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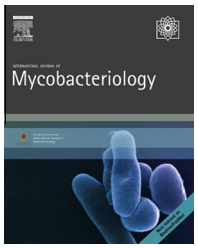

Interferon-gamma release assays and tuberculin skin testing for diagnosing latent *Mycobacterium tuberculosis* infection in at-risk groups in Poland

Katarzyna Kruczak *, *Lucyna Mastalerz*, *Krzysztof Sładek*

Department of Pulmonology, Jagiellonian University Medical College, Skawinska 8, 31-066 Kraków, Poland

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ABSTRACT

Objective/Background: The diagnostics of latent tuberculosis infection in Poland using the tuberculin skin test is challenging due to the obligatory Bacillus Calmette–Guérin vaccinations. Interferon-gamma release assays are still very rarely used for diagnostics. We compared the tuberculin skin test and the QuantiFERON-TB Gold In-Tube test to evaluate the degree of latent tuberculosis infection in at-risk groups for tuberculosis (homeless, close contacts, periodic contacts, nursing-home attendees) and in healthy individuals.

Methods: QuantiFERON-TB Gold In-Tube tests were carried out on 785 individuals from the homeless (n = 150), close contacts (n = 171), periodic contacts (n = 163), nursing-home attendees (n = 152), and healthy individuals (n = 149). The tuberculin skin test was performed on 129, 156, 147, 148, and 121 participants, respectively. We evaluated the (a) correlation between serum concentrations of interferon gamma and the tuberculin-skin-test induration diameter; (b) between the number of QuantiFERON-TB Gold In-Tube-positive results and the tuberculin-skin-test diameter in the studied groups; and (c) agreement between both tests and the kappa coefficient using the tuberculin-skin-test diameters of 5, 10, and 15 mm.

Results: Larger tuberculin-skin-test induration diameters were associated with elevated serum concentrations of interferon gamma. We found a positive correlation between the number of positive QuantiFERON-TB Gold In-Tube screening results and the tuberculin-skin-test induration diameter. The agreement between QuantiFERON-TB Gold In-Tube and tuberculin-skin-test screening results improved with increasing tuberculin-skin-test induration diameter.

Conclusion: Based on measures of tuberculin-skin-test induration diameter alone, it is difficult to diagnose latent tuberculosis infection with certainty. The agreement of the QuantiFERON-TB Gold In-Tube test increases with the tuberculin-skin-test diameter. Tuberculin-skin-test diameters larger than 15 mm are more likely to be associated with active infection.

* Corresponding author.
E-mail address: krucza@gmail.com (K. Kruczak).

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Introduction

It is estimated that at least 32% of the world's population (approximately 1.8 billion) is infected with *Mycobacterium tuberculosis*; the majority have asymptomatic, latent tuberculosis infection (LTBI). In countries with low and medium incidence of tuberculosis, the treatment of LTBI serves to halt the progression of this infection into the active form of the disease. This is particularly important for at-risk individuals, such as those recently infected, in close contact with a contagious patient, infected with human immunodeficiency virus (HIV), and people with previously untreated pulmonary fibrosis [1].

The tuberculin skin test (TST) is the standard method used to determine whether an individual is infected with *M. tuberculosis*. However, instances of tuberculin allergy can occur after a natural infection with the mycobacteria, or as a result of the Bacillus Calmette–Guérin (BCG) vaccine administration. For this reason, in countries (including Poland) where vaccinations, especially repeated ones, so-called revaccinations, were mandatory, the precise evaluation of the rate of mycobacterium infection using the tuberculin test is practically impossible. The immunological basis of the tuberculin test is the presence of a subset of T lymphocytes, known as *central memory cells*, which, in the event of repeated antigen presentation using tuberculin (a mixture of approximately 200 mycobacterial antigens), quickly gather and replicate to control the pathogen. The tuberculin test is not particularly specific, as the purified protein derivative is a mixture of proteins, whose antigen determinants are present in most species of mycobacteria, including the BCG vaccine. With BCG vaccinated patients and those infected with atypical mycobacteria, the so-called environmental mycobacteria, the TST result may yield a false positive [1–3]. In Poland, the National Program for Combating Tuberculosis recommends an induration diameter of at least 10 mm as positive tuberculosis skin test for the entire population (with the exception of HIV-positive patients, for whom a reaction of at least 5 mm is considered to be positive) [1–4].

Within the last few years, new methods have been developed to detect latent *M. tuberculosis* infection, such as QuantiFERON-TB Gold (Cellestis Ltd., Carnegie, Australia, now part of Qiagen) and T-SPOT.TB (Oxford Immunotec, Abingdon, Great Britain), which are highly specific and sensitive for detecting *M. tuberculosis* infection. These tests are typically peptide-based interferon-gamma (IFN-γ) assays, which measure the amount of serum IFN-γ released by specific T lymphocytes in response to antigen presentation by *M. tuberculosis* and a small number of other species of mycobacteria (*Mycobacterium kansasii*, *Mycobacterium szulgai*, and *Mycobacterium microti*). The diagnostic antigenic peptides used to induce IFN-γ release are early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10), and the most recent version of the QuantiFERON-TB Gold In-Tube (QFT-GIT) test also includes the *M. tuberculosis*-specific antigen, TB7.7 [3–13]. These antigens are not present in the BCG strain bacteria, which increase the diagnostic value of these tests.

Most investigators have concluded that interferon-gamma release assays (IGRAs) have high specificity (around 62–100%, greater than TST), since these assays are not influenced by a history of BCG vaccination and infection by environmental mycobacteria. The tests are also highly sensitive; for example, the sensitivity of the enzyme-linked immunospot varies between 83% and 97%, and was higher than the enzyme-linked-immunosorbent-assay method (70–89%) [3–13]. Most studies have reported concordance at 60–90% for TST and IGRA screening tests [3,10–13]. Discordant results, TST positive/IGRA negative, are most likely associated with prior BCG vaccinations, as well as immunization with nontuberculosis environmental mycobacteria. However, it is also possible that IGRA has reduced sensitivity in specific clinical situations, as well as the limited ability to detect the immune response during certain stages of infection, for example, a recent infection that is quickly subject to spontaneous remission or responded quickly to treatment [3]. It is possible that discordant results (TST positive/IGRA negative) are associated with short incubation time of the specific mycobacterial antigens. In support of this, several studies have demonstrated that, when the incubation time using the QuantiFERON test was extended for several days, the initially negative test result returned a positive result [14]. The second type of discordant result, TST negative/IGRA positive, is more difficult to understand. It may be necessary to change the cutoff points applied so far, in order to increase the sensitivity of IGRA tests. It is also probable that the IGRA tests, as stated previously, detect recent mycobacterial infections, due to the effector T cells. (The TST screen remains negative, as central memory cells, which take part in this reaction, are not yet stimulated and do not respond with an IFN-γ release [14].) Additionally, it must be taken into consideration that, in countries with a high tuberculosis incidence, there are many factors that modulate the immunological balance of Th1/Th2, for example, malnutrition, environmental mycobacteriosis, leprosy, verminous diseases, and tropical infections [7].

In this study, we compared the QFT-GIT and TST screening methods used to detect *M. tuberculosis* in at-risk groups. The at-risk groups were the homeless, close contacts, periodic contacts, and care homeworkers, and were compared to healthy people selected at random. We analyzed the following: (a) the relationship between serum concentrations of IFN-γ and the TST induration diameters; (b) the relationship between the number of QFT-GIT-positive results and the TST induration diameter; and (c) the concordance between QFT-GIT screening results and TST induration diameter at thresholds of 5, 10, and 15 mm.

Materials and methods

Between July 2007 and September 2009, *M. tuberculosis* infection was assessed using the QFT-GIT test in four at-risk groups: 145 homeless people, 171 close contacts, 163 periodic contacts, and 152 nursing-home attendees and personnel. We tested 149 randomly selected healthy inhabitants of Kraków to serve as our control group. In total, 785 people were tested (Table 1). The tuberculin-reaction test was carried out for 129, 156, 147, 148, and 121 individuals from the aforementioned groups, respectively.

Table 1 – Group characteristics.

	Group 1: homeless	Group 2: close contacts	Group 3: periodic contacts	Group 4: nursing-home attendees	Group 5: healthy
Total	150	171	163	152	149
Age: median (lower–upper)	50 (20–81)	49.5 (1–96)	45 (22–67)	47 (23–85)	44 (16–81)
Males	114	80	29	71	49
Females	30	91	134	81	100

The study protocol was accepted by the Bioethics Committee of the Jagiellonian University (agreement number KBET/66/B/2008; September 25, 2008). During the period July 2007 to September 2008, the participants were examined within the preventive program led by the authors and funded by the Municipal Office of Kraków. The implementation of the program did not require the approval of the local bioethics committee. After the decision to initiate the clinical study from 2008, the approval was received from the Bioethics Committee of the Jagiellonian University. The study was continued with the participation of people who provided informed consent, including the use of the results of tests obtained in the years 2007–2008. The participants provided a written informed consent for their results to be used in the clinical study and for enrollment in the study groups.

All of the people tested completed a questionnaire specially designed for this study, which details any current and prior illnesses, addictions/habits, and social status. In addition to a physical examination, the scar after a BCG vaccination was assessed for all the study participants.

All of the test participants had TST applied by administering 0.1 mL of tuberculin (2 cc purified protein derivative RT-23) intradermally into the ventral aspect of the forearm by an experienced nurse. The induration diameter who measured assessed after 72 h later, a diameter of at least 10 mm was considered a positive test result. We also used the QFT-GIT test (Cellestis Ltd.) as the IGRA test of choice.

On the first day of the TST, 1 mL of blood was sampled from each test participant, and divided between three test tubes: the first coated with ESAT-6, CFP-10, and TB7.7 antigens; the second coated with a mitogen, which consisted of phytohemagglutinin A (positive control); and the third, which constituted a negative control (a buffered solution of salt with 0.1% thimerosal).

Statistical analysis

The relationship between TST induration diameter and plasma IFN-γ concentration was evaluated using a Spearman's correlation coefficient. This value was reported, along with its relevance, and dispersion charts indicating the threshold values for the given tests.

A chi-square test of independence was performed between the QFT-GIT results and the TST diameter.

The concordance of the tests was carried out by calculating the kappa coefficient value (κ) with a 95% confidence interval. Data are presented in the form of tables of contingency, additionally providing p for the independence test (chi-square test).

Two-tailed tests were used throughout this study, and a critical value of $p < .05$ was used to determine statistical significance.

Results

Relationship between serum concentrations of IFN-γ and induration diameter in the TST

The relationship, assessed using Spearman's rank correlation coefficient, between IFN-γ concentrations and TST induration diameters in the tested groups was between .3 and .6, which indicated a moderate correlation (Table 2). This is in line with the expected outcome (i.e., the larger induration diameters resulted in higher greater serum IFN-γ concentrations).

QFT-GIT positive results are associated with TST induration diameter

We next examined whether the correlations between serum IFN-γ concentrations and TST induction diameter hold true when different diameters were examined at different thresholds. We grouped the induration diameters into the following groups: (a) <4 mm, (b) 5–9 mm, (c) 10–14 mm, and (d) $\geqslant$15 mm.

We evaluated the number of positive results based on the QFT-GIT test screen in relation to the TST induration diameter for all the at-risk groups and healthy individuals, and the number of positive TST results in these ranges (Figs. 1–5). In all the groups, greater numbers of QFT-GIT-positive results were found with increasing TST induration diameter.

The chi-square test independence test for all groups revealed statistically significant differences in the number of positive and negative QFT-GIF results depending on the TST diameter (in the healthy group $p = .02$, in at-risk groups $p < .001$).

Table 2 – Spearman's rank correlation coefficients between serum interferon-gamma levels and the tuberculin-skin-test induration diameter.

Group	Spearman's coefficient	p
Homeless	.55	<.001
Close contacts	.31	<.001
Periodic contacts	.41	<.001
Nursing-home attendees	.41	<.001
Healthy	.29	<.001

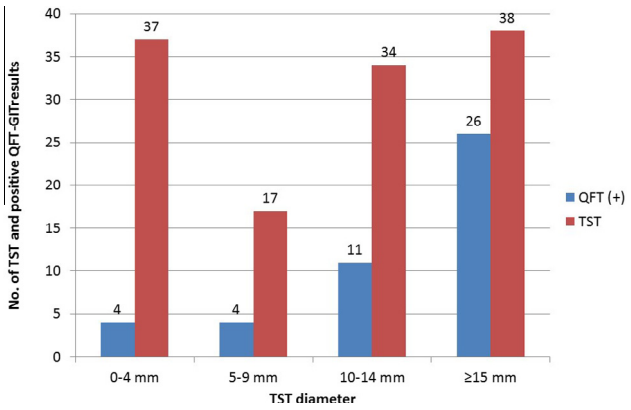

Fig. 1 – Distribution of positive QuantiFERON-TB test results grouped according to tuberculin-skin-test induration diameter in homeless people. QFT-GIT = QuantiFERON-TB Gold In-Tube; TST = tuberculin skin test.

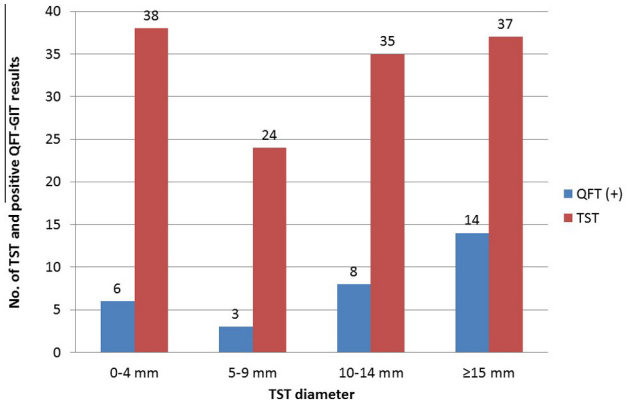

Fig. 2 – Distribution of positive QuantiFERON-TB test results grouped according to tuberculin-skin-test induration diameter for the close-contact individuals. QFT-GIT = QuantiFERON-TB Gold In-Tube; TST = tuberculin skin test.

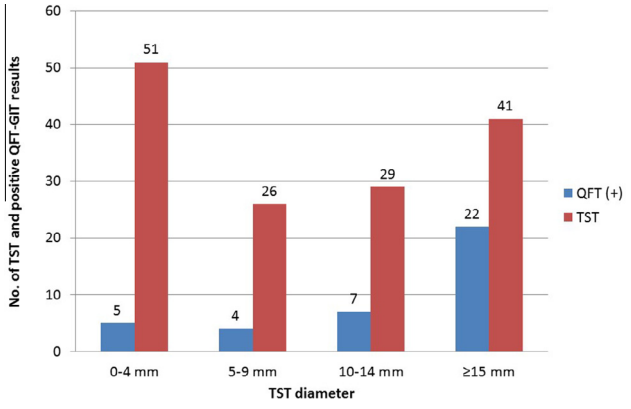

Fig. 3 – Distribution of positive QuantiFERON-TB test results grouped according to tuberculin-skin-test induration diameter for periodic contacts. QFT-GIT = QuantiFERON-TB Gold In-Tube; TST = tuberculin skin test.

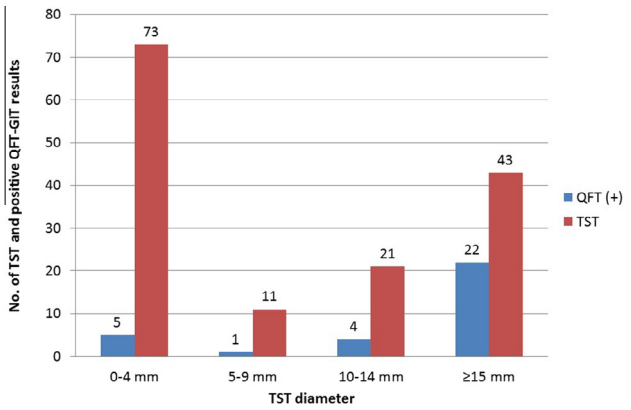

Fig. 4 – Distribution of positive QuantiFERON-TB test results grouped according to tuberculin-skin-test induration diameter for nursing-home attendees. QFT-GIT = QuantiFERON-TB Gold In-Tube; TST = tuberculin skin test.

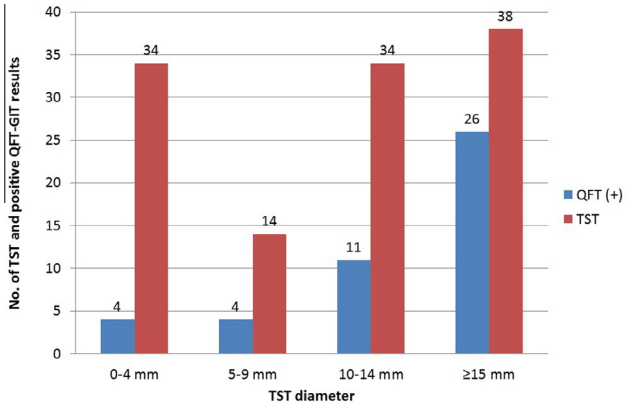

Fig. 5 – Distribution of positive QuantiFERON-TB test results grouped according to tuberculin-skin-test induration diameter for healthy individuals. QFT-GIT = QuantiFERON-TB Gold In-Tube; TST = tuberculin skin test.

Agreement of QFT-GIT and TST

We initially considered a positive TST value to be diameters 10 mm or greater, but also considered values above 15 mm. We compared the number positive results based on the QFT-GIT screen with positive from the TST using the two diameter measures (⩾10 mm and ⩾15 mm). Fig. 6 shows the percentage distribution of positive QFT-GIT test results and positive TST results using both induration diameters.

The percentage frequency of positive TST results was almost twice as large when a positive result was considered to be an induration diameter equal or larger than 10 mm. When it was assumed that a positive TST result was a diameter equal or larger than 15 mm, the frequency of positive results was similar to that of the QFT-GIT test.

Based on the analyses of the concordant results of these tests, we observed TST positive/QFT-GIT positive and TST negative/QFT-GIT negative. We also identified two discordant results: TST positive/QFT-GIT negative and TST

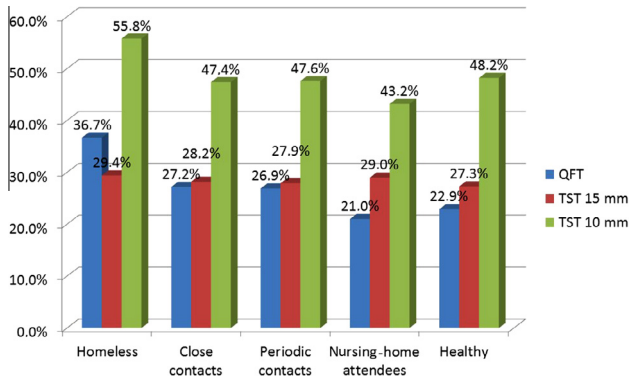

Fig. 6 – *Mycobacterium tuberculosis* infection based on QuantiFERON-TB test results and tuberculin reaction (⩾10 mm and ⩾15 mm) in at-risk and healthy individuals. QFT = QuantiFERON-TB; TST = tuberculin skin test.

negative/QFT-GIT positive. The majority of the discordant results were TST positive/QFT-GIT negative, which may be accounted for by the influence of multiple BCG vaccinations on the TST result. Subsequently, the agreement between QFT-GIT and TST screens was assessed taking TST induration values as positive at thresholds of 5, 10, and 15 mm (Table 3).

For the majority of groups, we found the agreement between QFT-GIT and TST screening results (κ coefficients) improved when larger TST induration diameters were used. In no instance did κ coefficient values exceed .6.

Discussion

Investigations of latent *M. tuberculosis* infection in at-risk groups aim to provide quick identification of individuals with an active form of the disease. This is particularly important for the management of tuberculosis in countries with a relatively low incidence rate, which may soon also include Poland. Such action is intended to prevent further transmission of tuberculosis by quickly diagnosing and treating its active form, while the detection of LTBI in people who come in contact with tuberculosis patients makes it obligatory for doctors to carry out regular control examinations and consider the incorporation of chemoprophylaxis treatment. The incidence of tuberculosis among people infected with *M. tuberculosis* is highest shortly following infection, and may reach 30–40% of infected among small children and about 2% among primary schoolchildren [15]. As stated previously, in Poland, chemoprophylaxis treatment currently only includes children and adolescents, and HIV-positive patients. The relatively high incidence of tuberculosis in Poland (18.6/100,000), in 2013, did not enable the effective treatment of latent tuberculosis in a larger group of people. This was complicated by the difficulty in diagnosing patients due to the ineffectiveness of TSTs, caused by repeated BCG vaccinations. Until recently, a BCG vaccination calendar was in effect in Poland. As well as vaccinations of newborns, there were obligatory booster vaccinations in the 1st, 6th, 12th, and 18th years of life. After a single administration of the BCG vaccine, the immunization is maintained for 10–15 years; after this time, if revaccination does not take place, all the positive tuberculin reactions could be treated as signs of infection (with the exception of the minimal influence of environmental mycobacteria on the tuberculin reaction in Poland). Unfortunately, numerous revaccinations after the infancy period result in the maintenance of a particularly high tuberculin response for many years [16,17]; as a result, there has so far been no sure way to differentiate a postvaccination reaction from a post-infectious one. Currently, the IGRA tests (including the QFT-GIT test used in this study), whose specificity reaches nearly 98–100%, fulfills the primary role of determining the degree of *M. tuberculosis* infection [18]. These assays are especially diagnostically significant in those countries (including Poland) where numerous BCG revaccinations are or were in effect. Unfortunately, we are currently unable to determine whether a positive IGRA test result is due to the presence of living, "dormant" bacteria in the organism, or only due to the activation of central memory cells, which were in contact with mycobacteria in the past. This also applies to the tuberculin reaction as a diagnostic test of LTBI [19].

In this study, two types of concordant results of these tests were identified: TST positive with QFT-GIT positive and TST negative with QFT-GIT negative, as well as two discordant

Table 3 – Agreement between QuantiFERON-TB Gold In-Tube and tuberculin-skin-test results at the 10-mm and 15-mm threshold.

Risk groups		TST 5 mm	TST 10 mm	TST 15 mm
Homeless	Correspondence (%) Kappa	58.7 .26	60.7 .34	75.4 .45
Close contacts	Correspondence (%) Kappa	55.8 .20	73.0 .29	73.4 .32
Periodic contacts	Correspondence (%) Kappa	53.7 .19	75.0 .30	76.1 .40
Nursing-home attendees	Correspondence (%) Kappa	64.0 .29	78.8 .36	79.0 .45
Healthy	Correspondence (%) Kappa	55.8 .20	60.9 .24	70.0 .23

TST = tuberculin skin test.

results TST positive with QFT-GIT negative and TST negative with QFT-GIT positive. Most instances of disagreement were QFT-GIT-negative with TST-positive results. This appears to be associated primarily with the influence of multiple BCG vaccinations on the TST result. In certain groups, increased tuberculin-reaction induration diameter showed good agreement with the QFT-GIT screen, based on the κ coefficients. Our findings are broadly in line with those reported by other investigators. Diel et al. [20] observed concordance for both tests at a level of 63.8–69.2% (κ = .2–.28) in a group of patients with BCG vaccinations, and 89.5–90.7% (κ = .58–.62) in the group of unvaccinated patients. Similarly, Ferrara et al. [21] observed an agreement of 41% (κ = .09) in the vaccinated group, and 80% (κ = .56) in the unvaccinated group. In a large cohort study, conducted by Arend et al. [22], an agreement of 75.4% (κ = .33) was reported when using a cutoff point of 10-mm TST induration diameter in the unvaccinated group. Using TST of at least 15 mm, the agreement reached 86.5% (κ = .49). It is commonly accepted that the larger the TST induration diameter, the more likely it is for infection to occur, and is commonly accepted to be 15 mm or more. Such an induration diameter is considered to be an infectious reaction, rather than a postvaccination response [16]. In an earlier study, we [23] and Kuś et al. [24] assessed the degree of *M. tuberculosis* infection among the Polish population. The QFT-GIT test was more useful for diagnosing *M. tuberculosis* infection [23,24], and found to have greater predictive accuracy for assessing the development of an active form of tuberculosis [25].

In the present study, we compared TST and QFT-GIT screening methods for the diagnosis of *M. tuberculosis* infection in at-risk groups and in healthy individuals. Consistent with the findings of others, and in line with the expected outcome, we found that TST induration diameters correlated with serum IFN-γ concentrations, analyzed using the QFT-GIT test. This is to be expected, since the same cytokines participate in both types of reactions; in the case of QFT-GIT, cytokines are released by T cells into the serum following stimulation by specific mycobacterial antigens, while in the TST, cytokines are released at the tubulin injection site. Spearman's rank-correlation-coefficient values .3–.6 indicate low (homeless group) or medium (other groups) correlation between IFN-γ concentrations and TST induration diameter. These findings suggest that patients with a large TST induration diameter have higher serum concentrations of IFN-γ. Additionally, based on the positive correlation between the number of positive QFT-GIT test results and the tuberculin-reaction induration diameter, it follows that the probability of a positive QFT-GIT result for a tested patient rises with increasing TST diameter. However, our results do not allow us to determine whether high TST values (e.g., over 15-mm induration diameter) will always yield QFT-GIT-positive results for a given patient. This is because QFT-GIT-positive test results (as well as high levels of IFN-γ) can be observed in patients with a TST diameter of 0 mm, as well as 20 mm. Therefore, we have shown that a positive TST result (over 15 mm) indicates a high probability of *M. tuberculosis* infection, while at the same time a negative result should not automatically rule out infection.

The results of this study demonstrate the rising diagnostic value of TST induration diameter in vaccinated patients. It should be noted, however, that the observed results and κ coefficients never reached a high agreement level in any of the groups included in this study. Our data indicate that TST induration diameters of at least 15 mm are suggestive, but not necessarily indicative, of *M. tuberculosis* infection. Particular care must be taken when adopting specific TST induration-diameter thresholds used to indicate *M. tuberculosis* infection.

Conflicts of interest

None declared.

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