*Dagmara Borkowska, Ewa Augustynowicz-Kopeć

Can Interferon-Gamma Release Assays be helpful for the diagnosis of pulmonary and extrapulmonary tuberculosis?

Czy testy IGRA mogą okazać się pomocne w diagnostyce gruźlicy płuc i postaci pozapłucnych?

Department of Microbiology, National Tuberculosis and Lung Diseases Research Institute, Warsaw
Head of Department: prof. Ewa Augustynowicz-Kopeć, MD, PhD

Key words

Interferon-Gamma Release Assays, pleural effusion, bronchoalveolar lavage fluid, tuberculosis

Słowa kluczowe

testy IGRA, płyn z opłucnej, popłuczyny oskrzelowo-pęcherzykowe, gruźlica

Summary

The diagnosis of pulmonary tuberculosis and tuberculous pleurisy standard methods is difficult and slow. Now, high hopes to cells of T cells secreting interferon-gamma in response to antigens of *Mycobacterium tuberculosis*. Use of the immune response to TB diagnosis. A systematic review was performed of latest publications. Interferon-γ release assays (IGRA) on peripheral blood mononuclear cells cannot distinguish active from latent tuberculosis infection. But detection of *Mycobacterium tuberculosis*-specific T-cells in the bronchoalveolar lavage or pleural effusion using IGRA (ELISPOT assay) is a promising tool for the diagnosis of active pulmonary or extrapulmonary tuberculosis in patients with negative acid-fast bacilli smears and suspected tuberculosis patients. In TB, antigen-specific T-cells expand and are recruited to the site of infection. Positive T-SPOT.TB performed in BALf and another fluid could be helpful in rapid TB diagnosis. At present, there is little research. Further studies are needed to confirm the usefulness of IGRA to detect active TB.

Streszczenie

Rozpoznanie gruźlicy płuc i gruźliczego zapalenia opłucnej standardowymi metodami diagnostyki mikrobiologicznej jest trudne i długotrwałe. Obecnie, duże nadzieje wiąże się z komórkami limfocytów T wydzielającymi interferon-gamma w odpowiedzi na antygeny *Mycobacterium tuberculosis* i wykorzystanie tej reakcji immunologicznej do diagnostyki gruźlicy. W pracy przedstawiono przegląd najnowszych publikacji dotyczących tego tematu. Testy IGRA wykonane z krwi obwodowej nie rozróżniają aktywnej postaci choroby od latentnego zakażenia prątkami gruźlicy. Wykrywanie limfocytów T w BALf i płynie z opłucnej przy użyciu testu T-SPOT.TB (technika ELISPOT) jest obiecującym narzędziem do diagnostyki zarówno gruźlicy płuc, jak i postaci pozapłucnych. Takie wykorzystanie testu może okazać się istotne dla chorych z negatywnym wynikiem bakterioskopii plwociny lub chorych podejrzanych o gruźlicę niepotwierdzoną bakteriologicznie. W aktywnej gruźlicy limfocyty T wydzielają interferon-gamma i wędrują do miejsca zmienionego chorobowo. Dodatni wynik T-SPOT.TB z BALf i innych płynów może być pomocny w szybkiej diagnostyce gruźlicy. W chwili obecnej ilość opublikowanych wyników badań uniemożliwia jednoznaczną ocenę przydatność IGRA do wykrywania aktywnej gruźlicy z materiałów diagnostycznych takich jak BALf i płyn z opłucnej.

Address/adres:

*Dagmara Borkowska
Department of Microbiology
National Tuberculosis and Lung Diseases Research Institute
ul. Płocka 26, 01-138 Warszawa
tel. +48 (22) 431-21-79
fax +48 (22) 431-21-82
d.borkowska@igichp.edu.pl

INTRODUCTION

Tuberculosis remains a major health problem, approximately 8 million of newly detected patients are recorded each year in the world and approximately 1.5 million deaths (1, 2). In Poland, 7250 cases of tuberculosis were recorded in 2013 and the incidence of all forms of tuberculosis was 18.8 and it is still higher than the average incidence rate in the European Union. Pulmonary tuberculosis was the dominant form of tuberculosis in Poland, 94.3%, and extrapulmonary tuberculosis accounted for approximately 5.7% of the registered cases. The most common form of extrapulmonary location was tuberculous pleurisy – 34.2% of all cases. In accordance with the recommendations of the WHO obtaining cultures of *M. tuberculosis* complex from diagnostic material of patients still remains the gold standard for the diagnosis of tuberculosis. In Poland, bacteriological confirmation of TB was ob-

tained for 4825 patients in 2013 which accounted for 66.6% of all patients (3).

Due to the sparsely mycobacterial nature of diagnostic materials obtained from patients with suspected tuberculosis, achieving a positive microbiological test result is, in many cases, not possible. Despite the application of modern diagnostic methods, obtaining a microbiological result confirming a TB case sometimes lasts for several weeks (4, 5). Therefore, the whole time we are searching for methods of approximating diagnosis and putting them into routine diagnostics. Serological diagnostics, that is, detection of antibodies against a pathogen, plays a supporting role in many infectious diseases. In the case of tuberculosis, due to a marginal part of the humoral response as a response to infection by a mycobacterium, these methods have not been applied in the diagnostic panel.

The cutaneous tuberculin test still plays an auxiliary role in the diagnosis. Tuberculin RT 23 is a mixture of 200 non-specific antigens that can be administered intradermally in the volar surface of the forearm. The skin reaction result is read after 48-72 hours, measuring the diameter of infiltration. Positive TST result does not decide whether an infection is caused by a tuberculosis mycobacterium, Bacille Calmette-Guerin vaccination (BCG), non-tuberculosis mycobacteria (4, 6). Results of numerous studies have shown limited specificity of the tuberculin test, particularly in populations vaccinated with BCG.

At present, high hopes are associated with the release of innovative interferon-γ release assays (IGRA). WHO and ECDC recommend performing IGRAs of peripheral blood for the diagnosis of latent tuberculosis infection, even before clinical symptoms (1, 7). As a re sult of infection with *Mycobacterium tuberculosis*, T cells producing interferon-gamma appear in blood. During IGRAs, another T-cell stimulation happens with antigens encoded in the RD1 region of the genome of *Mycobacterium tuberculosis*: ESAT-6, CFP10. Measuring the amount of interferon-gamma release is an indicator of infection. IGRA tests from peripheral blood cannot distinguish between the active form of tuberculosis and latent infection with *Mycobacterium tuberculosis*. Results of the IGRD T-SPOT. TB test used in the case of other diagnostic materials than blood, such as cerebrospinal fluid, bronchoalveolar lavage fluid and pleural fluid, point to new diagnostic possibilities of tuberculosis and its extrapulmonary form (8).

IGRA TESTS

Interferon-Gamma Release Assays (IGRA) from peripheral blood for the diagnosis of latent tuberculosis infection have were approved for clinical use in 2001 (5). The advantage of IGRA tests over the tuberculin test is a lack of cross-reactivity with BCG vaccine and most environmental mycobacteria (6, 7, 9-12). The assays used antigens specific for *Mycobacterium tuberculosis*: ESAT-6, CFP 10, TB7.7, RD1 encoded in the region of the genome of *Mycobacterium tuberculosis*. The region does not exist in the strains belonging to the BCG vaccine strain species *Mycobacterium bovis* and environmental mycobacteria, excluding *M. marinum*, *M. kansasii* and *M. szulgai* (6, 7, 9-12). In Poland, there are two IGRAs available: QuantiFERON and T-SPOT TB. The principle of operation is based on the measurement of interferon-gamma (IFN-γ), as produced by T lymphocytes in response to antigens of *Mycobacterium tuberculosis* or identification of T cells secreting IFN-γ (7, 9-12). The advantage of IGRA is to get a result in less than 2 days, simple interpretation is based on standardized methods and there is no need to re-visit a patient in a healthcare facility, as is the case of TST (tab. 1) (4, 6, 12).

Table 1. Brief characteristics of IGRAs of peripheral blood.

IGRA blood tests	T-SPOT.TB	QuantiFERON (TB GOLD or TB GOLD IN-TUBE)
Measurement technique	ELISPOT	ELISA
RD1 region antigens	ESAT-6, CFP10	ESAT-6, CFP10 (TB7.7, IN-TUBE version)
Material and amount	Peripheral blood, the amount depends on the age of the subject: 2 ml children under 2 years of age, children 2-4 years 4 ml, 6 ml older children and adults. One tube of lithium heparin	1 ml of peripheral blood in each of 3 special test tubes from the manufacturer
Visit to the facility	1	1
Time to get a result	Two days	Two days or longer
Sensitivity	78-95%	46-78%
Specificity	87-100%	98-100%
BCG and environmental mycobacteria	No cross-reaction	No cross-reaction
Principle of reading	Number of single cells secreting IFN-γ, or dark blue spots – spots on the well membrane	Concentration of IFN-γ in the tube
Interpretation – positive result	Number of spots ≥ 6 at least in one of two wells with antigens, negative control ≤ 10 spots, positive control ≥ 20 spots	Conc. of IFN-γ ≥ 0.35 IU/ml and ≥ 25% of the negative control, negative control ≤ 8.0 IU/ml, positive control of each range

Recently, in the world literature, there have been reports on the use of IGRAs for the diagnosis of active lung tuberculosis with BALf and the extrapulmonary form with pleural fluid. The phenomenon of cellular compartmentalization of immune response occurs during the active tuberculosis (4). In the peripheral blood, immune cells, particularly T-lymphocytes, after antigen recognition, proliferate and are transformed into memory cells, migrating to the lesion site. On the surface of the memory cells, there are homing receptors that

enable targeted cell transfer. As a result of the selective recruitment of leukocytes, the cells necessary for effective defense reaction are attracted to inflammatory sites (13). In connection with that, the number of T cells is significantly higher in the lesion site, e.g. in the lungs, pleura, than in the peripheral blood. This may be one reason why IGRA has negative results in patients with severe forms of tuberculosis (4, 14, 15). Jafari et al. showed in their study that the active form of tuberculosis from bronchoalveolar lavage fluid with negative smear result can be quickly confirmed using the T-SPOT.TB test (16-19). Results of other studies have shown the ability to confirm the tuberculous pleurisy using the T-SPOT.TB test of pleural fluid (2, 20-23).

THE USE OF THE T-SPOT.TB IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS AND THE EXTRAPULMONARY FORM – REVIEW OF THE LITERATURE

The hypothesis about the possibility of the use of IGRA for the diagnosis of tuberculosis has led to the creation of a series of studies on the use of materials other than blood for IGRA testing (T-SPOT.TB). Identification of antigen-specific lymphocytes at the site of infection of the cells of bronchoalveolar fluid or the fluid from the pleura may improve the diagnosis of tuberculosis in patients with negative smear results, and can severely affect the decision to start antituberculous treatment.

Application of IGRA testing of body fluids in the diagnosis of tuberculosis (BALf) and the extrapulmonary form (pleural fluid, peritoneal fluid, cerebrospinal fluid PMR) is connected with the possibility of measuring the gamma-interferon produced in response to contact with tuberculosis in affected areas. Losi et al., in their study, used the T-SPOT.TB test for the diagnosis of tuberculous pleurisy. They tested 20 patients suspected of having tuberculosis pleurisy, simultaneously performing the T-SPOT.TB assay of peripheral blood and fluid from the pleural in this group of patients. The sensitivity and specificity of the T-SPOT.TB test for the diagnosis of tuberculous pleurisy was estimated, respectively, at 95 and 76%. They concluded in the results that the T-SPOT.TB assay in clinical practice may be used for quick and accurate diagnosis of pleural tuberculosis (21). Strassburg et al. reported a clinical case of a 38 year-old woman who received immunosuppressive therapy lasting more than 8 months. The patient complained of malaise, shortness of breath, fever, and cough. The X-ray and computed tomography of the chest revealed changes in both lower lobes of the lungs. TST result was negative and amounted to 0 mm, microbiological testing of sputum and bronchial secretions showed tuberculosis. Bronchoscopy was performed and the patient BALf analysis resulted in the positive T-SPOT.TB test. Antimycobacterial treatment was administered 18 days before obtaining the *M. tuberculosis* complex culture of diagnostic materials. In the summary of the results of the publication the authors found that the ELISPOT technique of BALF could be a useful auxiliary diagnostic assay for active pulmonary TB in immunosuppressed patients (24). Trajman et al., due to the many limitations of conventional diagnostic methods for the diagnosis of tuberculous pleurisy, presented a series of modern research aimed at improving the diagnosis. One of them was the detection of cytokines, in particular interferon-gamma in the pleural effusion. The sensitivity and specificity of their research results estimated at 89 vs 97%. Measuring IFN-γ levels, they found it to be a better marker for the detection of tuberculosis in pleural effusion than interleukins (IL-2, 6, 8, 12, 18) (22). Jafari et al., in subsequent papers, on the usefulness of the T-SPOT.TB of BALf, concluded that the T-SPOT.TB may facilitate the diagnosis of pulmonary tuberculosis. Despite the fact that the specificity of the test was lower than that of molecular studies, the authors suggest that routinely performing bronchoscopy in patients with suspected tuberculosis with AFB (-) should be accompanied by IGRAs the result of which can improve the diagnosis of tuberculosis and the inclusion of antituberculous treatment (17-19).

Zhou et al., on the basis of a meta-analysis for the diagnosis of tuberculous pleurisy, using the ELISPOT technique of peripheral blood and the pleural, found that the yield of the T-SPOT.TB test, including the sensitivity and specificity of this method, was much lower than expected. They pointed out that, due to the high cost of IGRAs and technical difficulties during testing, these tests can only be used as an additional auxiliary tool. Given the current state of knowledge and lack of experience, the authors do not support the use of IGRA in the routine diagnosis of tuberculous pleurisy (23).

Cho et al., in their results of studies on the use of the ELISPOT method for the diagnosis of tuberculous peritonitis, concluded that the test may be a promising tool for the diagnosis of this form of extrapulmonary tuberculosis, but more research is needed on the methodology concerning primarily the proper separation of T cells. At the present stage of the research, of a large number of indeterminate results prevents the introduction of IGRAs of peritoneal fluid for the diagnosis of tuberculosis (25). Cattamanchi et al. analyzed the results of the T-SPOT.TB test of BALf in patients infected with HIV or AIDS in populations with high rates of TB incidence. The largest percentage of the studied pool of patients accounted for indeterminate results. The results of these studies differed considerably from the research conducted in Europe, because the sensitivity and specificity of the tests were unsatisfactory (16-19, 21, 26).

In a study on improving the diagnosis of tuberculous pleurisy, ADA and ADA2 isoenzyme concentrations were assessed, and the level of IFN-γ in the markers of inflammation. In addition, results of the T-SPOT.TB test were assessed in the study group. A group of 88 pa-

tients were examined, including 31 with confirmed pleural tuberculosis. It was found that the concentrations of ADA, ADA2 and the levels of interferon-gamma in the pleural fluid may be a determinant of tuberculosis and the results of the test T-SPOT.TB were unsatisfactory. Too many indeterminate test results discriminated the tool as a tool for the diagnosis of tuberculosis with pleural effusion (27). Similarly, in the paper by Dhed et al., too high reactivity of the foreign antigen in the negative control was found which prevented the unambiguous interpretation of negative or positive results (28).

The sensitivity and specificity of the T-SPOT.TB test varied depending on the study. Material type probably affected results, as well as the country of origin of patients. Sensitivity was calculated at 73-95% and specificity at 48-100% (18, 21, 25-27, 29, 30) (tab. 2).

CONCLUSIONS

The presented overview of recent literature shows that IGRA tests are of interest to many scientists, trying to use them both for a rapid diagnosis of pulmonary tuberculosis and the extrapulmonary form.

Tests performed using a commercial T-SPOT.TB kit are likely to accelerate the diagnosis of tuberculosis. However, not all of the results of this study confirm the usefulness of IGRA as an auxiliary test for the diagnosis of tuberculosis (27, 28). The current state of knowledge and discrepancies within research findings prevent an unambiguous assessment of the suitability of IGRA tests for the detection of active tuberculosis. They cannot be introduced into the panel of diagnostic tests. Research should continue on a larger, more diverse group of patients with suspected tuberculosis.

Table 2. Sensitivity and specificity of the T-SPOT.TB test, review of the literature.

Papers	Country	Sensitivity (%)	Specificity (%)	Pool (n)	Material
Losi et al. 2007	Italy, Germany, the Netherlands	95	76	20	Pleural fluid
Lee et al. 2008	Taiwan	94.7	85.7	40	Pleural fluid
Jafari et al. 2009	Germany	91	79	347	BALf
Kim et al. 2009	South Korea	83	100	48	Pleural fluid
Cho et al. 2011	South Korea	86	67	64	Peritoneal fluid
Cattamachi et al. 2012	Uganda	73	48	94	BALf
Keng et al. 2013	Taiwan	85.7	55.3	88	Pleural fluid

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received/otrzymano: 30.01.2015
accepted/zaakceptowano: 05.03.2015