Dagmara Borkowska[1], Zofia Zwolska[1], Dorota Michałowska-Mitczuk[2], Maria Korzeniewska-Koseła[3], Anna Zabost[1], Agnieszka Napiórkowska[1], Monika Kozińska[1], Sylwia Brzezińska[1], Ewa Augustynowicz-Kopeć[1]

[1]Department of Microbiology, Institute of Tuberculosis and Lung Diseases in Warsaw
Head: Prof. Z. Zwolska, MD PhD
[2]Outpatient Clinic, Institute of Tuberculosis and Lung Diseases in Warsaw
Head: D. Michałowska-Mitczuk, MD PhD
[3]Department of Epidemiology and Organisation of Fight Against Tuberculosis, Institute of Tuberculosis and Lung Diseases in Warsaw
Head: Prof. M. Korzeniewska-Koseła, MD PhD

Interferon-gamma assay T-SPOT.TB in the diagnostics of latent tuberculosis infection

Interferonowy test T-SPOT.TB w diagnostyce latentnego zakażenia prątkiem gruźlicy

Abstract

Introduction: Diagnostics of latent tuberculosis infection (LTBI) has been based on a century-old tuberculin skin test (TST). However, a positive reaction can result not only from infection with *Mycobacterium tuberculosis*, but also from BCG vaccination or cross-reaction with nontuberculous mycobacteria. T-SPOT.TB assay is a new test to diagnose tuberculosis infection by measuring in vitro T-cell interferon-gamma release in response to two *Mycobacterium tuberculosis*-specific antigens: ESAT-6 and CFP-10.

Material and methods: T-SPOT.TB assay was performed on samples of whole blood (n = 137) from March to September 2010. Tuberculin skin test was carried out in 96 participants. A positive TST result was considered to be an induration of 10 mm or more.

Results: Of the 137 patients tested, T-SPOT.TB assay results were positive in 37 (27%), negative in 98 (71.5%) and indeterminate in only 2 (1.5%) persons. We analyzed T-SPOT.TB and TST results in 96 patients who were subjected to both tests. Concordance between T-SPOT.TB and TST results (a 10-mm skin reaction interpreted as positive) was 79%. Fifteen (15.6%) patients had a positive TST result and a negative T-SPOT.TB, and 5 (5.2%) patients had a negative TST result and a positive T-SPOT.TB. We observed a good correlation between positive T-SPOT.TB results and the diameter of induration of ≥ 15 mm in TST results.

Conclusions: T-SPOT.TB offers a more accurate approach than TST in the identification of tuberculosis infection. The study showed that the test T-SPOT.TB is a good diagnostic tool in identifying persons with tuberculosis infection. For a full confirmation of this assessment, it is necessary to examine more cases.

Key words: latent tuberculosis infection, IGRA tests, tuberculin skin test, interferon-γ, antigens ESAT-6 and CFP-10

Pneumonol. Alergol. Pol. 2011; 79, 4: 264–271

Introduction

Tuberculin has been applied for over 100 years and was introduced in times when the discoverer of *Mycobacteria tuberculosis*, Nobel Prize winner Robert Koch conducted studies aimed at finding a medicine against tuberculosis. As the year 2010 was celebrated as the centenary year of his death, it is worth devoting a few words to tuberculin discovery.

When Koch started searching for a drug against tuberculosis, he had already been a worldwide famous physician-bacteriologist and discoverer of *Vibrio cholerae*, the life cycle of *Bacillus anthracis* and *Mycobacterium tuberculosis*. He was an authority in the medical world and was highly re-

Address for correspondence: Prof. Ewa Augustynowicz-Kopeć, MD PhD, Department of Microbiology, Institute of Tuberculosis and Lung Diseases, Płocka 26, 01–138 Warsaw, tel./fax: 22 43 12 182; e-mail: e.kopec@igichp.edu.pl

The article was submitted to edition on 18 November 2010

ISSN 0867–7077

spected by German authorities. A full success in the fight against 'white plague' — tuberculosis — required the development of a drug which would save the human kind. Robert Koch prepared a liquid culture of mycobacteria in a bullion with glycerine and subjected it to a high temperature. Administered to Guinea pigs, the substance reversed the TB-related lesions. The extract seemed to be an anti-tuberculosis drug. When German authorities got to know about successful results of the studies on tuberculin, they 'induced' Koch to present his discovery in public. In 1890, he presented the results of his first clinical studies on humans. It was commonly believed that the studies were cross-checked and included a good clinical documentation. However, the reports of physicians and anatomopathologists disparaged tuberculin as a potential medicine [1–4]. 'Koch's lymph', which received its final name 'tuberculin' at initiative of a Polish physician and microbiologist, Odo Feliks Bujwid, found its application as a diagnostic test, later on in history [1]. In 1906, Clemens Peter Freiherr von Pirquet was the first to describe the application of tuberculin in the diagnostics of a current or a past infection with *M. tuberculosis*. He showed that subcutaneous injection of tuberculin causes a strong reaction in those individuals who had already come in contact with mycobacteria. In 1907, Charles Mantoux modified the method of tuberculin administration and introduced an intradermal injection, used until today [1, 4].

In Poland, an RT23 type (renset tuberculin, 23 series) of PPD tuberculin (purified protein derivative) has been used since 1966, produced at the Institute of Serum and Vaccine in Copenhagen. It is injected intradermally, on the palmar surface of the forearm, at a volume of 2 units [5, 6]. The result is read off after 48–72 hours, by measuring the diameter of the reaction, transversely to the long axis of the forearm [6]. Tuberculin induces DTH (delayed-type hypersensitivity), which is mediated by T cells and macrophages able to produce lymphokines that cause oedema, fibrin deposition, and inflow of other inflammatory cells. Delayed-type hypersensitivity can be detected within 2–4 weeks following infection [7, 8]. Tuberculin is a mixture of over 200 proteins, with antigen determinants common for the majority of pathogenic mycobacteria, MOTT (mycobacteria other than tuberculosis), and BCG strain (Bacillus Calmette-Guerin). That is why the sensitivity of the skin test is low. Positive results are only suggestive of a previous contact with mycobacteria and are not able to distinguish between *M. tuberculosis* or atypical mycobacteria [8–10]. In populations vaccinated with BCG, the skin reaction may be positive in some individuals even after 15 years from vaccination [11]. The results of tuberculin test may be false negative if the patient is too young or too old, suffers from immune diseases, cancer, HIV infection, or is treated with immunosuppressants. Pregnancy and severe forms of tuberculosis. e.g. acute miliary tuberculosis, may also constitute factors leading to no reaction to tuberculin [5, 8, 12, 13].

In Poland, where the whole population is vaccinated with BCG, it is important to establish whether the positive result of tuberculin skin test (TST) is connected with a previous vaccination or with the ongoing infection with *M. tuberculosis*.

In the recent years, new methods have appeared, diagnosing latent MTB infections, i.e. before disease development. These are immunological tests IGRA (interferon-gamma release assays), evaluating the release of IFN-γ (interferon gamma) by T cells [13, 14]. As opposed to TST, these assays use specific antigens of *M. tuberculosis* for stimulation. These antigens were isolated and obtained in a recombined form owing to a significant advancement in mycobacterial genetics [8]. They include: ESAT-6 (early secreted antigenic target 6 kDa) and CFP-10 (culture filtrate protein 10 kDa) [14]. These antigens are coded by the RD1 region (region of difference 1) present in the genome of *M. tuberculosis* and absent in *M. bovis* BCG or in MOTT strains, with a few exceptions: *M. kansasii, M. szulgai* and *M. mariunum* [15–17]. ESAT-6 antigen is a small secretory protein composed of 95 amino acids. It was observed that it strongly activates the response of T cells and is secreted by mycobacterium cells together with the CFP-10 protein [17–19].

There are two IGRA assays currently available on the market, used for a fast diagnosis of MTB infection: QuantiFERON-TbGold (Cellestis, Australia) and T-SPOT.TB (Oxford Immunotec, Great Britain). Their methodology is based on the measurement of the level of IFN-γ, a cytokine secreted by T cells in response to antigens ESAT-6 and CFP-10, with ELISA method (enzyme-linked immunosorbent assay) or on the identification of T cells secreting IFN-γ, with the ELISpot method (enzyme-linked immuno-spot assay) [15].

A new-generation test is the T-SPOT.TB assay, approved in 2005 by FDA Agency (Food and Drug Administration). It uses the ELISpot technique [15, 20] which combines the immunoensimatic method with a short-term culture of T cells. One of the advantages of ELISpot technique is its high sensitivity, which allows for a detection of a single T cell, activated by *M. tuberculosis* antigens [17].

The aim of this work was to assess the utility of the new IGRA T-SPOT.TB assay in the detection of latent MTB infection among healthy individuals and patients with suspected TB.

Material and methods

In the period from March to September 2010, 137 individuals were examined with the T-SPOT.TB assay. The IGRA test was performed in compliance with the producer's protocol. The amount of peripheral blood collected in heparinised test tubes (lithium heparin) depended on the patient's age. This was 6 ml in adults and children aged over 9 years. The tuberculin test was carried out in 96 out of 137 individuals examined with T-SPOT.TB assay. Those 96 patients (who had both tests) were divided into 3 groups, according to the diagnosis of the clinician, included in the referral letter: I group — 17 healthy volunteers, employees of the health care system, aged 27–73 years, II group — 49 patients with a suspicion of tuberculosis, aged 13–67 years, and III group — 30 patients with a suspicion of other diseases than tuberculosis, aged 25–63 years. The induration diameter was measured after 48–72 hours. Induration of at least 10 mm was considered as a positive result.

In the T-SPOT.TB assay, the isolated PBMC cells (peripheral blood mononuclear cells) are incubated for 16–20 hours in the presence of ESAT-6 and CFP-10 antigens. In individuals infected with *M. tuberculosis,* the sensitised T cells secrete IFN-γ, which binds to its specific primary antibodies. Next, enzyme-labeled secondary antibodies are added, which bind to other epitopes of the examined cytokine — IFN-γ. The colorimetric reaction of an enzyme with a colour substrate (chromogen), leads to the formation of an image of dark blue round spots [8, 12, 16, 20] (Fig. 1).

The result of the T-SPOT.TB assay may be considered as positive if the spot count, in at least one of the panels — A or B — amounts to minimum 6. The result may be considered as negative if in both panels, the spot count is lower than 5 (Fig. 2).

Results

In the group of 137 patients subjected to the T-SPOT.TB assay, a positive result was obtained in 37 cases (27%), and a negative result in 98 (71.5%) subjects. The result was indeterminate in 2 cases (1.5%) due to a low number of T cells (Fig. 3).

Further analysis included 96 out of 137 subjects who were also subjected to the TST test. There were 44 TST-positive subjects (45.8%); negative results were found in 52 individuals (54.2%). Positive results of both tests were obtained in 29 patients (30.2%). Disconcordance between both tests — i.e. positive TST and negative T-SPOT.TB — was found in 15 cases (15.6%). The second type of disconcordance — negative TST/positive T-SPOT.TB was revealed in 5 subjects (5.2%), while concordantly negative results were obtained in 47 patients (48.9%). Concordant results of both tests were obtained in 76/96 patients. The concordance between T-SPOT.TB and TST results was 79% (Fig. 4).

The group of 96 individuals who had a T-SPOT.TB assay and a tuberculin skin test was

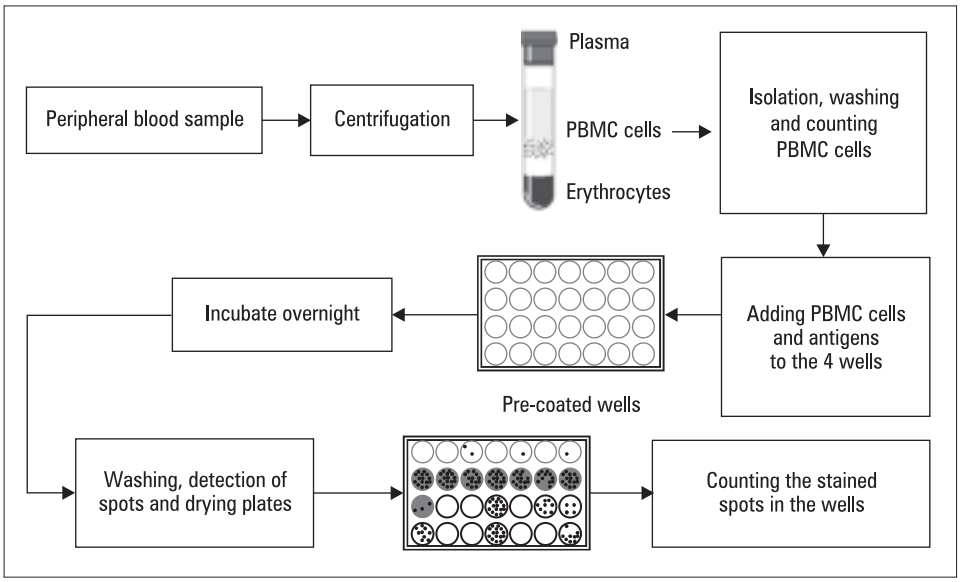

Figure 1. The main steps of the T-SPOT. TB assay (www.oxfordimmunotec.com)

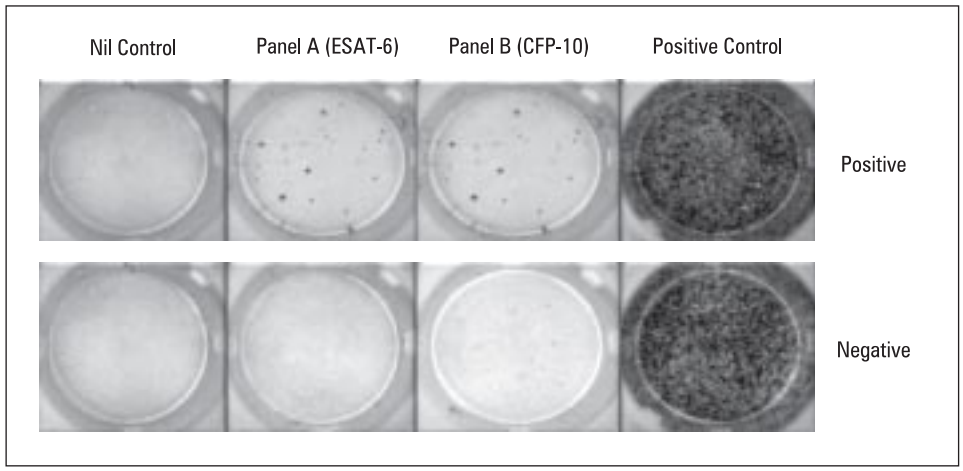

Figure 2. T-SPOT.TB assay results interpretation

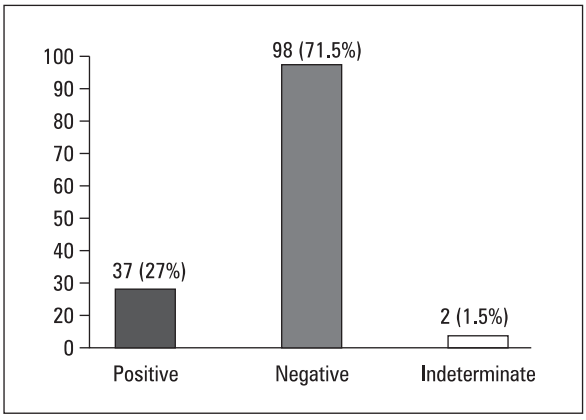

Figure 3. T-SPOT.TB results for tested group (n = 137)

divided into 3 study subgroups: healthy volunteers (health care workers), patients with suspected tuberculosis and patients with suspicion of other diseases than tuberculosis. From among 17 examined volunteers, 7 (41.2%) with a positive result of T-SPOT.TB test were also TST-positive. In 4 T-SPOT.TB-negative patients (23.5%), there was a positive result of TST test. Six individuals (35.3%) were negative for both tests. The concordance between test results in the group of healthy volunteers amounted to 76.5% (Table 1).

In the group of 49 patients with suspected tuberculosis, 26 (53.1%) were T-SPOT.TB-positive, four of which (8.2%) were TST-negative. In the group of 23 T-SPOT.TB-negative subjects (46.9%), 8 patients were TST-positive (16.3%). Positive results of both tests were found in 22 patients with suspected tuberculosis (44.9%) and negative results in 15 patients (30.6%). The concordance between T-SPOT.TB and TST results was 75.5% (Table 2).

In the group of 30 patients with suspicion of other diseases than tuberculosis, none of the examined patients obtained a positive result of both tests. Only 1 person (3.3%) was T-SPOT.TB-positive and TST-negative. Other patients were IGRA-negative, and only 3 of them were TST-positive. The concordance between test results in this group amounted to 86.7% (Table 3).

In the group of 96 patients who underwent IGRA and TST test, positive results of the T-SPOT.TB test correlated with the diameter of tuberculin induration. The result of the T-SPOT.TB test was assigned to one of 3 groups, depending on the diameter of the induration: below 10 mm, 10–15 mm, above 15 mm. In the group with induration diameter < 10 mm, there were 6 (6.2%) positive results of IGRA test. In the group of induration diameter of 10–15 mm, there were 8 (8.3%) IGRA-positive patients. The highest number of T-SPOT.TB-positive results (21, i.e. 21.9%) was reported for the induration diameter of over 15 mm. A positive correlation was found between the number of positive T-SPOT.TB results and the diameter of induration of over 15 mm (Fig. 5).

Discussion

According to the estimations of the WHO (World Health Organization), one third of the world's population is infected with *M. tuberculosis*. In approximately 10% of LTBI cases (latent tuberculosis infection), the infection develops into an active disease [16, 20]. The risk of the disease increases in individuals with recent infections or with a comprised immune system [9, 21].

So far, the *M. tuberculosis* infection has been diagnosed with tuberculin reaction showing a relatively low specificity [18]. Moreover, it is diffi-

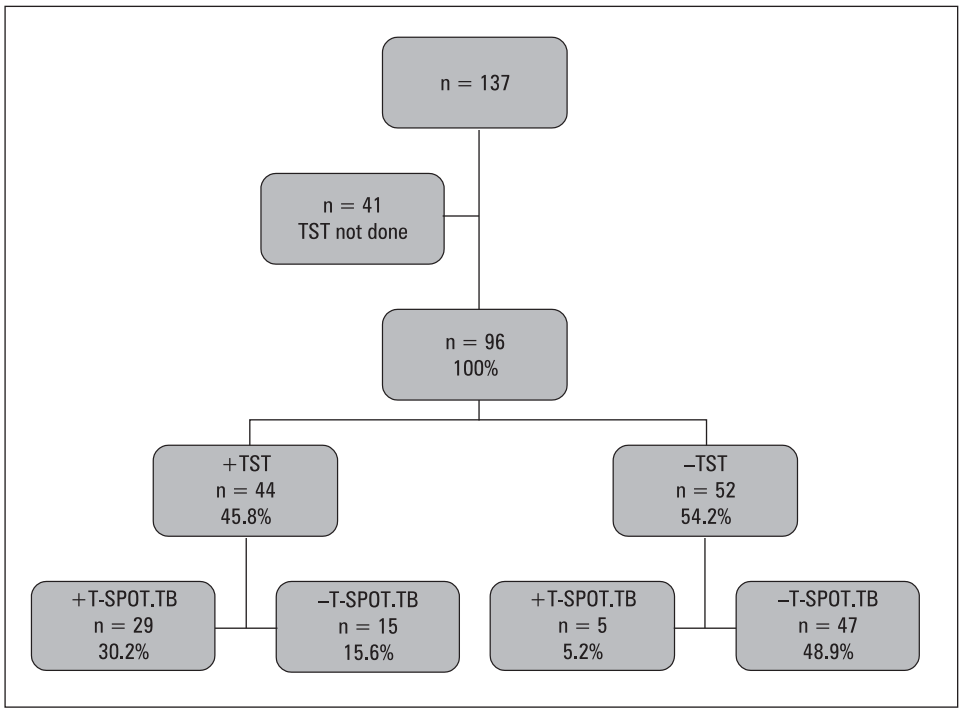

Figure 4. Distribution of T-SPOT.TB results in TST-positive and TST-negative patients (n = 96)

Table 1. Comparison between the results of the T-SPOT.TB assay and TST in health care workers group (n = 17)

T-SPOT.TB	TST					
	Positive		Negative		Total	
	n	%	n	%	n	%
Positive	7	41.2	0	0	7	41.2
Negative	4	23.5	6	35.3	10	58.8
Total	11	64.7	6	35.3	17	100

cult to conduct the test and interpret its results correctly, and the patient must visit the health care facility twice [9, 22, 23]. In Poland, with the population being subjected to BCG vaccinations, positive TST results may be connected with vaccinations. In the T-SPOT.TB test, the applied complex of selected antigens of *M. tuberculosis* increased the specificity of the test by reducing the cross-reaction with BCG vaccine and with the majority of the environmental mycobacteria [7, 9, 10]. The test includes two separate panels — A and B, to which antigens ESAT-6 and CFP-10 are added, which guarantees an optimal sensitivity of the test and allows for a detection of TB infection among individuals with a negative result of tuberculin test. Due to the lack of a golden standard in LTBI diagnostics [24], it is impossible to establish the sensitivity or specificity of T-SPOT.TB and TST tests. This correlation was not defined in any of the studies describing the correlation of both tests. Therefore, our work analysed the concordance between the results of both tests, which could possibly be considered as a substitute method used for estimation of the risk of TB infection. Results of multiple studies have confirmed that the concordance between IGRA and TST test amounts to 60–80% [16].

According to Brodie et al., a general concordance between T-SPOT.TB and TST results among immigrants vaccinated with BCG amounted to 64%, and to 82% in a group of unvaccinated individuals [25]. Mazurek et al. reported that the results of the QuantiFERON-TB test (the second one from among IGRA assays) and of the TST test are concordant in 83%. The study was conducted among 1226 adults, with different risk rates of TB infection. Disconcordance of the following type: negative IGRA and positive TST results, was 7 ti-

Table 2. Comparison between the results of the T-SPOT.TB assay and TST in patients with clinical suspition of tuberculosis (n = 49)

T-SPOT.TB	TST					
	Positive		Negative		Total	
	n	%	n	%	n	%
Positive	22	44.9	4	8.2	26	53.1
Negative	8	16.3	15	30.6	23	46.9
Total	30	61.2	19	38.8	49	100

Table 3. Comparison between the results of the T-SPOT.TB assay and TST in patients with diseases other than tuberculosis (n = 30)

T-SPOT.TB	TST					
	Positive		Negative		Total	
	n	%	n	%	n	%
Positive	0	0	1	3.3	1	3.3
Negative	3	10	26	86.7	29	96.7
Total	3	10	27	90	30	100

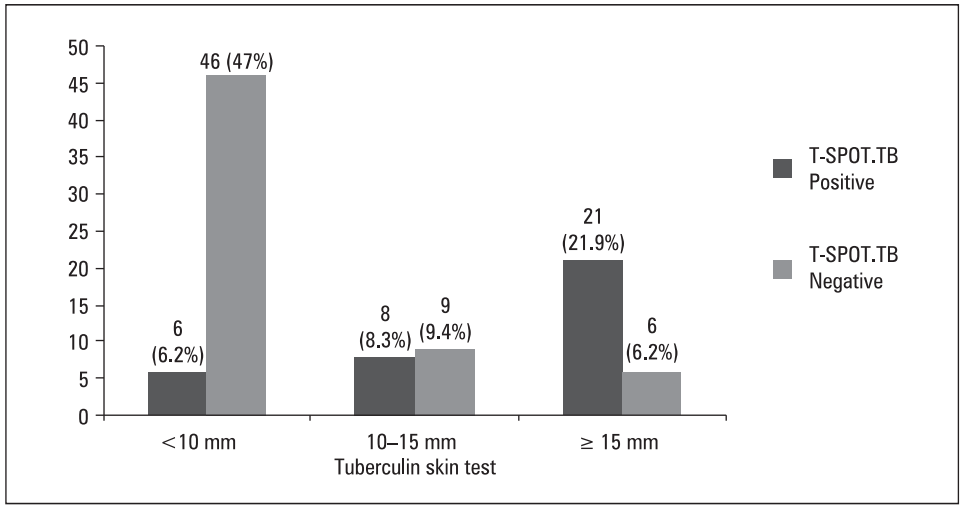

Figure 5. Distribution of T-SPOT.TB results and tuberculin skin test results-stratified according to the size of induration (n = 96)

mes more frequent among patients vaccinated with BCG than in unvaccinated individuals [26].

Fietta et al. found a concordance between the results of the above mentioned tests of 78%, among 258 patients with a different rate of risk of *M. tuberculosis* infection. The IGRA test showed the presence of infection in 91% of the patients, and the TST test in merely 65% of them. Most of the IGRA-positive and TST-negative patients had at least one factor increasing the risk of tuberculosis, but also the risk of false negative result of tuberculin test: older age, hepatitis C, alcohol addiction, cancer, use of steroids or renal insufficiency [27].

In own studies, a general concordance between T-SPOT.TB and TST results of 96 individuals amounted to 79%. Ewer et al. studied latent TB infections among pupils of one of the British schools. They applied the T-SPOT.TB and the HEAF test (an equivalent of TST, used in the population of children in Great Britain). A general concordance of the tests amounted to 89%. It was revealed that the fact of BCG vaccination did not influence the results of the T-SPOT.TB test. However, induration diameters in HEAF tests in BCG-vaccinated children were much higher than in those who had not been vaccinated. Positive IGRA results with

negative TST results pointed to a higher probability of TB infection than positive TST results with negative IGRA results [10].

Own studies have shown both types of disconcordance between test results: positive TST and negative T-SPOT.TB results in 15 cases (15.6%), which could be related to BCG vaccination, and negative TST with positive T-SPOT.TB results in 5 subjects (5.2%), which could be caused by the lack of skin reaction to tuberculin, due to patient's age or a poor health state. Nienhaus et al. found a similar disconcordance rate. The disconcordance — positive TST and negative T-SPOT.TB results — was revealed in 12.1% of subjects, while the disconcordance — negative TST and positive T-SPOT.TB results — was found only in 3.1% [28].

In the conducted analysis, every of three groups (healthy volunteers — health care workers, patients with suspected tuberculosis and patients with suspicion of other diseases than tuberculosis) included a higher rate of individuals with a positive TST result than with a positive T-SPOT.TB result.

Ferrara et al. found that among 255 patients who underwent the QuariFERON-TB Gold and TST test, the number of positive IGRA results increased with an increasing diameter of the induration [9]. Own studies conducted among 96 individuals showed similar results. The lowest number of positive T-SPOT.BT results, i.e. 6 (6.2%) was obtained in individuals with the diameter of tuberculin induration of less than 10 mm, while the highest number — 21 (21.9%) — in individuals with a reaction size of over 15 mm.

IGRA tests did not show false positive results in BCG-vaccinated patients. They may be frequently repeated, as there is no boosting effect, observed with repeated TST tests, leading to false positive results [14]. Tuberculin includes ESAT-6 and CFP-10 antigens used in T-SPOT.TB test. Therefore, tuberculin test conducted before blood sampling for IGRA assays may lead to unreliable results of interferon assays. This can be confirmed by the results of some recently conducted studies which showed that sensitised T cells produce interferon-γ for up to 6 months following TST [13, 30].

An advantage of the T-SPOT.TB test is a short time-to-result (1–2 days) and no need for patient's revisit to the health care facility. Blood for the test is sampled only once from the patient. Interpretation of test results is simple and indeterminate results are extremely rare. In own studies, there were only two cases (in 137) of such results, which constituted 1.5%. T-SPOT.TB may be helpful in TB risk detection among employees of laboratories, prisoners, the homeless, people in close contact with patients with TB, especially children, and patients qualified to biological treatment [8, 9, 13, 29, 30]. Latent infections, although asymptomatic and not posing any direct epidemiological risk, are an obstacle for patients with chronic infectious diseases to be qualified to biological treatment. Patients with psoriasis, rheumatoid arthritis, Crohn's disease, treated with TNF (tumour necrosis factor) antagonists undergo a higher risk of tuberculosis. Therefore, it is necessary to perform both the TST and the IGRA test in those patients, to identify LTBI and to introduce a prophylactic treatment of TB [30].

Conclusions

1. The conducted studies showed that the T-SPOT.TB test is a good diagnostic tool in the identification of individuals infected with TB. For a complete confirmation of this thesis, it is necessary to conduct a study with a larger sample size.
2. When comparing two tests (TST and T-SPOT.TB), the highest concordance of results was found for the induration of at least 15 mm in diameter.
3. In the conducted studies, the concordance of both tests amounted to approximately 79%.

References

1. Zwolska Z. Robert Koch — twórca bakteriologii chorób zakaźnych. Via Medica, Gdańsk 2006.
2. Augustynowicz-Kopeć E., Zwolska Z. Postępy w diagnostyce i epidemiologii molekularnej *Mycobacterium tuberculosis*. Konferencja Naukowa „Mikrobiologia 100 lat po Robercie Kochu" PTM, Warszawa 2010.
3. Zwolska Z. Koch i jego dokonania — rys historyczny. Konferencja Naukowa „Mikrobiologia 100 lat po Robercie Kochu" PTM, Warszawa 2010.
4. Gradmann C. Robert Koch and the white death: from tuberculosis to tuberculin. Microbes Infect. 2006; 8: 294–301.
5. Kruczak K., Skucha W., Duplaga. M., Sanak M., Niżankowska-Mogilnicka E. Assessment of the latent tuberculosis infection (LTBI) with QuantiFERON-GIT (QFT-GIT) assay in selected risk groups in Krakow. Borgis — Nowa Medycyna 2009; 1: 37–42.
6. Kang Y. A., Lee H. W., Yoon H. I. et al. Discrepancy between the tuberculin skin test and the whole-blood interferon γ assay for the diagnosis of latent tuberculosis infection in an intermediate tuberculosis-burden country. JAMA 2005; 293: 2756–2785.
7. Kruczak K., Niżankowska-Mogilnicka E. The new diagnostics methods of latent tuberculosis infection. Pneumol. Alergol. Pol. 2008; 76: 446–450.
8. Lalvani A. Diagnosing tuberculosis infection in the 21st century. New tools to tackle an old enemy. Chest 2007; 131: 1898–1906.
9. Ferrara G., Losi M., Meacci M. et al. Routine hospital use of a new commercial whole blood interferon-γ assay for the diagnosis of tuberculosis infection. Am. J. Respir. Crit. Care Med. 2005; 172: 631–635.
10. Ewer K., Deeks J., Alvarez L. et al. Comparison of T-cell-based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak. The Lancet 2003; 361: 1168–1173.
11. Paluch-Oleś J., Kozioł-Montewka M. Evaluation of usefulness of QuantiFERON-TB Gold in tube assay and tuberculin skin test for immunodiagnosis of *Mycobacterium tuberculosis* infection. Borgis — Nowa Medycyna 2009; 1: 32–36.

12. Ferrara G., Losi M., D'Amico R. et al. Use in routine clinical practice of two commercial blood tests for diagnosis of infection with *Mycobacterium tuberculosis*: a prospective study. The Lancet 2006; 367: 1328–1134.
13. Demkow U. Commentary to the article of K. Kruczak and E. Niżankowska-Moglicka: "The new diagnostic methods of latent tuberculosis infection". Pneumonol. Alergol. Pol. 2008; 76: 468–471.
14. Ziołkowski J. Gruźlica dziecięca. Borgis, Warszawa 2010.
15. Van Leeuwen R.M.L., Bossinka W.J., Thijsen S.F.T. Exclusion of active *Mycobacterium tuberculosis* complex infection with the T-SPOT.TB assay. Eur. Respir. J. 2007; 29: 605–607.
16. Pai M., Riley L. W., Colford J.M. Jr Interferon-γ assay in the immunodiagnosis of tuberculosis: a systematic review. Lancet Infect. Dis. 2004; 4: 761–776.
17. Lalvani A. Counting antigen-specific T cells: a new approach for monitoring response to tuberculosis treatment? CID 2004; 38: 757–759.
18. van Pinxteren L.A.H., Ravn P., Agger E.M., Pollock J., Andersen P. Diagnosis of tuberculosis based on the two specific antigens ESAT-6 and CFP 10. Clin. Diagn. Lab. Immunol. 2000; 2: 155–160.
19. Harboe M., Oettinger T., Wiker H. G. et al. Evidence for occurrence of the ESAT-6 protein in *Mycobacterium tuberculosis* and virulent *Mycobacterium bovis* and for its absence in *Mycobacterium bovis BCG*. Infect. Immun. 1996; 64: 16–22.
20. Pai M., Kalantri S., Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: part I. Latent tuberculosis. Expert Rev. Mol. Diagn. 2006; 3: 413–422.
21. Stefan D.C., Dippenaar A., Detjen A.K. et al. Interferon-gamma release assays for the detection of *Mycobacterium tuberculosis* infection in children with cancer. Int. J. Tuberc. Lung Dis. 2010; 6: 689–694.
22. Demkow U., Filewska M., Białaś B. et al. Antimycobacterial antibody level in pleural, pericardial and cerebrospinal fluid of patients with tuberculosis. Pneumonol. Alergol. Pol. 2004; 72: 105–109.
23. Leung C.C., Yam W.C., Yew W.W. et al. T-Spot.TB outperforms tuberkulin skin test in predicting tuberculosis disease. AJRCCM 2010; 182: 834–840.
24. Shams H., Weis S.E., Klucar P. et al. Enzyme-linked immunospot and tuberculin skin testing to detect latent tuberculosis infection. Am. J. Respir. Crit. Care Med. 2005; 1161–1168.
25. Brodie D., Lederer D.J., Gallardo J.S., Trivedi S.H., Burzynski J.N., Schluger N.W. Use of a interferon-γ release assay to diagnose latent tuberculosis infection in foreign-born patients. Chest 2008; 4: 869–874.
26. Mazurek G.H., LoBue P.A., Daley C.L. et al. Comparison of a whole-blood interferon gamma assay with tuberculin skin testing for detecting latent *Mycobacterium tuberculosis* infection. JAMA 2001; 286: 1740–1747.
27. Fietta A., Meloni F., Cascina A. et al. Comparison of a whole-blood interferon-gamma assay and tuberculin skin testing in patients with active tuberculosis and individuals at high or low risk of *Mycobacterium tuberculosis* infection. Am. J. Infect. Control. 2003; 31: 347–353.
28. Nienhaus A., Schablon A., Diel R. Interferon-gamma release assay for the diagnosis of latent TB infection — analysis of discordant results when compared to the tuberculin skin test. PLoS One. 2008; 7: e2665.
29. Lalvani A. Counting antigen-specific T cells: a new approach for monitoring response to tuberculosis treatment? CID 2004; 38: 757–758.
30. Korzeniewska-Koseła M. Prevention of tuberculosis in patients treated with tumour necrosis factor antagonists. Reumatologia 2010; 48: 4–13.