

# Immunosurveillance for *Mycobacterium tuberculosis* of health care personnel in a third level care hospital

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## KEY WORDS

Tuberculosis surveillance; health care workers; tuberculosis skin test

## PAROLE CHIAVE

Immunosorveglianza tubercolare; personale sanitario; test cutaneo alla tubercolina

## SUMMARY

**Background:** Health care workers are at risk for *Mycobacterium tuberculosis* (MTB) infection. **Objectives:** To perform an occupational health survey among 621 employees of a 800-bed third level care hospital covered by MTB surveillance. **Methods:** Statistical analysis was applied to results from tuberculin skin test (TST), QuantiFERON® -TB Gold in tube assay (QFT), PPD-ELISA for serum antibodies, and occupational or vaccine data. **Results:** 29.1% of subjects were TST positive, 18.5% were QFT positive. In 23% of subjects no correlation between these tests was found, presumably linked to BCG-vaccination, since TST positivity was 4 times higher among vaccinated subjects, whereas both tests correlated well in unvaccinated subjects. QFT values above 2 IU/ml were significantly associated with positive TST and age over 40 years. Working in MTB risk level 4 was significantly associated with QFT, TST, and PPD-antibody levels, suggesting booster effects by repeated exposure. No clear correlation was observed with medical specializations but significantly higher QFT positivity was found in subjects not assigned to the classical medical professions and originating from MTB high risk areas. **Conclusions:** These results shift the focus on maintenance personnel, who mostly worked in MTB risk level 2 areas. The less positive QFT results in vaccinated subjects highlight QFT's advantage as a screening tool and argue for a protective effect of the BCG-vaccine, although percentages of vaccinated persons varied largely between different medical professions. Interestingly, the percentage of QFT positive persons was lower among subjects reporting MTB exposure than those who were not aware of exposure events.

## RIASSUNTO

«**Immunosorveglianza tubercolare del personale sanitario in un ospedale di terzo livello**». **Background:** Il personale sanitario è a rischio di infezione da *Mycobacterium tuberculosis* (MTB). **Obiettivi:** Compilare un'indagine di Medicina del Lavoro su 621 lavoratori/trici sottoposti/e a sorveglianza per infezione tubercolare in un ospedale di terzo livello con 800 posti letto. **Metodi:** Si è eseguita l'analisi statistica dei risultati del test cutaneo alla tubercolina secondo Mantoux (TCT), del test QuantiFERON®-TB Gold in tube assay (QFT) e del test PPD-ELISA

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per la ricerca di anticorpi sierici e dei dati relativi alla categoria professionale di appartenenza e alla vaccinazione. **Risultati:** Il 29,1% dei soggetti mostra positività per TCT, il 18,5% per QFT. Nel 23% dei casi questi test non correlano, presumibilmente a causa della pregressa vaccinazione con BCG; infatti, mentre nel personale vaccinato la positività per TCT è quattro volte maggiore di quella per QFT, in quello non vaccinato vi è una buona correlazione tra i due test. Livelli di QFT al di sopra di 2 IU/ml sono significativamente associati a positività per TCT e ad età maggiore di 40 anni. Il lavoro in reparti con livello di rischio 4 di esposizione a MTB è associato significativamente sia a positività per QFT e TCT sia ad un aumentato livello di anticorpi anti-PPD, suggerendo l'ipotesi di un effetto booster dovuto ad esposizioni ripetute a MTB. Non si evidenzia nessuna chiara correlazione nell'ambito delle specializzazioni mediche, mentre positività di QFT significativamente più alta è presente nel personale non appartenente alle professioni sanitarie ma proveniente da aree ad alto rischio di tubercolosi. **Conclusioni:** I dati ottenuti fanno porre particolare attenzione al personale ausiliario che lavora in ambiente con di livello di rischio 2 di esposizione a MTB. Una minor positività di QFT nel personale vaccinato evidenzia il vantaggio di utilizzare QFT come test di screening e depone per un effetto protettivo del vaccino BCG, sebbene la percentuale di persone vaccinate differisca sensibilmente nell'ambito dei diversi profili professionali. Interessante notare che la percentuale di positività per QFT è minore tra il personale che riferisce un evento specifico di esposizione a MTC rispetto a quello non conscio di essere stato esposto.

#### Abbreviations

MTB:	<i>Mycobacterium tuberculosis</i>
LTBI:	latent tuberculosis infection
IGRA:	Interferon-gamma release assay
QFT:	QuantiFERON® - TB Gold (in tube) assay
IU/ml:	international units per millilitre
TST:	tuberculin skin test according to Mendel Mantoux
mm:	millimetre
PPD:	purified protein derivative/tuberculin from <i>MTB</i>
ELISA:	enzyme linked immunoassay
OD:	optical density
BCG:	Bacillus Calmette Guerin ( <i>MTB</i> vaccine strain)
p:	statistical significance

#### INTRODUCTION

The Italian Health Ministry (Ministro della Salute, Rome, Italy) and the Centers for Disease Control and Prevention, (CDC, Atlanta, USA) have provided guidelines for the control of nosocomial transmission of *Mycobacterium tuberculosis* (*MTB*) in health care workers (4, 13).

In these manuals the tuberculin skin test (TST) according to Mendel-Mantoux using intra-dermal application of purified protein derivative (PPD) from *MTB* is the method of choice. For employees in public facilities (e.g., health-care facilities, schools, and child-care facilities) where many susceptible persons are at risk due to the presence of

individuals with active *MTB*-infection, an increase in the PPD-provoked skin induration of greater than 10 mm are to be considered positive. In the meantime, also regarding the use of the relatively new interferon gamma release assays (IGRA) additional recommendations have been included, which are not considered definitive due to the lack of relevant information (5). Although diagnostic sensitivity of IGRAs was found higher compared to TST in numerous studies, it is still not high enough to rule out tuberculosis and in addition the reported specificities are rather variable. Thus further evaluations have to be made to reach definite conclusions about the overall predictive capacity of those tests (for review see 7, 19).

IGRAs are represented by the QuantiFERON® principle (Cellestis Inc.) which measures the interferon gamma released from patient lymphocytes in whole blood after incubation with defined *MTB* antigens. A second principle is applied by the respective ELISpot methods, which are more "open source" and can be used with different antigens. These tests measure the interferon-gamma which is released by activated lymphocytes and bound to antibody-coated membranes in cell culture plates. The latter tests seem to have some advantages in terms of sensitivity and perhaps also specificity if they are used to determine immune responses also in compartments other than blood (19). However,

they may be more complicated due to sampling and transport requirements as well as the need for purification and cell culture of (viable) patient lymphocytes.

In the Azienda Sanitaria di Bolzano, a third level care hospital with improved facilities serving an area of about 1 million people, a survey of *MTB* has therefore been ongoing for some time using a commercially available QuantiFERON® - TB Gold -in tube assay (QFT). This test was shown in a recent meta-analysis of the literature to be the first choice blood test for diagnosing latent tuberculosis infection (LTBI) (6, 7) and updated recommendations for its use and interpretation were published very recently (5, 15). TST is also still performed but this test is regarded as a less reliable marker for infection since BCG (Bacillus Calmette- Guerin) vaccination of health care personnel was used up to the year 2000 in the above facility.

One of the aims of this study was an investigation of *MTB* exposure of the different groups of health care professionals. An occupational classification was set in place as well as evaluation of the likelihood of *MTB* exposure within the different medical disciplines involved. The data obtained since introduction of QFT testing were statistically analyzed and additionally also testing for serum antibodies against PPD (tuberculin) as a possible marker for repeated *MTB* exposure was performed in a student project. The questions the project proposed to answer included: agreement of QFT data with TST results, meaning of the interferon gamma titer of the QFT results, influence of age, vaccination status, records of exposure and investigation of association with different risk groups and/or medical professions.

## METHODS

### Study population

Data on 621 out of about 3000 employees of the Hospital (Azienda Sanitaria di Bolzano) including students and subcontracted maintenance staff with available QFT results were collected and evaluated

for different parameters. These included age, sex, occupation, the clinical department and its risk classification as well as the BCG vaccine status of the subject. TST results (in mm) were also included with an induration of more than 10 mm considered as positive. Previous *MTB* exposure records were also considered as well as available medical records of the occupational health physician such as X-ray results or chemoprophylaxis. It was also verified whether a subject originating from an area of high risk for *MTB*, *i.e.* countries with an incidences of more than 40 per 100000 as reported to the World Health Organization (WHO).

The medical professions were sub-grouped in:

- (1) doctors
- (2) nurses
- (3) specialized supporting health care providers
- (4) medical students, health assistants, psychologists
- (5) physical therapists, dietary assistants, speech therapists and occupational therapists
- (6) biologists and medical technical assistants
- (7) others - these included all other personnel present in the hospital environment who it was not possible to assign to any of the above listed occupations (e.g. cleaning and maintenance staff, technicians, etc. The priest was also included here)

The groups were set up not only by grouping related professions but also according to the intensity of close patient contact, which was considered to be less in students and psychologists than, for instance, in physical therapists.

### Evaluation of *MTB* exposure

In the "individual surveillance programme for health care personnel 2006/2007" of the Hospital the operative units (UO) were rated according to their estimated exposure risk at 5 levels. These ratings were mainly based on the number of patients diagnosed with *MTB* disease in the respective medical disciplines since the preceding year, also considering possible increasing incidences in certain units. According to the estimated risk level appropriate measures of surveillance were set in place (18).

Risk level 5 - UO in which the *MTB* incidence was significantly higher than in the previous years. This situation was not observed in the covered study time.

Risk level 4 - UO in which a risk of contact with more than 6 *MTB* patients per year exists. This included the infectious diseases department, the pneumology department, the pneumological service of the Hospital, the microbiology laboratory and the pathology department.

Risk level 3 - departments with less than 6 *MTB* patients in the previous year. The respective departments were: geriatrics, orthopaedics, nephrology, internal medicine, surgery, urology, ear, nose and throat department and the intensive care unit.

Surveillance measures for the above risk groups 3 and 4: annual QFT testing.

Risk level 2 - conditions in which no contact with a *MTB* patient has been observed. This includes all other medical disciplines.

Surveillance measures: QFT only at the start of employment and where there is a suspicion or signs and symptoms of *MTB* infection.

Risk level 1 - this would be the risk for the general population not working in a hospital environment. As the present study was restricted to people working in the hospital no-one was assigned to this risk group.

### **Mendel Mantoux tuberculin skin test (TST)**

Despite its cross reactivity with BCG vaccine strain and several other non-tuberculous mycobacteria, this method is still the approved standard test for *MTB* screening. It tests the cellular immune reaction against PPD (tuberculin) in the skin by applying 5 IU of PPD intradermally and subsequent measuring skin induration in mm. As recommended, a diameter of skin induration of more than 10 mm is considered a positive test result.

### **QuantiFERON® - TB Gold in tube assay (QFT)**

The commercially available QFT uses whole blood as specimen which is stimulated by incubation with defined *MTB* antigens like ESAT-6 or CFP-10. This ensures an increased specificity for

germs of the *MTB*-complex as those antigens are missing in the BCG vaccine strain or *M. avium* and several other atypical mycobacteria. After stimulation in whole blood the amount of interferon-gamma produced by reactive T-cells is measured in the plasma by an enzyme linked immuno-sorbent assay (ELISA). As a control reaction, mitogen stimulation is used to evaluate the overall lymphocyte reactivity, e.g., of immuno-suppressed patients. This test was performed, validated and interpreted according to the prescriptions of the manufacturer (Cellestis Inc.). As suggested by the supplier, the quantity of interferon-gamma of 0.35 units per ml (IU/ml) was used as the "cut-off" value for a positive test result.

### **ELISA testing for serum antibodies against PPD (tuberculin)**

This was done with a home-made enzyme linked immunoassay. Purified protein derivative (PPD) form *MTB* was obtained from the Institute of Animal Health, Pirbright, UK. Dilutions of PPD in 0.1M NaHCO<sub>3</sub> pH: 9.6 were coated in 96-well polystyrene plates with high binding capacity (Maxisorp®, Nunc, Denmark) with a total amount of 100pg/well overnight at 4°C. Thereafter unspecific binding sites were saturated for one hour at room temperature with phosphate buffered saline (PBS, pH: 7.4) containing 1% bovine serum albumin (SIGMA-Aldrich). Serial dilutions of test sera were made in PBS-1% BSA containing 0.05% of detergent Tween-20 and the dilutions incubated for another 2 hours at room temperature under shaking conditions. Plates then were washed 3 times with PBS/0.05% Tween-20 with an automated plate washer (Multiwash®, Labsystems, Finland). Thereafter a horse radish peroxidase-labelled secondary antibody (rabbit anti-human, DAKO, Denmark) was used for another 2 hours at a dilution of 1:5000 in the same buffer/conditions. After the final wash (4x) the plates were developed with o-Phenylenediamine Dihydrochloride (OPD, Sigmafaste™ - SIGMA-Aldrich) and the colour reaction measured at a wavelength of 450 nm in a photometric plate reader (Multiskan®, Labsystems, Finland). From the obtained optical density (OD)



the respective background values (i.e., identical test setup without PPD antigen) were subtracted and the means of the summarized OD differences obtained. Although cross reactivity is expected due to the use of PPD and such antibody tests certainly are not useable in routine MTB diagnostics, this test gives an overall picture of the humoral immune response in addition to the above listed cellular immune response tests.

### Statistical analysis

All available data were included in a database using the freely available epidemiological diagnostic tool Epi-Info™ Version 3.4.3 ([www.cdc.gov/epiinfo/](http://www.cdc.gov/epiinfo/)) using parametric as well as non-parametric measurements of the above indicated variables. Statistical significance was calculated by different methods including Student's T-test,  $\chi^2$  (chi-square) and Fisher's Exact test. "p"-values lower than 0.05 were considered as statistically significant. Forma-

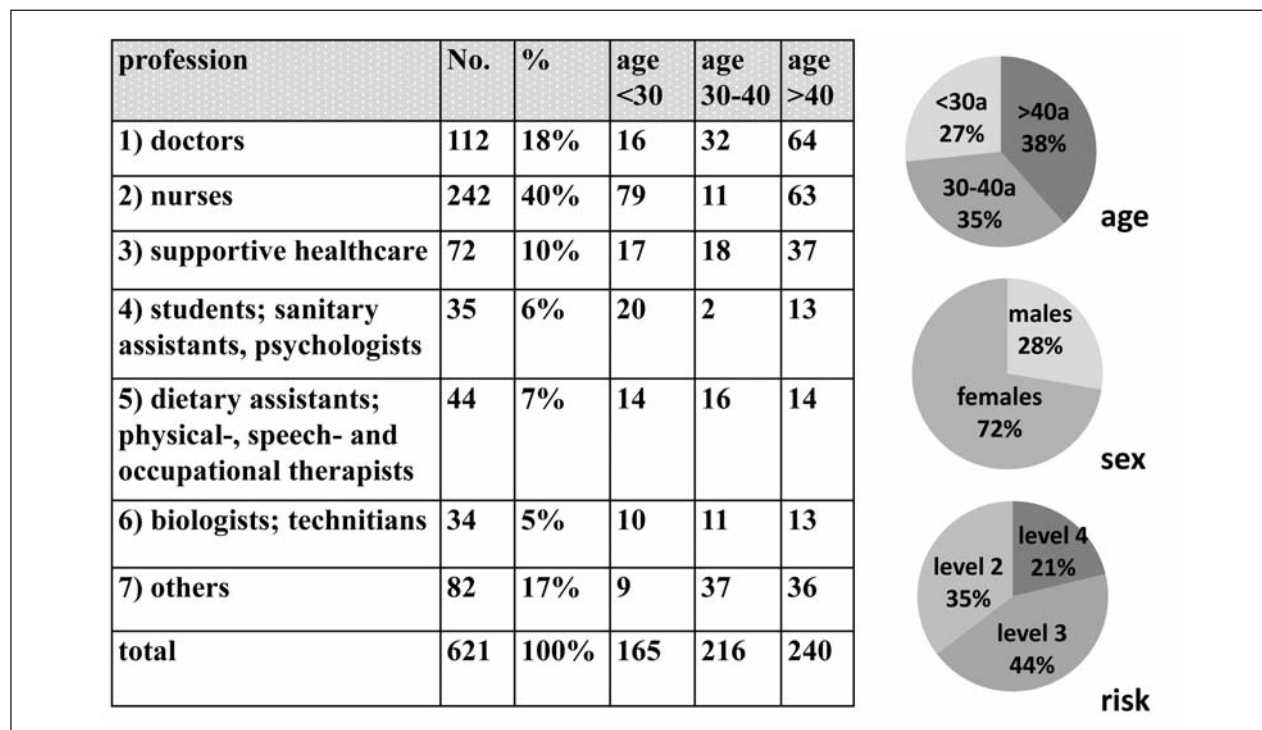
tion of contingency tables and calculation of odds ratios were performed by the computer programme Prism 5.0. (Graphpad Software Inc).

## RESULTS

### Description of the study population

The mean age of the test population was 38.3 years (19-64). The distribution of age groups is shown in Figure 1 - upper part. More than two thirds (72.3 %) were females, which emphasizes the preponderance of females in care giving. A total of 57 persons (9.2%) originated from areas with enhanced *MTB* risk, including Romania, Peru, Georgia, India, Thailand, Panama and Morocco.

The distribution of professions within the tested population was as shown in Figure 1 - centre part. Doctors (Group 1) and nurses (Group 2) formed the biggest groups followed by group 7 ("others")



**Figure 1** - Statistics of the study population. The percentage proportion of the different professions in the study population (No.=621) is shown on the left side. The age distribution (less than 30, 30 to 40 and above 40 years) of the tested health care employees covered by the MTB surveillance and the percentage of subjects assigned to the prevalent risk levels is shown on the right side

not assigned to the classical health care professions and Group 3 (auxiliary health care personnel).

Concerning the risk levels for possible *MTB* exposure, 21% of the test subjects were in risk group 4, 44% were in risk group 3 and 35% were in risk group 2 (Figure 1).

### Vaccine status

A total of 236 individuals (38% of the test population) had a record of vaccination with the BCG vaccine, which was administered between 1967 and 2000. Vaccine coverage varied between the different professions, with the highest numbers observed among physical therapists, dietary assistants, speech therapists and occupational therapists (group 5). Group 7 (others), which included all job profiles not assigned to any of the classical medical professions, had a significantly lower vaccination rate compared to the average (Table 1). The latter group also contained more than 50% of the personnel of foreign origin from *MTB* high risk areas; 2/3 of these were employed at risk level 2 work only, and nearly all of them were unvaccinated.

### MTB test results

Of the 612 subjects with available QFT results 115 (18.5%) had a positive QFT test.

The mean age of the quantiferon positives was significantly higher than the mean age of the QFT negatives (44 versus 37 years), indicating an age-dependent risk of contracting *MTB* infection.

In 549 subjects TST results were also available with 160 (29.1%) of these Mantoux positive. A positive QFT test correlated with TST results in 77%, but in 127 (23%) of the tested subjects no agreement between TST and QFT was seen; 10.4% had a positive result in both tests (Table 2).

In 81 subjects both the QFT titer and information on the diameter of the induration of the TST test were available. We found no clear correlation of high QFT interferon gamma readouts with risk groups or certain professions. However QFT values above 2 IU/ml were significantly associated with TST positivity and subjects over 40 years of age had significantly more frequent QFT titers above 2 IU/ml (53/14) than subjects under 40 years (20/27;  $p=0.0001$ ) (Table 3).

### Correlation of QFT with profession, MTB risk level

Analyzing the different groups of medical professionals, association with QFT in most cases was not significant (n.s.). Interestingly Group 5 consisting of physiotherapists, dietary assistants, speech therapists and occupational therapists had a lower frequency of positive QFT results, whereas Group 7 (others) had a much higher rate compared to the average (Table 4).

Regarding the *MTB* risk levels, we found an elevated rate of QFT and TST positivity associated with work at risk level 4 (Table 5)

**Table 1** - Professional profile and vaccine coverage

Profession	Vaccinated - y/n (%)	Odds ratio	Significance
(1) doctors	41/71 (37%)	0.941	n.s.
(2) nurses	104/138 (43%)	1.229	n.s.
(3) supporting health care	23/41 (36%)	0.915	n.s.
(4) students; health assistants, psychologists	7/28 (20%)	0.408	$p=0.0214$
(5) dietary assistants; physical-, speech- and occupational therapists	29/15 (66%)	2.175	$p=0.0003$
(6) biologists; technicians	16/18 (47%)	1.45	n.s.
(7) others	16/74 (18%)	0.353	$p<0.0001$
Total	236/385 (38%)	-	-

Numbers and percentage of BCG vaccinated people in the different groups. Odds ratio and statistical significance of the vaccine coverage compared to average is shown.  $p<0.05$  is regarded as statistically significant. n.s.=not significant

**Table 2** - Correlation of QFT positivity with TST positivity and age

Parameter	QFT pos.	QFT neg.	Odds ratio	Significance
TST 0-4 mm	7	271	-	-
TST 5-9 mm	17	94	7.002	p<0.0001
TST >10 mm	57	103	24.424	p<0.0001

Parameter	QFT pos.	QFT neg.	Odds ratio	Significance
Age < 30a	11	154	-	-
Age 31-40a	37	179	2.894	p=0.0028
Age >40a	67	173	5.422	p<0.0001

Numbers of positive (>0,35 IU/ml) and negative QuantiFERON®-TB Gold in tube (QFT) test results are compared with the documented diameter (in mm) of skin indurations observed in the tuberculin skin test (TST). The highlighted cells indicate the discordant test results between both tests (No.=127; 23%) of the persons in whom both parameters were available (No.=549). The distribution of QFT test results in different age groups is shown in the lower part. Odds ratio and statistical significances compared to the distribution in the youngest age group are shown

**Table 3** - Correlation of elevated QFT titers with TST positivity and age

Parameter	TST >10 mm	TST <10 mm	Odds ratio	Significance
QFT <2 IU/ml	13	13	-	-
QFT 2 -10 IU/ml	29	9	3.222	p=0.0359
QFT >10 IU/ml	15	2	7.5	p=0.0202

Parameter	Age < 30 y	Age 31-40 y	Age > 40y	Significance
QFT <2 IU/ml	6	21	14	-
QFT 2 -10 IU/ml	4	10	33	p=0.0753
QFT >10 IU/ml	1	5	20	p=0.0397

QuantiFERON®-TB Gold in tube (QFT) titers >2 IU/ml showed a positive correlation with a positive (induration >10 mm) tuberculosis skin test (TST). In addition, a higher prevalence of more elevated QFT titres was also detected in the older age group. Statistical significances between the two TST groups and comparison of the oldest age group with the distribution in the youngest age group are shown. p<0.05 is regarded as statistically significant

### Correlation of QFT and TST with vaccination status or proven exposure

Of the 236 persons with a record of BCG vaccination, 50.3% had a positive TST result, whereas only 9.3% were QFT positive. In the 385 persons with no available record of BCG vaccination both tests were equally distributed.

As expected, the relative QFT positivity was lower in the BCG vaccinated (20/216) than in the unvaccinated group (95/290). Using TST, less impressive differences were seen: (78/157 in vaccinated versus 81/232 in unvaccinated).

The origin of an area of high *MTB* incidence

was strongly associated with positive QFT results, as was the record of an X-ray investigation by the occupational health service. Only 4 subjects had a clear positive X-ray result, all of whom were also QFT positive. It is interesting to note that a documented record of *MTB* exposure in the hospital was not associated with a higher probability of QFT positivity (Table 6).

### Anti PPD antibodies

The amount of antibodies against *MTB* found in sera of subjects working in risk level 4 was significantly higher than of those in risk level 2. Fig-

**Table 4** - Distribution of QFT positivity within medical professions

Profession	QFT pos./neg. (%)	Odds ratio	Significance
(1) doctors	27/85 (24.1%)	1.397	p=0.0635
(2) nurses	39/203 (16.1%)	0.845	n.s.
(3) supporting health care	8/56 (12.5%)	0.628	n.s.
(4) students, health assistants, psychologists	4/31 (11.4%)	0.568	n.s.
(5) dietary assistants; physical-, speech- and occupational therapists	3/41 (6.8%)	0.322	p=0.0225
(6) biologists, technicians	7/27 (20.6%)	1.14	n.s.
(7) others	27/63 (30%)	1.885	p=0.0028
Total	115/505 (18.5%)	-	-

Numbers and percentage of positive (cut off=0.35 IU/ml) and negative test results of the QuantiFERON®-TB Gold-in tube assay (QFT) are shown for the different professions. Odds ratio and statistical significance compared to the average is shown (p<0.05 is regarded as statistically significant, n.s.=not significant)

**Table 5** - Association of MTB risk levels and positivity for QFT, TST and vaccination status

MTB risk level	QFT pos. (%)	TST pos. (%)	Vaccinated (%)
4 - infectious disease, pneumological service, microbiology, pathology	37/96 (28%) OR=1.624 p=0.041	51/82 (41%) OR=2.962 p<0.0001	63/70 (47%) OR=2.132 p=0.0009
3 - geriatrics, orthopaedics, nephrology, internal medicine, surgery, urology, ear, nose&throat, intensive care	36/233 (13%) OR=0.651 p=0.0536	71/198 (29%) OR=1.708 p=0.032	108/161 (40%) OR=1.589 p=0.0175
2 - all other disciplines	42/177 (19%)	38/181 (21%)	65/154 (30%)

The numbers (positive/negative) and percentage of positive test results of the QuantiFERON®-TB Gold -in tube assay (QFT, >0.35 IU/ml) and tuberculosis skin test (TST, induration >10 mm) are shown for the defined risk groups. The vaccine coverage with the BSG-vaccine among the 3 groups is also shown. Odds ratio (OR) and statistical significance of positive test results and vaccine status as compared to the lowest risk group 2 is shown (p<0.05 is regarded statistically significant)

ure 2 depicts the difference of optical density (OD) of the mean ELISA values of sera from subjects working in risk level 4 and risk level 2. Unpaired t-test revealed significance (p=0.0093).

## DISCUSSION

We performed a survey of *MTB* in defined groups of health care workers in a third level care hospital in northern Italy. The rather special situation in this group is that 38% of these subjects had been vaccinated with BCG vaccine up to the year

2000 upon entry into employment in the hospital. The relatively high vaccine coverage is an explanation for the rate of tuberculin skin test positivity being four times that of QFT in the vaccinated people whereas comparable values were found in the unvaccinated group with both tests. This indicates a higher specificity of the Quantiferon assay in vaccinated populations which has been proven by a series of recent publications (9, 12, 17, 19).

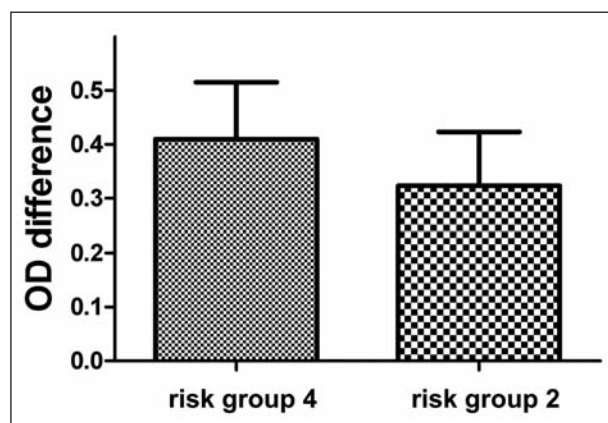
The combination of QFT positive and TST negative test results was 5 times less frequent than the combination of TST positive and QFT negative. Even if there is no "gold standard" for early



**Table 6** - Parameters with significant association with QFT and TST positivity or negativity

Tested variable (y/n)	QFT pos. (%)	QFT neg. (%)	Odds ratio	Significance
Record of exposure	18/97 (16%)	138/349 (31%)	0.469	p=0.0004
High risk area origin	23/92 (20%)	34/472 (7%)	3.47	p<0.0001
BCG vaccination	20/95 (17%)	216/290 (43%)	0.283	p<0.0001
X-ray performed	81/34 (70%)	89/417 (18%)	11.162	p<0.0001
Positive X-ray	4/77 (5%)	0/89 (n.a.)	n.a.	p=0.0495
Drug treatment	9/106 (5.6%)	15/491 (3%)	2.779	p=0.0205
Tested variable (y/n)	TST pos. (%)	TST neg. (%)	Odds ratio	Significance
Record of exposure	48/111 (30%)	94/291 (24%)	1.339	n.s.
High risk area origin	20/140 (14%)	23/366 (6%)	2.273	p=0.0091
BCG vaccination	79/81 (49%)	157/232 (40%)	1.441	n.s.
X-ray performed	99/61 (62%)	44/345 (11%)	12.725	p<0.0001

Numbers and percentage of positive (>0.35 IU/ml) and negative test results of the QuantiFERON®-TB Gold -in tube assay (QFT) and tuberculosis skin test (TST, cut off=10 mm skin induration) are shown for selected variables leading to significant results. The first number represents the existence of the variable, the second its absence (“y” and “n” signify yes and no). Increased or decreased prevalence of a positive test result in the presence of the tested variable are expressed by odds ratios. Statistical significance is shown (p<0.05 is regarded statistically significant, n.s.=not significant)



**Figure 2** - Different antibody titres against PPD according to risk level. The difference in optical density (OD) of the mean PPD-ELISA values of sera from subjects working in risk level 4 (left column) and risk level 2 (right column) is depicted. Error bars indicate the standard error of the mean (SEM). Unpaired T-test revealed a significant (p=0,0093) difference between the two groups

detection of LTBI this suggests that the negative predictive value of QFT is higher than the TST, which is also supported by the majority of the most recent papers indicating a higher specificity of the IGRA (1, 7, 16, 20).

Radiological examination is recommended to exclude active lung disease in the presence of LTBI. Of the 170 subjects who had to undergo X-ray examination only 4 had positive X-ray results, all 4 were also positive in the QFT; 24 subjects received treatment for LTBI, and one third of them tested QFT positive.

Although there was a discordant result between QFT and TST in 23% of the test results, which seems relatively low compared to other studies (11, 14, 17), the QFT in summary proved more suitable for detection of *MTB* infection in the hospital environment as it is not hampered by immunity caused by infections with non-tuberculous mycobacteria or a vaccination background. Given the relatively high local vaccine coverage in the older age groups and certain professional subgroups this justifies the higher costs of the QFT test as compared to TST testing only.

Concerning the BCG vaccine coverage, we found remarkable differences between the different groups with physical therapists and occupational therapists having a much higher rate of vaccination as compared to the average. This could also explain the significantly lower rate of QFT positives in this group. Also the significantly lower overall rate

of QFT positive results in the whole vaccinated group would support a protective effect of the BCG vaccine which, presumably due to genetic differences of the vaccine preparations used, has been described as between 80% and nil in different studies (3). But it also has to be considered that a much higher rate of QFT positivity was found in subjects of foreign origin from areas with high risk for *MTB* infection and nearly all of these subjects were unvaccinated. Although according to the WHO definitions, an incidence of *MTB* infection above 40/100000 is regarded as high risk, many of the countries of origin of the tested subjects had incidences well above 100/100000 whereas recently reported data for Italy were 7.5/100000 (8). Analyzing occupational status, the subjects of foreign origin were found predominately in group 7, i.e., workers not assigned to any of the listed medical professions, who are focused on auxiliary, maintenance and cleaning personnel. The fact that most of them worked in areas rated as risk level 2 could be a possible explanation for the finding that the overall rate of QFT positivity was higher in risk level 2 than in risk level 3.

As could be expected, the rate of QFT positivity was significantly higher among people working in risk level 4 disciplines and also elevated mean antibody levels against PPD were found in risk level 4. This could reflect a booster of the immune response by repeated *MTB* exposure (2) as well as possible booster effects by repeated routine testing with intradermal application of PPD (tuberculin) (10).

Regarding the high titers of QFT there was no clear association between the elevated interferon gamma level and risk groups or certain professions. However QFT values above 2 IU/ml significantly correlated with TST values above 5 mm, which would argue against recently published concerns that the QFT might not be sufficiently sensitive (14).

Also, age over 40 years was significantly associated with higher QFT values and more than 50% of the positive QFT results were found in this age group.

Interestingly, subjects with a documented record of *MTB* exposure in the hospital had a lower rate

of positive QFT test results compared to subjects where no such record was listed. Even if self-reporting of exposure at the workplace seems to be a very unreliable marker, and presumably many likely exposure events are not reported to the occupational health service, such data could serve as additional indirect markers reflecting exposure. Thus we can affirm that our results suggest that subjects who did not feel exposed or did not report an exposure had a higher likelihood of being QFT positive than subjects who were aware of the risk.

NO POTENTIAL CONFLICT OF INTEREST RELEVANT TO THIS ARTICLE WAS REPORTED

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