

QuantiFERON-TB Gold Plus evaluation for latent tuberculosis infection among Italian healthcare workers: a cross-sectional study

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ABSTRACT

Background: healthcare workers (HCWs) are at higher risk of being exposed to tuberculosis (TB). The aim of this study was to estimate LTBI prevalence and evaluate associated risk factors among Italian HCWs using a new generation IGRA test, QuantiFERON Plus (QTF-Plus), providing also an analytical evaluation of this test.

Methods: this cross-sectional study was conducted during the annual health surveillance program performed in 2017, evaluating 1470 HCWs of a teaching hospital in Rome, tested with QTF-Plus. A standardized questionnaire was collected for multivariate risk analysis. The independent variables associated with a higher frequency of LTBI were tested in a multivariate logistic regression model.

Results: LTBI was diagnosed in 50 out of 1470 (3.4%) of HCWs. The male gender and age >40 years were significantly associated with the risk of LTBI. The levels of interferon- γ were significantly lower in subjects with a recent conversion than in those with an older positivity. Moreover, interferon- γ values exceeding 8 IU/ml were recorded in 8 out of 35 remote subjects but in no recent subjects. The concordance between TB1 and TB2 results was 88%, while in 6 out of 50 subjects we found a discrepancy.

Conclusion: the LTBI condition among Italian HCWs was very low. In subjects with discordant outcomes between TB1 and TB2, especially when the test values are close to the cut-off, is recommended a repetition of the test to confirm its positivity.

Key words: tuberculosis, latent tuberculosis infection, interferon-gamma release assay, quantiferon plus, healthcare workers

INTRODUCTION

Healthcare workers (HCWs) are at higher risk of being exposed to tuberculosis (TB) compared to the general population [1]. In these workers, the assessment of latent (LTBI) and active (TBA) tuberculosis is part of the annual health surveillance program aimed to prevent occupational diseases [2].

Various diagnostic protocols, mostly based on the tuberculin skin test (TST) have been proposed to detect LTBI among HCWs. Recently, the joint guidelines of the American Thoracic Society / Infectious Diseases Society of America and Center for Disease Control and Prevention recommended the use of interferon-gamma release assay (IGRA) rather than TST, for the screening of LTBI in HCWs [3]. QuantiFERON-TB Gold Plus (QFT-Plus) is a new generation IGRA test available since 2015. The test includes two tubes: TB1 and TB2. TB1 contains tubercular antigens (ESAT-6 and CPT-10) designed to elicit the response of CD4 T-lymphocytes, while the TB2 tube contains, in addition, a set of short peptides that stimulate a CD8 T-lymphocyte response. This implementation would result in an improved sensitivity and specificity compared to the previous generation test (Quantiferon Gold in tube - GIT), in the identification of LTBI [4 - 10]. Moreover, difference in response between the TB1 and TB2 tubes can be used as a marker of CD8 response and/or of a recent exposure.

Only few studies are currently available in Italy about the prevalence of LTBI in a hospital setting, and none based on QFT-Plus. Given the higher frequency of reactivation of LTBI, in HCWs, knowledge of the prevalence of this condition in this population represents a crucial information.

Furthermore, recent studies show that an analytical evaluation can add useful information to assess the risk of TB progression in LTBI patients [12]: indeed, some authors observed that the discrepancy between TB1 and TB2 responses can help to identify a recent contact / infection (and therefore a population with a higher risk of progression) and that the values of interferon γ (INF- γ) both in TB1 and in TB2 are significantly different in LTBI subjects compared to TBA subjects [5, 12]. In fact, a high lymphocyte response is expected in subjects with LTBI, based on the antigenic stimulation due to the persistence of the vital mycobacterium. These suggestions, however, need confirmation. In our study we performed an analytical evaluation to compare the immunological response in recent LTBI subject and workers with old infection using the new IGRA test in order to estimate the prevalence of this subclinical condition among Italian healthcare workers.

METHODS

Study design, setting and subjects

A cross-sectional study, using routine demographic,

clinical, and laboratory data, was performed. The health workers in the study (doctors, nurses, technicians and other operators employed in the various hospital departments) were evaluated during the annual health surveillance program performed from January to December 2017. The prevalence of the LTBI was retrospectively evaluated by analyzing the results of the QFT-Plus tests performed in the HCWs of a teaching hospital in Rome. The tests results were classified according to the interpretative guidelines provided by the manufacturer. Tests were classified as "positive" or "negative", respectively, if higher or lower response than the cut-off value of 0.35 IU / ml of INF- γ , was detected in at least one test tube (TB1 or TB2). All the workers with a "positive" result had to perform diagnostic tests to exclude active tuberculosis such as chest X-ray, blood chemistry. The final medical evaluation was done by a specialist in infectious diseases. A subject was diagnosed as affected by LTBI in the case of positivity to the QFT-Plus test in the absence of clinical-radiological and serological signs of active infection. On the basis of the results of the previous IGRA test LTBI subjects were classified as "recent LTBI" (those with a negative previous test) or "remote LTBI" (those with a previous positive test).

Data collection

For all subjects, we collected information on gender, age, nationality, worker seniority, department and task assignment and any previous documented professional contact with TBA patients. The employment departments were classified according to the exposure risk, based on the guidelines of the Italian Ministry of Health [2] which defines five categories of incremental risk (A;B;C;D;E); the category D and E areas were considered at higher risk of transmission.

Data analysis

The prevalence of the LTBI condition was posed in relation to the main demographic, occupational and exposure characteristics. Data were analyzed using the statistical software Stata (Release 11). A p-value of < 0.05 was taken as the level of statistical significance. The inference of the analyzed factors was expressed as OR and respective 95% confidence intervals (95% C.I.) and p-value. We also tested the correlation test between TB1 and TB2 using the non-parametric Spearman test and performed a comparative analytical evaluation of the QFT-Plus tests in LTBI subjects in relation to the "recent" or "remote" LTBI status means of Student's t-Test.

RESULTS

The study involved 1470 healthcare workers. Table 1 shows the main characteristics of the study population. LTBI was diagnosed in 50 out of 1470 (3.4%) of the workers enrolled in the study. It was more common in males than in females (24 out of 484: 5.0% vs 26 out of 986: 2.6%, respectively; $p < 0.01$). In 15 cases the prior test result was negative (recent LTBI), whereas the remaining 35 cases had a previous positive test (remote LTBI). The outcome of the test was also stratified in relation to the main demographic, exposure and employment characteristics (table 2). The independent variables associated with a higher frequency of LTBI were tested in a multivariate logistic regression model showing that only the male gender and an age > 40 years remained statistically significant (table 3). Regarding the analytical evaluation of the test, there was a high rate of concordance between TB1 and TB2 results (88%). The tests showed a discrepancy in 6 out of 50 subjects. Most of these subjects, (5 out of 6), showed a reversion of the test result in the following year (i.e. they tested negative in both tubes at the follow-up). The quantitative analysis of the TB1 and TB2 response showed levels of interferon γ (INF- γ) significantly lower in subjects with a "recent" conversion than in those with an older ("remote") positivity (t test, $p < 0.01$). In particular, outcomes exceeding 8 IU / ml were recorded in 8 out of 35 "remote" subjects but in no "recent" subjects (Figure 1).

DISCUSSION

In Italy, the rate of TB in the general population is 7.2 / 100000 according to the Italian Institute of Health [11] but the prevalence of LTBI among the general population has not been established yet. Moreover, sparse information is available on the rate of LTBI infection in HCWs in Italy, due to the low number of studies, the methodological problems associated with the different diagnostic methods used, and in particular to the challenges of interpreting TST results in a population that is widely immunized for BCG. In the present study, we evaluated the rate of LTBI infection among HCWs using the IGRA test, a novel in vitro IFN- γ plus assay [8 - 10]. This method has been developed to improve the accuracy of the TB testing and to overcome some of the limitations of the previous generation' tests. In previous studies, QFP Plus was more sensitive than the QFT GIT in the diagnosis of latent tuberculosis, although the agreement between the two tests was estimated to be good [12 - 13]. To our knowledge, this is the first study performed in a European country that systematically evaluates the prevalence of the LTBI using the QFT Plus test as recommended by the CDC / ATS 2017 guidelines [3]. In our study, the only factors associated with LTBI are the

TABLE 1. Main characteristics of the study population

| VARIABLES | N | % |
|--|--------------|------|
| Subjects | 1470 | 100 |
| Mean (SD) age, year | 40.98 (0.49) | |
| Age | | |
| ≤40 years old | 743 | 50,5 |
| >40 years old | 727 | 49,5 |
| Sex | | |
| Male | 484 | 33 |
| Female | 986 | 67 |
| Born in a high incidence country* | | |
| No | 1435 | 97,7 |
| Yes | 35 | 2,3 |
| Seniority | | |
| ≤10 years | 430 | 29,2 |
| >10 years | 1040 | 70,8 |
| Job | | |
| Nurse | 660 | 44,9 |
| Medical Doctor | 561 | 38,2 |
| Laboratory Staff | 127 | 8,6 |
| Other Technical Staff | 48 | 3,3 |
| Dentist | 11 | 0,7 |
| Others | 63 | 4,3 |
| Working Area§ | | |
| Low-Average risk (groups A,B,C) | 1333 | 90,7 |
| High risk (groups D,E) | 137 | 9,3 |
| Reported exposure to active TB | | |
| Yes | 54 | 3,7 |
| No | 1416 | 96,3 |
| IGRA Test Outcomes | | |
| Negative | 1420 | 96,6 |
| Positive | 50 | 3,4 |

* High Incidence : ≥ 20 cases per 100,000 population

§ *Prevenzione della tubercolosi negli operatori sanitari e soggetti ad essi equiparati. Ministero della Salute 2013 (Tuberculosis prevention in healthcare workers and similar. Italian Ministry of Public Health 2013)*

male gender and being older than 40 years, while the origin from high-endemic countries, the job assignment, the seniority and working in high-risk environments were not statistically associated with LTBI. In 2013, Durando et al. in a TST based study performed in Italy found an association between coming from high TB endemic nations and LTBI in medical students [14]. Similar findings were reported in a QFT Plus test performed in Germany

TABLE 2. IGRA Test Outcomes by the main characteristic of the study population

| VARIABLES | POSITIVE IGRA | % | P VALUE |
|--|---------------|------|---------|
| Sex | | | |
| Male | 24/484 | 5,0 | <0,05 |
| Female | 26/986 | 2,6 | |
| Age | | | |
| ≤40 years old | 11/743 | 1,5 | <0,001 |
| >40 years old | 39/727 | 5,4 | |
| Born in a high incidence country* | | | |
| No | 49/1435 | 3,4 | NS |
| Yes | 1/35 | 2,9 | |
| Seniority | | | |
| ≤10 years | 4/430 | 0,9 | <0,001 |
| >10 years | 46/1040 | 4,4 | |
| Job | | | |
| Nurse | 28/660 | 4,2 | NS |
| Medical Doctor | 16/561 | 2,9 | |
| Laboratory Technician | 3/127 | 2,4 | |
| Other Technical Staff | 1/48 | 2,0 | |
| Dentist | 0/11 | 0,0 | |
| Others | 2/63 | 3,17 | |
| Working Area§ | | | |
| Low-Average risk (groups A,B,C) | 44/1333 | 3,3 | NS |
| High risk (groups D,E) | 6/137 | 4,4 | |
| Reported exposure to active TB | | | |
| Yes | 4/54 | 7,41 | NS |
| No | 46/1416 | 3,2 | |

* High Incidence : ≥20 cases per 100,000 population
 § Prevenzione della tubercolosi negli operatori sanitari e soggetti ad essi equiparati. Ministero della Salute 2013 (Tuberculosis prevention in healthcare workers and similar. Italian Ministry of Public Health 2013)

[15]. In our opinion, the survey methodology (TST was used in one study) and the low age of the population in both studies could justify the difference with our study. In addition, in some European countries, including Germany, the vaccination with BCG is routinely carried out in the pediatric age, while in Italy it is not.

In a study performed in South Korea in 2017 on 626 HCWs, the prevalence of latent infection diagnosed by QFT Plus test was similar to ours [16].

The hospital where this study was performed carries out a tuberculosis annual surveillance program since 2006. This prevention program allows, in the suspected or diagnosed cases, to early identify and adequately

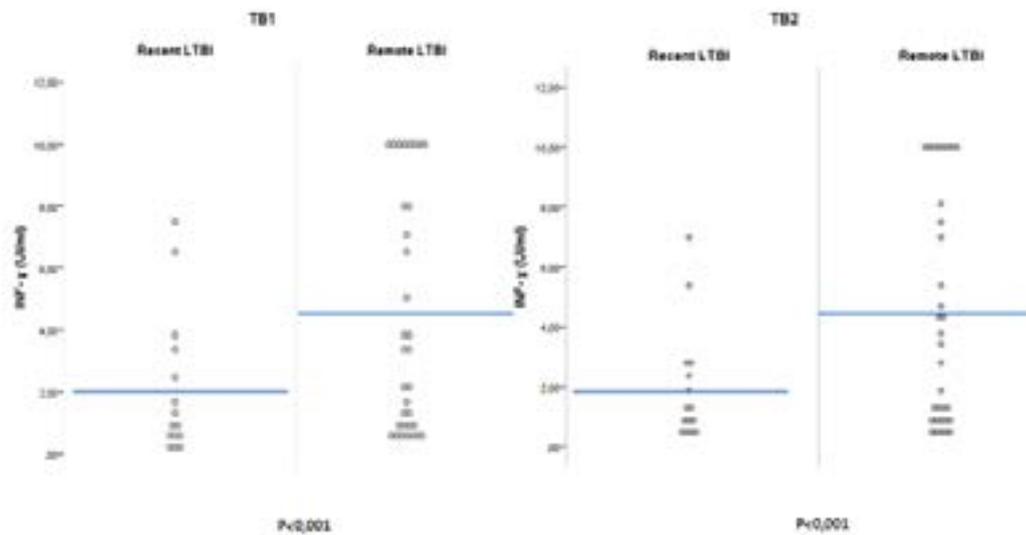
TABLE 3. Risk Factors for LTBI in the study population (multivariate analysis)

| VARIABLES | O.R. | 95% C.I. | P VALUE |
|-----------------------------|------|-------------|---------|
| Male | 1,91 | (1,05-3,49) | <0,05 |
| Age>40 years old | 2,5 | (1,10-5,69) | <0,05 |
| Seniority>10years | 2,42 | (0,68-8,60) | NS |

O.R. odd ratio
 95% CI: confidence interval at 95%

isolate (in rooms with negative pressure) subjects that are at higher risk of transmission. The effective implementation of this procedure would justify, in our opinion, the poor correlation of the LTBI with the seniority, with the job task and with the setting of employment. The foregoing points out the effectiveness of the implementation of procedural and environmental measures, in reducing the risk of nosocomial transmission of tuberculosis. Regarding the analytical evaluation of the test, the response in TB1 and TB2 (IU / ml) was significantly higher in the "remote LTBI" compared to the "recent LTBI" and was statistically associated with the years since the first diagnosis. In recent studies, it has been found that the production of INF- γ in the IGRA test, in subjects with LTBI, is on average higher than in subjects with active TB and treated TB. This finding suggests that the containment of the infection is the result of a better immune response to tuberculosis with respect to what occurs in subjects who develop an active form [10, 12]. Another possible hypothesis is that the prolonged antigenic stimulation due to the persistence of the infection in the latency phase is able to induce a persistent activation of the CD4 [17 - 18]. This would justify the results of our study showing a high production of INF- γ both in TB1 and in TB2 (both containing peptides capable of evaluating the CD4 response) in "remote LTBI" subjects. Moreover, we did not find a response exceeding 8 IU / ml in any "recent LTBI" subjects. This finding, if confirmed in subsequent studies, could represent a useful threshold to decision making in the pre-employment phase. In fact, these subjects (once a TBA has been clinically excluded), are unlikely to be recent converters if values are found above such a threshold. In a recent study [12], authors suggested that a more conservative definition of QFT-Plus positivity, based on double-positive antigen tube results (TB1 and TB2), is a useful strategy for increasing QFT-Plus specificity in the low-risk setting, based on the high positive to negative reversion rate among HCW with discordant QFT-Plus T1 and T2 results test. In our study, follow-up investigation of 6 HCWs showed that all but one follow-up testing reverted to

FIGURE 1. Dot plots of TB1 and TB2 IFN- γ values in the Recent LTBI and Remote LTBI



negative. Our results therefore confirm that discordant TB1 and TB2 results might indicate false positive results when the results are near the cut-off value [19 - 21]. Retesting should be recommended under these circumstances.

Strengths and limitations

Due to the low prevalence of LTBI, the analytical evaluation of the QFT-Plus test was conducted on a fairly limited number of positive cases. Another limit of our study was the cross-sectional study design because could not be monitored changes over time.

CONCLUSIONS

Our survey confirms the low prevalence of the LTBI condition among Italian HCWs (3.4% of the population). Among the risk factors analyzed, only the male gender and age > 40 years were significantly associated with the risk of LTBI. In relation to the quantitative outcome of the test, the response in TB1 and TB2 was higher in those with "remote LTBI" than in the "recent LTBI". A value above 8 IU / ml appears indicative of a remote infection. In subjects with discordant outcomes between TB1 and TB2, especially when the test values are close to the cut-off, given the high frequency of reversions it is recommended a repetition of the test to confirm its positivity.

Availability of data and materials

The raw data are available upon request by the

head of the department Prof. Dr. Andrea Magrini (andrea.magrini@uniroma2.it).

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Ethics approval and consent to participate

The study was approved by the ethics committee of Policlinico Tor Vergata (committee's reference number: 194/18).

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