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Prevalence of latent infection with Mycobacterium tuberculosis in Mazowieckie province using interferon gamma release assay after stimulation with specific antigens ESAT-6 and CFP-10

Ocena częstości występowania zakażenia prątkiem gruźlicy w populacji województwa mazowieckiego na podstawie wyniku testu mierzącego uwalnianie interferonu gamma po stymulacji swoistymi antygenami ESAT-6 i CFP-10

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Abstract

Introduction: Over 8000 cases of tuberculosis (TB) are diagnosed annually in Poland. People infected with *Mycobacterium tuberculosis* (MTB) have a risk of active disease of around 10% during their whole life, and the risk is highest in the first two years after infection. Recognizing infection before TB disease development enables prophylaxis against its activation and ceases transmission of infection. Knowledge about the proportion of infected people in the population is crucial to predict the number of new cases of active disease.

Materials and methods: The prevalence of latent TB infection (LTBI) was tested in 700 healthy adult inhabitants of the Mazowieckie province in different age groups, using both tuberculin skin test (TST) and interferon gamma release assay (IGRA). Commercial test QuantiFERON®-TB-Gold In Tube (QFT) was used. All participants were mandatorily BCG vaccinated according to the Polish vaccination schedule.

Results: Twenty-three per cent of participants tested positively for QFT, which was significantly less than for TST (50.3%). The prevalence of positive QFT results increased with age, as well as the incidence of TB in Poland. Positive QFT was most frequent in the oldest age group (48.8%) and rarest in the youngest (7.1%). Conversely, positive TST occurred more often in younger participants (45%), who rarely suffer from TB. Among people over 60, with the highest TB incidence rate, only 33.8% tested positively with TST. Concordance between both tests was low, with a kappa value of 0.198. The prevalence of LTBI defined as positive QFT among health care workers (HCW) was significantly higher than among other participants (32.2% v. 20.4%, $p < 0.01$).

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Conclusions: LTBI was diagnosed in 23.3% of the tested population of the Mazowieckie province. QFT is a better tool for diagnosing LTBI as it shows a positive correlation with age (as the incidence of TB disease does). Concordance between both tests is low. The prevalence of LTBI in HCW is higher than in other participants.

Key words: latent tuberculosis infection, tuberculin skin test, interferon gamma release assay

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Introduction

Every year, about 8000 individuals in Poland are diagnosed with tuberculosis (TB). Nearly one half of this group is sputum-positive and constitutes a source of infection for other individuals. Individuals infected with *Mycobacterium tuberculosis* constitute the source pool for new cases of the disease. The most important aim of anti-tuberculosis programs is the detection of active TB as early as possible to inhibit the transmission of the infection to healthy individuals, by means of early treatment and isolation. The risk of the disease in an infected person does not exceed 10%, is at the highest level during the first two years following infection, and is sustained throughout their whole life. Along with the improvements in the epidemiological profile of tuberculosis, i.e. the reduction of incidence and morbidity, the anti-tuberculosis programs are directed towards the detection of infection in healthy individuals. This facilitates undertaking of prophylactic measures aimed at prevention of tuberculosis development in individuals already infected with *Mycobacterium tuberculosis*.

For 100 years, infection with *Mycobacterium tuberculosis* has been detected by tuberculin skin test (TST). Following intradermal injection of tuberculin, infected individuals react with infiltration at the injection site, i.e. with a positive TST result. The result of TST is scored as positive with a diameter of induration ranging from 5 to 15 mm, depend on population tested and national guidelines. The statement that a positive TST result is proof of tuberculosis infection is true only in populations in which no anti-tuberculosis vaccinations (bacillus Calmette-Guerin, BCG) are used [1]. Metaanalysis of many tests that have collected thousands of observations of both BCG-vaccinated and non-vaccinated individuals have shown that vaccination, particularly if performed after infancy (as it used to be until recently in Poland), leads to larger TST induration and this reactivity tend to persist for many years [2]. The main cause of falsepositive TST results is the fact that tuberculin is a mixture of about 200 antigens present not only in acid-fast bacilli of *M. tuberculosis*, but also in the BCG strain used for vaccination as well as in other non-tuberculous mycobacteria common in the human environment, e.g. in tap water.

A solution to this problem is to use, in the detection tests, such antigens that are specific to *M. tuberculosis* and are not present in the BCG strain or in most non-tuberculous bacilli. Such antigens are ESAT-6 and CFP-10.

The principle of the tests is stimulation of lymphocytes in a sample of blood collected from a person suspected to be infected with *M. tuberculosis* with ESAT-6 and CFP-10. The response is measured by the amount of produced interferon-gamma (IFNγ) and stimulated lymphocyte counts. Tests assessing IFNγ production are generally known as interferon-gamma release assays (IGRAs). An advantage of these assays is that a previous BCG vaccination has no impact on the result [3–6]. Determination of the incidence of *M. tuberculosis* infection in particular populations is of crucial importance for prognosis of future tuberculosis morbidity (incidence). Thus, it may facilitate planning the management aimed at the elimination of tuberculosis from our population. A reliable test for detection of *M. tuberculosis* infection in healthy individuals allows the selection of individuals in whom prophylactic management may be initiated with the purpose of preventing the infected person from developing active tuberculosis. Such management is increasingly common in countries with a small percentage of infected individuals in the population.

The aim of the present study was a prospective assessment of the incidence of *M. tuberculosis* infection in groups of healthy adults from various communities by means of interferon gamma release stimulated by specific, recombinant *M. tuberculosis* antigens (ESAT-6 and CFP-10) and TST results. Another goal was to evaluate the diagnostic usefulness of the QuantiFE-RON®-TB-Gold In Tube (QFT) test in the detection of *M. tuberculosis* infection in different study groups as compared to the TST.

Material and methods

The study was conducted in three groups of healthy adult residents of Mazowieckie province who, having obtained written and oral information regarding the purpose and the nature of the study, gave in writing their consent to participate. The authors of the study obtained the approval of the Bioethics Committee of the Institute of Tuberculosis and Lung Diseases in Warsaw.

The studied individuals originated from the following communities:

1. blood donors and healthy individuals registered at a family physician's practice (individual medical practice in Mazowieckie province);
2. healthcare professionals;
3. penitentiary inmates.

After obtaining consent, the following procedures were performed on the volunteers in the following order:

1. history including current diseases, HIV infection, intake of immunosuppressive drugs and contact with tuberculosis, history of tuberculosis, and BCG vaccinations;
2. blood collection for IGRA tests;
3. tuberculin skin test.

Individuals in whom diseases or conditions of potentially significant impact on immunity, such as pregnancy, insulin-dependent diabetes, renal insufficiency, cancer, HIV infection, intake of immunosuppressive drugs, including glucocorticosteroids were identified, were not included in the study.

QuantiFERON®-TB-Gold In Tube Test

Commercial QuantiFERON®-TB-Gold In Tube tests available from Cellestis (Australia) were used. All test procedures were performed in accordance with the manufacturer's instructions.

Vein blood was collected by a qualified personnel into two heparinized tubes from the kit: one tube with walls coated with ESAT-6, CFP-10 and TB7.7 antigens, and the other tube containing pure physiological saline with phosphate buffer (negative control). Samples were numerically coded. The diagnostic technician performing the IGRA test and the nurse reading the TST results did not know the results of other tests or survey data.

Following blood collections, tubes were flipped upside down several times so that blood thoroughly washed the tube walls coated with antigens specific to *Mycobacterium tuberculosis*. Immediately after collection, samples were placed in a portable heater (MOBICOOL TROPICOOL-CLASSIC model TC-18UG-12), where they were subjected to incubation at 37°C for 16-24 hours in an upright position. Next, the blood was centrifuged for 15 minutes at +4°C and at a speed of 3000 RPM (High Speed Brushless Centrifuge MPW-350R from MPW Med. Instruments). Samples were stored at –40°C until IFNγ levels were determined by ELISA. The assay was performed by a qualified laboratory diagnostic technician according to the manufacturer's recommendations.

IFNγ concentrations in tested samples were calculated from a reference curve using KC junior software from Bio-Tek Instruments 1998–2001. Final results were obtained after subtraction of a blank sample value from the test sample value. A zero value was assumed for negative results. A positive test result was defined as an IFNγ concentration of 0.35 IU/ml or more, according to the manufacturer's recommendations. An indeterminate result was given if the IFNγ concentration in the negative control was 8.0 IU/ml or more.

Tuberculin skin test

The tuberculin skin test was performed by intradermal injection of 0.1 ml — 2 units of tuberculin PPD RT23 into the dorsal surface of the forearm. Tuberculin was manufactured by Statens Serum Institute (Copenhagen, Denmark). The result was read after 72 hours by measuring the diameter of the infiltration transversely to the long axis of the forearm. Tuberculin was injected and the results were read by the same person, with many years of experience, routinely performing TSTs in the Institute of Tuberculosis and Lung Diseases in Warsaw.

Statistical analysis

Descriptive statistics for the tested variables are presented in tables as absolute numbers and percentages.

Incidences of positive tests in groups determined by the studied factors were compared by means of χ^2 test or Fisher's exact test (in case of cell counts being too low in the contingency tables, preventing the use of the χ^2 test). An analysis of factors affecting the risk of infection was also performed. Logistic regression analysis was used for this purpose. The analysis was performed in three groups: 1) ZDR — healthy persons with no association with healthcare; 2) SŁZ — healthcare professionals; and 3) ZKR — penitentiary inmates (prisoners). At the first stage of the analysis, the impact of gender and age was studied. The impacts of the remaining factors were assessed in models taking into consideration the age and gender of patients. The results were presented as hazard ratios with 95% confidence intervals and p values. Statistical significance was defined as $p \leq 0.05$.

The assessment of the level of significance for the relationship between TST and QFT test results was performed using the concordance coefficient κ. Values of κ below 0 indicate lack of conformity, values in the range of 0–0.2 indicate very poor agreement, values in the range of 0.21–0.4 indicate poor agreement, values in the range of 0.41–0.6 indicate moderate agreement, values in the range of 0.61-0.8 indicate significant agreement, and values above 0.81 indicate excellent agreement.

Calculations were performed using SPSS statistical packages for Windows 12.0 (SPSS, Inc., Chicago, Illinois).

Results

A total of 700 subjects took part in the study. Due to incomplete demographical data, 43 subjects were excluded from analysis. Among the remaining 657 subjects, QFT test results were obtained in 621 individuals. The reasons for missing results in 36 subjects were inability to collect blood or accidental sample destruction (29 cases) and indeterminate test results (7 cases). TST results were obtained in 487 of 657 subjects. The lack of TST results in 170 patients was due to the lack of consent for tuberculin injection (154 patients) and not reporting for test result reading despite having received the injection (16 patients). Both QFT test and TST results were obtained in 452 of 657 subjects.

The study group of 657 subjects included 404 females (61.5%) and 253 males (38.5%). Age groups of 0–24 years, 25–44 years, 45–59 years, and 60 and more years were represented by 12.9%, 44.3%, 30.1%, and 12.6% of subjects, respectively. The study subjects belonged to three different communities assigned with the following abbreviations for the sake of simplification of the text and tables:

1. ZDR: blood donors reporting at the Regional Centre for Blood Donation and Treatment in Warsaw and healthy individuals registered at a family physician's practice (individual medical practice in the Mazowieckie province) — 374 subjects, accounting for 56.9% of the entire study population. The age of subjects in this group (mean ± SD) was 43.8 ± 17.0 years;
2. SŁZ: Healthcare professionals — employees of microbiology labs processing material collected from tuberculosis patients and physicians in contact with tuberculosis patients — 145 subjects, accounting for 22.1% of the entire study population. The age of the subjects (mean ± SD) was 42.1 ± 9.8 years;
3. ZKR: inmates of a penitentiary institution in Warsaw — 138 subjects, accounting for 21.0% of the entire study group. The age of the prisoners (mean ± SD) was 36.8 ± 12.8 years.

All study subjects were considered BCG-vaccinated. A scar on the arm that might indicate antituberculosis vaccination was found in 95% individuals. In the remaining 5% of subjects, BCG vaccination could not be excluded based on medical history. It must be noted that in Poland starting from 1955, the vaccination schedule provided multiple BCG vaccinations from the neonatal age to the age of 18. Since 2006, a single vaccination has been administered on the 1st day of life, pursuant to the Ordinance of the Minister of Health.

Evaluation of *M. tuberculosis* infection incidence based on the QFT test

Positive QFT test results were observed in 23.3% of the entire study population. The incidence of positive QFT test results was compared between the study groups: inmates, healthcare professionals exposed to tuberculosis bacilli at work, and healthy individuals from outside these communities (Table 1). A positive QFT test result was observed in 20.4% of subjects in the ZDR group, 23.5% of subjects in the ZKR group, and 32.2% of subjects in the SŁZ group. The percentage of positive QFT test results in the SŁZ group (healthcare professionals) was significantly higher than in the ZDR group ($p < 0.01$), while the incidence of *M. tuberculosis* infections assessed by means of the QFT tests in the inmates group was the same as in the ZDR group, i.e. in individuals from outside healthcare professions or penitentiaries.

The analysis of the relationship between the QFT test result and the age in the entire study population is presented in Table 2. The percentage of subjects with positive QFT test results increased with age: from 7.1% in the youngest age group to 48.8% above the age of 60 ($p < 0.001$). Similar trends were observed in all study groups when analysed separately. The observation of *M. tuberculosis* infection being less common in younger subjects compared to older subjects is consistent with the tuberculosis incidence rates in the Polish population. The risk of infection assessed on the basis of positive QFT test result was also studied in the ZDR group in an age- and gender-dependent manner (Table 3). Gender was found to have no significant effect on the test result, while the probability of a positive QFT test result increased with age in a statistically significant manner. *M. tuberculosis* infection was nearly 5 times more common in subjects above the age of 45 (p =

Table 1. QFT results in different groups

			QFT test		Total
			Negative	Positive	
Group	Health care workers	Number	80	38	118
	(SŁZ)	%	67.8%	32.2%	100.0%
	Healthy voluntiers	Number	292	75	367
	(ZDR)	%	79.6%	20.4%	100.0%
	Correctional inmates	Number	104	32	136
	(ZKR)	%	76.5%	23.5%	100.0%
Total		Number	476	145	621
		%	76.7%	23.3%	100.0%

p = 0,03 SŁZ v. ZDR p < 0,01 ZKR v. ZDR p = 0,46
Abbreviations in the text

Table 2. QFT results in different age groups (all participants)

			QFT test		Total
			Negative	Positive	
Age group (years)	≤ 24	Number	78	6	84
		% of age group	92.9%	7.1%	100.0%
	25–44	Number	229	36	265
		% of age group	86.4%	13.6%	100.0%
	45–59	Number	127	63	190
		% of age group	66.8%	33.2%	100.0%
	≥ 60	Number	42	40	82
		% of age group	51.2%	48.8%	100.0%
	Total	Number	476	145	621
		% of age group	76.7%	23.3%	100.0%

p < 0,001
Abbreviations in the text

Table 3. Odds ratio of being infected based on positive QFT result in the group of healthy voluntiers (ZDR) adjusted for age and gender

	OR	95% CI for OR		p
		Lower limit	Upper limit	
Woman	1.000			
Man	1.006	0.58	1.747	0.982
Age ≤ 24 yrs	1.000			
Age 25–44 yrs	1.362	0.454	4.081	0.581
Age 45–59 yrs	4.626	1.672	12.803	0.003
Age ≥ 60 yrs	12.126	4.362	33.713	< 0.001

Abbreviations in the text

0.003) and 12 times more common in subjects above the age of 60 (p < 0.001) compared to subjects below the age of 24.

Tuberculosis incidence rates with positive smear results allowed the estimation of the annual infection risk and, consequently, the approximate percentage of infected individuals by age (year of birth). In line with the results of studies conducted by Styblo et al. [7], the annual risk of *M. tuberculosis* infection in populations with tuberculosis incidence rates confirmed by positive smear results of 50/100,000 — is 1%. Estimated percentages of individuals infected with tuberculosis in Poland, calculated from data in the Central Registry for Tuberculosis [8, 9], are presented in Table 4.

Assuming the estimated annual risk of infection, the percentage of individuals infected by the age of 20 should not exceed 5%, and by the age of 45 — 25–28%. These incidences are similar to data obtained from QFT test results in the study population.

Table 4. Incidence of smear-positive pulmonary tuberculosis and estimated annual risk of infection in the population

Year	Incidence of smear-positive cases/100.000/yr	Estimated annual risk of infection in %
1972	28.3	0.57
1980	21.9	0.44
1990	12.0	0.25
2000	8.7	0.17
2008	7.8	0.16

Table 5. Positive TST results (cut off 10 and 15 mm) in the whole group and subgroups

Group tested	Number (n)	TST ≥ 10 mm n (%)	TST ≥ 15 mm n (%)
Whole group	487	245 (50.3)	126 (25.9)
ZDR*	213	86 (40.4)	34 (16)
SŁZ*	143	88 (61.5)	52 (36.4)
ZKR*	131	71 (54.2)	40 (30.5)

Abbreviations as in Table 1

Tuberculin skin test

There are no universal guidelines for TST result interpretation. According to the Polish guidelines defined in the National Anti-Tuberculosis Program [10], the TST result is defined as positive starting from 10 mm, while certain studies [11] and US guidelines developed by Centers for Disease Control and Prevention (CDC) and the American Thoracic Society (ATS) assume that for individuals who are not in high risk groups, the result of the TST is considered positive starting from the induration diameter of 15 mm or more [12]. Table 5 presents the percentages of patients with TST induration larger than 10 mm and larger than 15 mm in the entire population and individual subgroups.

If TST infiltration with a diameter of 10 mm or more is to be assumed as the criterion for positive TST results in line with Polish guidelines, tuberculosis infection should be diagnosed in more than 50% of all study subjects, including 40.4% of subjects in the healthy group.

Table 6 presents the incidence of positive TST results for the criteria of more than 10 mm and more than 15 mm in individual age groups of the entire study population. Tuberculin infiltrations larger than 10 mm were comparably common in all age groups (33.8–54.4%). Infiltrations larger than 15 mm were less common, but also showed no significant differences between the age groups of under 24, 25–44, and 45–59 years (20–32.8%). Of note is the very low incidence of positive TST results with diameters of 15 mm and more in the age group of 60 and more years (14.1%), which shows that the test is not sufficiently sensitive in elderly subjects.

Table 6. Positive TST results (cut off 10 mm and 15 mm) in different age groups

Age group	Number (n)	TST ≥ 10 mm n (%)	TST ≥ 15 mm n (%)
≤ 24	43	22 (51.2)	9 (20.9)
25–44	192	105 (54.7)	49 (25.5)
45–59	174	91 (52.3)	57 (32.8)
≥ 60	78	27 (34.6)	11 (14.1)
Total	487	245 (50.3)	126 (25.9)

Analysis of concordance between 10 mm and 15 mm tuberculin skin test results and QFT test results

Analysis of concordance of both assays was performed in 452 subjects in whom both QFT and TST results were available. The concordance of the QFT and the TST results was analysed for the criterion of 10 mm or more (Table 7) and 15 mm or more (Table 8) in the entire patient population. The concordance between the TST and the QFT test in this study was very poor if TST results of 10 mm or more were considered positive (κ 0.198), and significantly better when cut-off value of positive result was 15 mm (κ 0.323).

Sensitivity and specificity of the TST in confirmation of infection defined as positive QFT test results was analysed (Figure 1). Test specificity increased, but sensitivity decreased significantly with the increase in TST infiltration diameter. The course of the ROC curve suggests that the TST is not the optimal test for diagnosing tuberculosis infection.

Concordance between the QFT test results and TST infiltrations was also analysed for the diameter of 10 or more millimetres (Table 9) and 15 or more millimetres (Table 10), in individual age groups. The poorest concordance between both tests was observed in younger age groups (0-24 and 25–44 years), in which the percentages of subjects with positive TST results were high and exceeded 50% for the cut-off diameter of 10 mm, while the per-

Table 7. Concordance between TST (cut off 10 mm) and QFT results

			QFT test		Total
			Negative	Positive	
TST [mm]	0–9	Number	186	41	227
		% with TST 10	81.9%	18.1%	100.0%
		% with QFT 0.35	57.1%	32.5%	50.2%
	10+	Number	140	85	225
		% with TST 10	62.2%	37.8%	100.0%
		% with QFT 0.35	42.9%	67.5%	49.8%
	Total	Number	326	126	452
		% with TST 10	72.1%	27.9%	100.0%
		% with QFT 0.35	100.0%	100.0%	100.0%

Assesment of agreement		Value	Standard error	T(b)	p
Assesment of agreement	Kappa	.198	.041	4.674	.000
N		452			

Table 8. Concordance between TST (cut off 15 mm) and QFT results

			QFT test		Total
			Negative	Positive	
TST [mm]	0–14	Number	271	65	336
		% with TST 15	80.7%	19.3%	100.0%
		% with QFT 0.35	83.1%	51.6%	74.3%
	15+	Number	55	61	116
		% with TST 15	47.4%	52.6%	100.0%
		% with QFT 0.35	16.9%	48.4%	25.7%
	Total	Number	326	126	452
		% with TST 15	72.1%	27.9%	100.0%
		% with QFT 0.35	100.0%	100.0%	100.0%

Assesment of agreement		Value	Standard error	p
Assesment of agreement	Kappa	.323	.049	.000
N		452		

centages of subjects with positive QFT test results was much lower and did not exceed 10% in the group under 24 years and 20% in the group 25–44 years old.

When TST diameter of 15 mm or more was considered a positive result, higher concordance between the TST and QFT test results was observed in individual age groups than in the case of diameter of 10 mm or more.

Discussion

One of the most important indicators of epidemiological status of tuberculosis are the annual infection risk and the percentage of individuals infected with *M. tuberculosis*, as they constitute the source pool for new cases of the disease. An efficient test for the detection of latent tuberculosis infection should identify individuals at high risk of tuberculosis in the future. This condition is met by tests based on the measurement of interferon secretion by the subject's lymphocytes stimulated by antigens specific to *M. tuberculosis* (Interferon-Gamma Release Assays , IGRAs). Higuchi et al. [13] observed that during a follow-up (lasting more than 3 years) of 84 subjects who had been

in close contact with tuberculosis patients and had positive TST results but negative QFT test results, none of the subjects develop active tuberculosis. In the study conducted by Diel et al. [4], only 2.3% of subjects with positive TST result and as much as 14.6% of subjects with positive QFT test results develop active tuberculosis during a 2-year follow-up of individuals exposed to tuberculosis. No cases of tuberculosis were observed in the group of subjects with negative QFT and positive TST results, despite the exposure to sputum-positive patients, with the QFT test results being independent of previous BCG vaccination of patients [4].

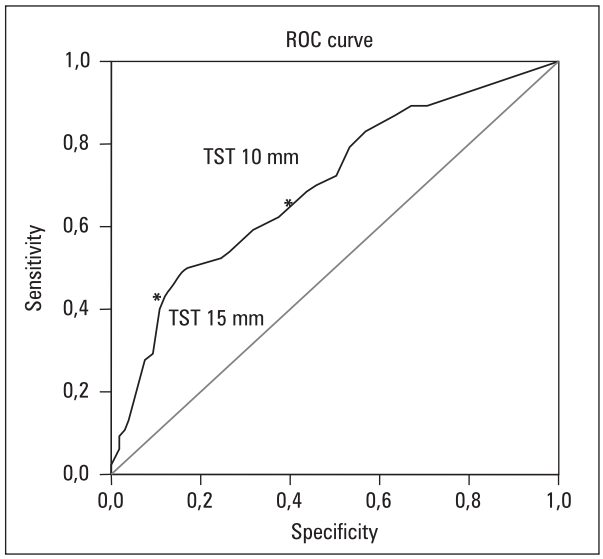

Figure 1. ROC curve for TST diameter *versus* QFT results

If the QFT test is specific for *M. tuberculosis* infection, it should be significantly age-dependent because in countries with tuberculosis incidence systematically decreasing, the incidence is shifted towards older age groups [8, 9]. Poland is one such country, where data acquired from the tuberculosis incidence registry show a decidedly lower incidence in younger age groups. The incidence rate in the Mazowieckie province is nearly 7 times lower in the age group of 15–19 years than in the age group of 65 years and more [8, 9].

Positive QFT test results were observed in 23.3% of subjects in the study population. The percentage of individuals with positive QFT test

Table 9. Condordance between TST (cut off 10 mm) and QFT results in different age groups

Assesment of agreement			Value	Standard error	p
Age group/Years	≤ 24	κ	.087	.086	.317
		N	43		
	25–44	κ	.163	.051	.002
		N	166		
	45–59	κ	.273	.070	.000
		N	166		
	≥ 60	κ	.322	.103	.003
		N	77		

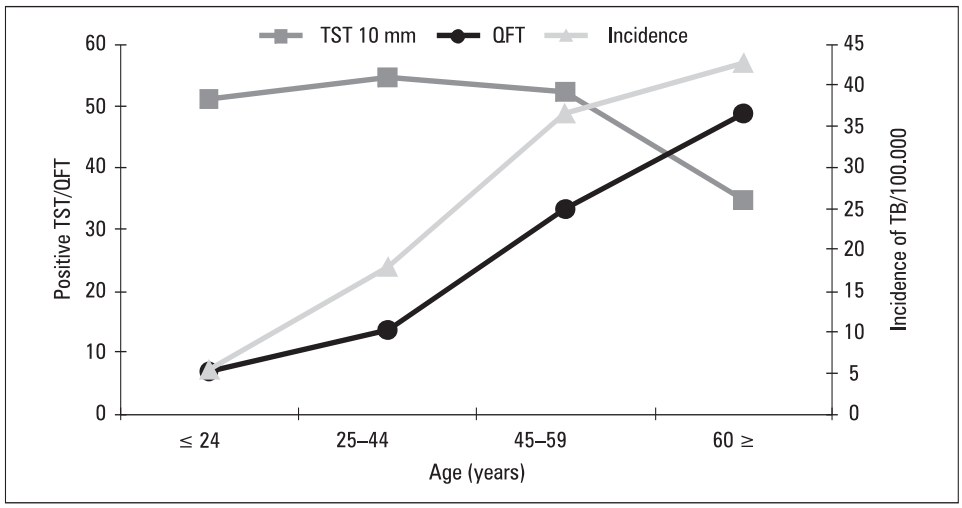

Figure 2. Percentage of infected based on positive QFT and TST results related to the incidence of TB different age groups

Table 10. Concordance between TST (cut off 15 mm) and QFT results in different age groups

Assesment of agreement			Value	Standard error	p
Age group/Years	≤ 24	κ	.205	.174	.133
		N	43		
	25–44	κ	.393	.083	.000
		N	166		
	45–59	κ	.364	.076	.000
		N	166		
	≥ 60	κ	.213	.076	.006
		N	77		

results increased with age in a statistically significant manner (from 7.1% in the under 24 group to 48.8% in the 60 and more group). Multiple regression analysis revealed that the odds ratio, i.e. the possibility of being infected with M. tuberculosis, was about 5 times higher in individuals above 45 years of age and 12 times higher in individuals above 60 years of age, compared to individuals under 24 years of age. The usefulness of the QFT test as a tool for the detection of tuberculosis infection is supported by the trend of increasing prevalence of positive results with age. This trend is consistent with the incidence of tuberculosis infections, while the opposite is true in the case of prevalence of positive TST results (Figure 2).

The results of studies conducted by the authors of this work suggest that the QFT test shows a high consistency with the estimated numbers of infected individuals in Poland.

The analysis of the usefulness of the TST in diagnosing tuberculosis infections was conducted in the study population for two cut-off values: 10 mm or above and 15 mm or above. Positive TST results for the diameter of 10 mm or above were much more common than positive QFT test results (50.3% vs. 23.3%). In addition, positive TST results were observed with similarly high frequencies in the youngest and the medium age groups. Similar results were obtained by other authors [14, 15], which may be explained by positive reaction to tuberculin due to exposure to non-tuberculous bacilli in the environment or due to previous multiple BCG vaccinations. Anti-tuberculosis vaccinations result in limitation of TST specificity [16]. In populations, in which multiple BCG vaccinations were performed in line with vaccination schedules, as was until recently the case in Poland, specificity of TSTs is particularly low. In studies conducted by Pai et al. [17], specificity was 59%. A very large meta-analysis of studies including a total of more than 250 000 subjects showed that TST rarely produces positive results at 10 years after vaccination in populations having received a single vaccination immediately after birth (1%), while positive results are found 10 years after vaccination in as much as 21.2% of subjects if vaccinations were repeated after the 1st year of life [18]. Based on these studies, one may draw the conclusion that BCG revaccination performed after the 1st year of life is the cause of positive TST results persisting for many years. Such is the case in Poland, where, according to the vaccination schedule in force until 2005, BCG vaccination was repeated every several years until the age of 18. Due to false-positive results resulting from post-vaccination reactions and exposure to non-tuberculous bacilli, the usefulness of TST in the detection of tuberculosis infection is a subject of scepticism.

Data are available suggesting that the larger the diameter of the infiltration, the higher the probability of infection; therefore, many authors consider infiltrations of 15 mm or above to indicate a positive result. The results of this study, showing a higher consistency with QFT test results for positive TST results of 15 mm than for 10 mm, confirm that the 15-mm cut-off value is more specific. Meta-analysis of studies of the impact of BCG vaccinations on TST diameter showed that infiltrations with a diameter of 15 mm or more are due to *M. tuberculosis* infection, and not BCG vaccination [2]. However, it must be mentioned that, although the specificity of the test increases with the increasing TST infiltration diameter as the criterion for the positive result, test sensitivity decreases significantly, as evidenced by ROC in the test material.

The study showed that, contrary to the QFT test, the tuberculin skin test produced more common negative results in the age group above 60 years of age compared to the remaining age groups. Infiltrations with the diameters of 15 mm or larger were observed only in 14.1% patients aged

60 or older. This result is markedly lower than the estimated infection rate.

The disappearance of tuberculin reactivity in elderly subjects is known in the literature [12, 19] and is explained by poorer efficiency of the immune system in the elderly and by poorer reactivity of skin itself [12, 19, 20]. In their study on silicose patients, Leung et al. [21] showed that in elderly subjects above the age of 65, TST results were negative more frequently than in the group of younger subjects, while the results of IGRA tests were positive in older and younger subjects with similar frequencies.

Therefore, the TST was not a good indicator of tuberculosis infection in the population of this study as the percentage of positive results significantly exceeded the percentage of infected patients estimated on the basis of the register of incidence, showed no age-dependent differentiation, and decreased significantly at the age of over 60, although theoretically, latent infections with *M. tuberculosis* should be more common in the elderly. This shows that in the study population, the usefulness of the positive TST result of 10 mm as an indicator of the infection is very limited.

The prognostic value of TST results depending on size of induration is unclear [22]. Canadian studies on contacts with sputum-positive patients showed that the risk of infection increases with the TST diameter, suggesting that the size of the infiltration may matter; however, a more thorough analysis showed that the exposure to a sputum-positive patient is most important. The risk of infection following a close, domestic exposure was high even for small infiltration sizes of 0-4 mm, while in the case of out-of-home exposure, significance could be attributed to infiltrations of 10 mm or more [23].

The concordance between the TST and the QFT test in this study was very poor if TST results of 10 mm or more were considered positive (κ 0.198), and significantly better when the positive result cut-off value was 15 mm (κ 0.323). The poorest concordance between both tests was observed in younger age groups (≥ 24 and 25–44 years), in which the percentages of subjects with positive TST results were high and exceeded 50% for the cut-off diameter of 10 mm, while the percentages of subjects with positive QFT test results in these groups was much lower and did not exceed 10% in the under 24 years group and 20% in the 25–44 years group. Very poor agreement between the TST and QFT test results was reported by other authors [3, 20, 24, 25].

One of the goals of the study was to evaluate the impact of environmental factors on the incidence of tuberculosis infections. The study showed that the highest percentage of individuals with positive QFT test and TST results (regardless of cut-off value) was observed in healthcare professionals and was significantly higher than in the ZDR group (QFT 32.2% vs. 20.4%; $p < 0.01$). The incidence of *M. tuberculosis* infection assessed by means of the QFT test in the group of inmates was the same as in the ZDR group, i.e. in individuals from outside the healthcare professions or penitentiaries.

Conclusions

1. Latent infections with *M. tuberculosis* defined by positive QFT test results were much less common in the study group compared to when a positive TST result was considered to be the diagnostic criterion. The incidence of latent infection defined by the positive QFT result was similar to estimates derived from the tuberculosis morbidity register according to the Styblo principle.
2. The incidence of infections with *M. tuberculosis* as assessed by the QFT test was age-dependent and significantly lower among young subjects (7.1%) compared to older subjects (48.8%) and thus in line with the tuberculosis incidence trends.
3. The usefulness of TST in diagnostics of latent tuberculosis infection was poor. This might be due to false-positive post-vaccination reactions, particularly in younger age groups, and false-negative reactions in elderly patients, most probably due to anergy.
4. The concordance between the TST and QFT test results was very low if TST infiltration of 10 mm or more was considered to be a positive result, and higher when the positive result cut-off value was 15 mm.
5. The incidence of latent tuberculosis infection in healthcare professionals as measured by the QFT test was significantly higher than in individuals from outside this group (32.2% vs. 20.4%; p 0.01).

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