely associated with presence of certain risk factors for LTBI than the TST. These findings support the high specificity of the QFT test, in agreement with studies by Matulis, *et al* among patients with inflammatory diseases in Switzerland[38]. Concordance between the QFT and TST tests was 81%, due to a high degree of concordant negative test results, while the kappa agreement was poor (kappa = 0.2) due to the discordant positive test results. None of the factors related to *M. tuberculosis* infection could explain the discordant test results in our study, whereas comparative studies have shown that corticosteroid treatment was associated with positive IGRA/negative TST discordant test results[28].

While several studies have shown an association between a positive TST result and previous BCG vaccination, especially when vaccination followed the first year of life[11,33,39], such an association could not be observed in our study. While a substantial proportion of patients (34%) did not recollect their prior BCG vaccination status, the study design did not allow us to clarify whether they were missing at random.

Immunosuppressive treatment, autoimmune disease, or other immuno-deficiencies may compromise the sensitivity of the TST and IGRA tests. Several studies have convincingly demonstrated that performance of the TST is unreliable in patients with immunosuppression[16,27,28], and to date, data on the IGRA tests suggest that they are less sensitive to immunosuppression. As documented in other studies[28,38], we established an inverse association between the use of systemic corticosteroid and a positive TST, but in contrast to previous studies, we also demonstrated that an inconclusive QFT result was associated with the use of corticosteroid treatment, although the overall prevalence of indeterminate QFT results was low (5%). It remains to be determined if and when this immunosuppression reverts after withdrawal of corticosteroids. Studies in people who are HIV-positive have demonstrated that the CD4 cell count correlates with ability to respond in the QFT test[40], and that the performance of the QFT seemed to deteriorate when CD4 counts were $< 200 \times 10^6$/l. We evaluated if the ability to perform an adequate response in the QFT test was associated to a low CD4 cell count among patients with inflammatory disease. Only a few patients (n = 22) presented with a low CD4 cell count ($< 500 \times 10^6$/l), and only one had a CD4 cell count $< 200 \times 10^6$/l. No association between QFT performance and CD4 cell count was established. From our results we cannot recommend the use of the CD4 cell count as a marker of validity of the QFT test in patients without HIV infection, and larger studies will be required to determine whether other markers may be useful. However, we were able to demonstrate that the CD4 cell count was correlated to PHA-induced production of IFN-γ, comparable to that seen among HIV-infected patients[40].

By including the *in vitro* PPD response we were able to compare an antigen-specific response from the T cells to the nonspecific T cell response generated by PHA. Further, the PPD response enabled us to compare the result with the *in vivo* PPD (the TST) as a control for technical error.

The PPD response was associated to the TST response, assuring that the TST results obtained in the study were not biased by interreader variability or a high degree of misclassification due to anergy. As well, the PPD response was correlated to PHA-induced IFN-γ production and to CD4 cell count, indicating that both PPD and PHA responses seemed to be dependent on the CD4 cell count, as demonstrated among HIV patients[40].

Limitations to our study must be considered. The study made use of an older version of the QuantiFeron TB Gold test, the Quantiferon TB Gold 2nd generation, which is now outdated. As the study had been started, we used the outdated test throughout the study to avoid changes in methodology.

The study was conducted in a country with low TB incidence with an overall low number of positive test results, thus results are not necessarily directly applicable to countries with high TB incidence.

The study was not designed to address the question of progression to disease, as the protocol recommended prophylactic treatment to test-positive patients.

Implementation of TST screening and chemoprophylactic treatment has helped in reducing the incidence of TB among patients with inflammatory diseases treated with TNF-α inhibitors[4,41]. The risk, however, has not been eliminated and we have reported one patient overlooked by procedures based on TST screening alone[42]. Our present study strongly indicates that current screening procedures are not sufficient since the attending physician, following current guidelines, missed the majority of those with positive QFT test results, and since 9 of 18 (50%) QFT-positive patients were TST-negative. None of the discordant QFT+/TST– results reported here had any other LTBI risk factors or chest radiographic findings that would have led to chemoprophylactic treatment according to the current screening procedures. Other studies have found similar high proportions of discordant IGRA-positive/TST-negative results among patients with inflammatory diseases[16,38], demonstrating that the TST, even using the > 5 mm cutoff, is not sensitive enough in this patient group and that the IGRA test may improve sensitivity. Whether the TST should be excluded when screening for LTBI among patients with inflammatory diseases is questionable, as evidence-based support of the superiority of the QFT in inflammatory diseases is limited[28,38].

We found the QFT to be more closely associated with risk factors for LTBI than the TST. The poor concordance between QFT and TST indicates that both tests should be taken into consideration when screening for LTBI in patients with inflammatory diseases. Clinical outcome-based validation studies are needed for evaluation of the predictive value

of positive test results in inflammatory diseases prior to treatment with TNF-α inhibitors.

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