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Interferon-gamma-based tests as a new tool in the diagnostics of latent tuberculosis

Testy uwalniania interferonu gamma nowym narzędziem do wykrywania ukrytego zakażenia prątkiem gruźlicy

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Despite increasing advances in the diagnostics and treatment of tuberculosis (TB), this condition remains a significant health and social problem [1–5]. According to the estimates of the World Health Organization, approximately 2 billion people are infected with tuberculosis [1, 5]. There are about 8 million new cases (including 1.5 million children) and 3 million deaths (500 000 children) every year [1, 5]. Spreading of HIV infections, as well as social and economic factors (homelessness, malnourishment, drug addiction, migrations) are associated with an increasing morbidity [1, 5–8].

Even though the incidence of tuberculosis in Poland is considerably lower than in Africa or South-East Asia, our epidemiological indices have been among the worst in the entire Europe for years [2, 3, 7].

The natural history of tuberculosis includes three stages: exposure, infection, and active disease [7, 8]. Following contact with the bacilli, only some of the exposed individuals get infected [2, 7, 8]. Latent tuberculosis is defined as a state in which there are no clinical, radiological, or bacteriological symptoms [9]. It has been estimated that with no additional risk factors, the risk of disease reactivation among latent cases amounts merely to 0.1% a year, which may be extrapolated to a lifetime risk of disease development of 10% [7, 8, 10].

Infected individuals constitute a group which will be a source of new tuberculosis infections for

many years [7]. Countries with low TB morbidity are very strict about monitoring the contact sources and prophylactic management of infected individuals [9, 10].

One of the important factors in qualification to chemoprophylaxis of tuberculosis, both in Poland and all over the world, is the result of the tuberculin sensitivity test [7, 8, 11]. However, a significant limitation of this diagnostic method is its low specificity in individuals vaccinated with BCG (*Bacillus Calmette-Guerin*), because a positive result of this test may in their case constitute an anamnestic response to BCG antigens [12–14]. Moreover, due to similarities between different types of bacillus antigens, the positive result may follow from cross-reactions between a mycobacterium tuberculosis antigen present in the tuberculin and antigens of nonpathogenic environmental bacilli [12, 15].

In the recent years, a new method has been developed to identify infected individuals with a latent form of the disease, before its conversion into an active form [10, 16–20]. New diagnostic tests are based on the assessment of INF- γ (interferon gamma) released by human peripheral blood leukocytes activated by specific TB antigens (IGRA, interferon gamma release assays) [17–19].

Two types of IGRA tests with different methodologies were developed:

- test evaluating the amount of released interferon in short-term cultures of full blood cells

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stimulated by TB antigens (Quantiferon-TB Gold — Cellestis);

- test in which the number of cells producing interferon after stimulation is counted (T-spot-TB — Oxford Immunotec).

Both IGRA tests used antigens of *Mycobacterium tuberculosis* — early secreted antigenic target 6kDa (ESAT-6) and culture filtrated protein (CFP-10) [21–28]. Both antigens are co-secreted in 1:1 ratio in short-term cultures of tubercle bacilli [23]. Antigens ESAT-6 and CFP-10 are encoded by genes of the RD-1 region, absent from all subtypes of BCG strain and most of the nontuberculous mycobacteria (except for *M. szulgai*, *M. marinum* and *M. kansasii*) [13, 27, 28]. Homologue of ESAT-6 exists in *M. leprae*. However, despite a homology of 36%, cross-reactions may be expected in countries where *M. leprae* infections are present. Both ESAT-6 and CFP-10 are antigens of low molecular mass, preferentially recognised and acting as main stimulators of INF- γ production in the early stages of experimental infections with *Mycobacterium tuberculosis* [29, 30]. Both antigens are found on the same operon, remain under control of the same promoter, are actively released by *M. tuberculosis*, and in the end form a dimer. Although they are two separate polypeptides, they are presented to the cells of the immune system in equimolar amounts and at the same time. Thus, they may be treated as one antigen. A limited diversity of T cells in the early period of infection determines high sensitivity of the tests based on the above mentioned antigens [22, 23]. In the later phases of infection, the lymphocytic immune response is more heterogeneous, and the reaction to these antigens may be less intense [22, 29, 30]. Therefore, tests based on them may be useful in the detection of *M. tuberculosis* infections in an early phase, long before the development of an active, clinically evident phase of the disease [29, 30]. Response to ESAT-6 and CFP-10 is complementary, and thus it seems rational to evaluate the response to both antigens [21, 22]. The available literature concludes that the diagnostic utility of the tests based on above mentioned antigens, applied in the detection of *M. tuberculosis* infections, is very high, with specificity amounting to 90–99% and sensitivity to approximately 72–97% [13, 20, 24–28]. Lack of cross-reactions with bacteria of the BCG strain and majority of the environmental mycobacteria, as well as the fact that it is an objective and quantitative diagnostic method with a possibility of frequent repetitions without causing the booster effect, constitute the main advantages of the IGRA test over the tuberculin sensitivity test [13, 17–19, 27, 28].

This test is also advantageous because it is inexpensive and does not require a lot of labour (only one patient visit is necessary) and is associated with a lower number of mistakes following from subjective performance and interpretation of test results [18].

Introduction of IGRA tests based on antigens that are not found in the BCG strains allows for a differentiation between the post-vaccination reactivity and a latent tuberculosis infection. With this test, we have a unique possibility to directly detect the presence of *M. tuberculosis* infection in the population of patients in which tuberculosis vaccinations are common. This may aid clinicians in making appropriate decisions considering chemoprophylaxis of tuberculosis in BCG-vaccinated subjects.

However, due to relatively small clinical experience with IGRA tests, enormous heterogeneity of the immune response to antigens of tuberculosis mycobacteria, and diversity of genetic and environmental factors that affect the course of infection, it is necessary to conduct wide-ranging studies that would include different populations.

The current issue of “Pneumonologia i Alergologia Polska” includes an article of Borkowska et al. [31], evaluating the interferon-gamma assays T-SPOT.TB in a group of 137 patients under care of the Outpatient Clinic at the Institute of Tuberculosis and Lung Diseases in Warsaw, and among healthy employees of the hospital laboratory.

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