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The IGRA tests: where are we now?

Testy IGRA — gdzie jesteśmy dzisiaj?

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In the early 1990s, WHO declared TB a global emergency [1]. Since that time after introduction of DOTS strategy and National TB Programs, the reduction of global incidence rate (2002), as well as of total number of TB cases (2006) were recorded. However, in 2012, about 8.6 million cases were established, of which only two third were registered. It means that 3 million cases were missed and untreated. According to the new-post 2015 Global TB Strategy, TB epidemic will end by 2035, when the incidence rate decrease to < 10 TB cases/100 000 population.

Can IGRAs be helpful to achieve this goal?

Approximately ten years ago, alternative to TST – IFN-gamma release assays (IGRAs) were introduced for diagnosis of latent TB infection (LTBI). Two commercial IGRA tests are currently available: QuantiFERON-TB GOLD IN Tube (QFN-GIT) (Cellestis, Carnegie, Australia) and T-SPOT.TB (Oxford, Immunotec, Abingdon, UK). They are based on the detection of IFN-gamma secreted by T cells stimulated by three antigens specific for M. tuberculosis: early secretory antigenic target (ESAT)-6, culture filtrate protein (CFP)-10 encoded in region of difference (RD)-1 and TB 7.7 encoded in RD 11. The advantage of IGRA tests over the tuberculin skin test (TST) is the lack of cross-reactivity with M. bovis BCG strains and with the majority of nontuberculous mycobacteria (NTM). Moreover, IFN-gamma assays, as in vitro tests, may be performed repeatedly without booster effect.

Although there is no gold standard for diagnosis of LTBI, IGRAs identify this population better, compared to TST. But in many areas in Europe, including Poland, identification of subjects with LTBI relies on a much cheaper and easier to perform (without the requirement of special laboratory equipment) TST. The analysis of four studies assessing IGRAs for the diagnosis of LTBI revealed the pooled specificity at 98% for T-SPOT.TB and at 100% for QFN-GIT in low risk population, compared to 89% for TST [2]. Moreover, negative predictive value (NPV) for progression to active disease among 1,442 healthy persons scored negative by QFN-GIT was 99.8% in 2 yrs follow-up and 97.8% for T-SPOT.TB (182 individuals). In the light of the above data, the likelihood of false negative results, although probable, is really low.

As one third of global population is infected with Mycobacterium tuberculosis, it means that 200 million of people will develop active TB during their life. Can IGRAs be helpful in identifying subjects for preventive treatment? Data from different studies including HIV-positive and HIV-negative patients demonstrated that though IGRAs may better predict–progression to active

disease than TST (positive predictive value — PPV 14% and 3%, respectively), still more than 85% of those with positive IGRAs did not develop active TB [2].

For–case detection and curative treatment remain the cornerstone of TB control, during the last decade, many studies have been conducted to assess the clinical utility of IGRAs in diagnosis of active TB, especially in smear and culture negative patients. However, sensitivity of IGRAs varies from 50−65%, with a specificity of about 80% depending on epidemiological background (low or high burden countries), age or immune conditions [3, 4]. Meta-analysis revealed that the pooled sensitivity of IGRAs was 80% for all spectrum of TB cases (smear positive, culture positive and culture negative) and the pooled specificity was 79%. Although the values were higher than those for TST (the pooled sensitivity and specificity were 65% and 75%, respectively [5]), but still not high enough to use these tests as rule out assays for active TB. Nevertheless, quite different to QFN-GIT, T-SPOT.TB performed on extrasanguinous samples such as pleural fluid, bronchoalveolar lavage fluid (BALF) reached high pooled sensitivity (88%) and high pooled specificity (82%) [5]. If these data are confirmed in larger studies, for the first time this IFN-gamma assay can be used as immunodiagnostic test for active TB.

Special consideration should be paid to immunocompromised patients: HIV-positive, those with renal failure, diabetes or on immunosuppressive drugs. It is well-known that HIV co-infection increases the risk of active TB by reactivation of latent infection or by favouring the progression of recently acquired infection towards active disease. Although it might be expected that IGRAs in HIV-positive population are less affected by immunosuppression than TST, it is not so evident. Meta-analysis data from the high and low-burden countries revealed that QFN-GIT sensitivity for active TB range between 61% and 68%, and between 65% and 72% for T-SPOT.TB [6]. But when QFN-GIT and TST were compared, the pooled sensitivity was similar [6]. However, like in immunocompetent population, the IGRAs should not be used for diagnosis of active TB, as one third of patients could be missed. Neither there is enough evident data for replacement of TST with IGRAs for identifying HIV-positive subjects with LTBI.

Surprisingly, the results of the studies assessing the impact of immunosuppression on IGRAs are inconclusive. Some studies indicated lower sensitivity of QFN-GIT with CD4 bellow 200 cells/ /mm^3 [7], while the others did not find any differences [8] or even reported higher sensitivity in patients with CD4 T lymphocytes below 200 cells/ /mm^3 (T-SPOT.TB) [9]. Also Gooovaerts et al., who examined IFN-gamma responses to ESAT-6 and CFP-10, did not find any differences between those with TB-associated immune reconstitution inflammatory syndrome (TB-IRIS) and non-IRIS controls [10].

Taking into account the results of recently published studies and meta-analysis, WHO expert panel advised against using IGRAs for diagnosing active TB irrespective of HIV status [11]. With no doubt, currently used IGRAs can not replace bacteriological methods or NAAT (nucleic acid amplification test).

Not typical immunosuppression, but a kind of systemic anergy is present in patients with sarcoidosis. In this issue of "Pneumonologia i Allergologia Polska", Kempisty et al. [12] contribute to our knowledge of IGRAs spectrum in such patients. In their study of 151 patients, BCG vaccinated in the past with confirmed sarcoidosis, QFN-GIT was performed in all of them, while T-SPOT.TB only in a subgroup of subjects. They represented all stages of sarcoidosis with different activity. It is worth noting that it is the first study in which both IGRA tests were performed in patients with sarcoidosis. In such population two aspects should be taken into consideration: decreased systemic immune reactions and the common mycobacterial antigens. Due to different populations of Treg cells: CD4+CD25+FOXP3+, CD4+CD39+ peripheral immune reactions, like TST are inhibited, while the local ones are enhanced (so-called immune paradox). However, the positive results of QFN-GIT were similar to that reported by the others, and reflected TB prevalence in different settings: low in Denmark [13] and high in India [14]. Also in our own study conducted by Piotrowski et al (under review), the frequency of positive QFN-GIT did not differ from that in age-matched control group with low exposure to M. tuberculosis infection. The activity of the disease was not associated with IGRAs results. Of note, authors did not demonstrate intermediate IGRAs results, which affected mainly QFN-GIT and to a lesser extent T-SPOT.TB in immunocompromised populations [15].

Secondly, it has been suspected for more than 100 years, that mycobacteria or more likely their products elicit immune responses leading to sarcoidosis. Mycobacterial proteins and DNA have been found within sarcoidosis granulomas,

as well as adoptive immune response to these targets were widely demonstrated [16−18]. Recently, ESAT-6 – one of the three proteins used in IGRAs has been detected in sarcoidosis tissue. Moreover, in half of the patients with sarcoidosis, the response of CD4 and CD8 T cells from BALF to ESAT-6 was noticed [16]. However, Kempisty et al. [12] found comparable IGRAs results for that of local population. So, from the diagnostic point of view, IFN-gamma assays, quite opposite to TST, seem to be reliable methods for detection of LTBI in sarcoidosis patients as well.

In conclusion, the indication for IGRA tests is still the diagnosis of LTBI, especially in BCG-vaccinated populations. These tests can not differentiate LTBI from active disease or those who would benefit from preventive treatment. As ESAT-6 is secreted in all stages of latency and in active TB, new immune-based tests with new infection-phases specific antigens are needed. High NPV of QFN-GIT shows the opportunity to use this test to exclude TB infection or even active disease in some clinical situations.

Conflict of interest

The author declares no conflict of interest.

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