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LATENT TUBERCULOSIS INFECTION

Renata Zrinski Topić

Department of Clinical Laboratory Diagnosis, Srebrnjak Children's Hospital, Zagreb, Croatia

Summary

Latent tuberculosis infection (LTBI) is a subclinical infection with *Mycobacterium (M.) tuberculosis*. Children and immunosuppressed individuals due to pathologic conditions or therapeutic procedures are at a high risk of reactivation of LTBI to active disease compared to general population and require timely identification. The diagnosis of LTBI is based on the measurement an adaptive immune response against *M. tuberculosis*. The use of tuberculin skin test (TST) has been associated with a number of interfering factors that cause false-positive or false-negative test results. The new *ex vivo* whole blood tests determining interferon-gamma (IFN-γ) released from T-lymphocytes (interferon-gamma release assays, IGRAs) upon stimulation with *M. tuberculosis* specific antigens.

The introduction of IGRAs to routine clinical practice has improved the diagnosis of LTBI, but recommendations and guidelines for the diagnosis of LTBI are not consistent between different European countries. Therefore, European centre for disease prevention and control developed guidance document based on the up-to-date scientific evidence.

Novel concepts of IGRAs and new studies should be designed so as to provide answers to all open questions.

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

Reports of the World Health Organisation (WHO) state that approximately one third of the world's population is infected with *Mycobacterium (M.) tuberculosis* [1]. However, a minority of those individuals will ever develop active tuberculosis (TB). It is estimated that approximately 5% of immunocompetent infected subjects develop clinical disease within two years of infection (primary TB), and another 5% over their lifetime (post-primary TB or "reactivation"). The remaining 90% of immunocompetent infected subjects never progress to active disease.

Corresponding author: Renata Zrinski Topić
e-mail: renata.zrinski-topic@zg.t-com.hr

Latent tuberculosis infection (LTBI) is a state of persistent *M. tuberculosis* specific cell-mediated immune response in the absence of active disease. Although individuals with LTBI do not manifest clinical symptoms and are not contagious to other, they are at increased risk for developing manifest disease. The increased reactivation of LTBI has been observed in immunocompromised situations such as acquired or chronic diseases, and therapeutically induced immunodeficiencies.

In the United States and Europe, a significant efficiency in control of TB was seen in the 1950s. However, increase of new cases of TB was seen in the 1980s. Although the migration of people from high-prevalence countries is significant epidemiological factor of TB in developed countries, the most cases of TB in the domestic population of the developed countries in the last 30 years occured as result of LTBI reactivation.

Therefore, the earliest possible diagnosis of LTBI is a precondition for control and potential elimination of TB. Preventive measures for LTBI are considered one of the crucial actions in the management of tuberculosis as a public health problem worldwide and development of new tools into TB control is one of component of the new global Stop TB Strategy [2].

PATHOMECHANISM OF TUBERCULOSIS INFECTION

Infection with *M. tuberculosis* starts with phagocytosis of mycobacterium by pulmonary dendritic cells. The recognition of the mycobacterium components (19 and 27 kDa lipoproteins, 38 kDa glycolipoprotein, lipomannan, mannose-capped lipoarabinomannan) are mediated by many host receptors (Toll-like receptors, C-type lectins, nucleotide-binding oligomerization domain-like receptors and the other). The interaction of mycobacterium with host receptors initiates an intracellular signaling cascade that results in a nonspecific proinflammatory response [3]. Dendritic cells migrate to regional lymph node and activate both $CD4^+$ and $CD8^+$ specific T lymphocyte. The important characteristic of cell-mediated immune response is production of interferon-gamma (IFN-γ), the key cytokine for a immune response against *M. tuberculosis*. IFN-γ induces the transcription of more than 400 genes [4]. Their activation contribute to activation of alveolar macrophages, production of additional cytokines and induction of additional immune cells, production of effectors such as oxygen radicals and reactive nitrogen intermediates. The granuloma formation at the site of infection separates *M. tuberculosis* from the rest part of the lung and controls its replication. TNF-α is another major cytokine for a immune response against *M. tuberculosis*. It stimulates the phagocyte capacity of macrophages synergising with IFN-γ. Moreover, TNF-α is also responsible for the granuloma for-

mation, and is involved in both immune and immunomodulatory responses [5,6]. In the majority of infected subjects, an effective cell-mediated immune response develops two to eight weeks after infecton with *M. tuberculosis*. However, the mycobacterium is completely removed in approximately only 10% subject, while in the remaining individuals the pathogen evades and modulates the host immune response. *M. tuberculosis* responds to the host immne system with transcriptional activity of many of its 4000 different genes that may lead to increased survival and persistence of the mycobacterium in the host. The early secretory antigenic target 6 (ESAT-6)/culture filtrate protein 10 (CFP-10) complex is example of *M. tuberculosis* protection mechanism [7]. The complex is secreted by live bacteria and is cruical for the virulence of *M. tuberculosis*. Moreover, ESAT-6/CFP-10 complex disociate at low pH (acidification of phagosome in alveolar macrophage), and ESAT-6 inserts itself into phagosome lipid bilayer, causing lisis of phagosome and escape *M. tuberculosis* from phagosome. ESAT-6 also induces apoptosis of macrophage by caspase dependent pathway, and causes cytolisis of alveolar epithelial cells causing dispersion of *M. tuberculosis* in extracellular milieu. These mechanisms together with additional *M. tuberculosis* factors may result in the latent infection. The current definition of LTBI is based on a dynamic model of infection where endogenous reactivation and effective cell-mediated immune response occur constantly in immunocompetent individuals [8]. On the other hand, if disruption of cell-mediated immunity (depletion or functional abnormalities of T lymphocytes, TNF-α absence) occurs the infected individuals develop active disease.

LTBI AND RISK FACTORS

There are two categories of individuals who are risk for tuberculosis infection.

The first group are individuals who are at increased risk for *M. tuberculosis* infection: direct exposure to tuberculous patient (a member of family, a healthcare worker), a foreign-born pearson from high-incidence TB areas and children [9-13]. The second group are individuals who are at higher risk of reactivation of LTBI to active disease compared to general population or are at higher risk for a poor clinical outcome (disseminated and extrapulmonary TB) [14]. It is documented that immune suppression is very important predispose factor for progression of LTBI to TB (Table 1).

Immunocompromised individuals represents a heterogeneous group which includes [15]:

- Children (immature immune system) [16]
- Immunodeficiency disorders (human immunodeficiency virus infection, HIV) [17]

- Chronic disorders (chronic kidney disease, diabetes mellitus) [18,19]
- Immunosuppresion associated with infection or malignancies [20]
- Immunosuppresion associated with therapeutic procedures (organ transplantation, chemotherapy, TNF-α antagonist, systemic corticosteroids) [21,22]

The most effective way to avoid reactivation of LTBI following immunosuppressive therapies is early diagnosis and treatment of the LTBI before treatment of the main disorder.

Table 1 Risk of LTBI reactivation in immunocompromised subjects

Immunodeficiency	Increased risk compared to general population
Disease	
HIV	50 - 200
Malignancies	10 - 15
Renal failure	6 - 50
Diabetes mellitus	3
Therapy	
TNF-α antagonist	> 25
Corticosteroids	> 5
Immunosuppressive	20 - 74

HIV; human immunodeficiency virus infection, TNF; tumour necrosis factor

DIAGNOSIS OF LTBI

The diagnosis of LTBI is based on the measurement an adaptive immune response against *M. tuberculosis* because direct measure of latent infection is not possible. This response may be measured as delayed type hypersensitivity response by the tuberculin skin test (Mantoux test) six to eight weeks after exposure to the *M. tuberculosis*, or as production T lymphocyte cytokine IFN-γ by interferon-gamma release assays two to eight weeks after exposure to *M. tuberculosis*.

For about a century, the diagnosis of tuberculosis infection, of both active and latent, was made by use of *in vivo* tuberculin skin test (TST). The test is based on the measurement of delayed hypersensitivity reaction upon intradermal administration of purified protein derivative (PPD). TST determines the activity of effector and memory T-lymphocytes and measures the reaction induced by IFN-γ, interleukin (IL) 4, IL 10, IL 12, TNF-α and granulocyte colony-stimulating factor (G-CSF). A significant limitation of the TST is heterogeneity of PPD preparation, a poorly defined

mixture of more than 200 mycobacterium proteins and protein fragments, including antigens from the vaccine strain *M. bovis* Bacillus Calmette-Guerin (BCG) and from non-tuberculosis mycobacteria. Therefore, the test does not differentiate an immune responses toward previous BCG vaccination, non-tuberculosis mycobacteria infection and *M. tuberculosis* infection [23,24]. Moreover, specificity and sensitivity of the TST are diminished by the variable cut off values used on positive TST determination. As no positive control is used, the test may produce false-negative results due to immunosuppressive conditions [25]. However, the TST does not require additional infrastructure to be performed.

LABORATORY DIAGNOSIS OF LTBI

The diagnosis of tuberculosis infection has been improved with the new *ex vivo* tests which measure antigen specific T lymphocyte function. The basis of this tests are effector T lymphocytes in blood of individuals infected with *M. tuberculosis* which could recognize mycobacterial antigens. Due to the short life of effector T lymphocytes, their presence in sample diagnose an ongoing infection. The new tests measure IFN-γ released from effector T lymphocytes (interferon-gamma release assays, IGRA) upon *ex vivo* stimulation with specific peptides to *M. tuberculosis*, ESAT-6, CFP-10 and TB7.7 (Rv2654) [26-28]. These peptides are encoded within the region of difference (RD) 1 (ESAT-6, CFP-10), and RD 11 (TB7.7) of the *M. tuberculosis* genome and belong to a group of 0.5% of all antigens to this bacterium, thus differentiating it from the majority of other mycobacteria (except *M. kansasii*, *M. szulgai*, *M. marinum*, *M. flavescens*, *M. gastrii*) [29,30].

Up to date, two IGRAs are commercially available, QuantiFERON-TB Gold In-Tube (QFT-IT, Cellestis Ltd., Carnegie, Victoria, Australia) and T-SPOT.TB (Oxford Immunotec Ltd., Oxford, UK). The primary differences between two IGRAs are the sample preparation and the method for detection of IFN-γ production. QFT-IT, an enzyme-linked immunosorbent assay, uses whole blood and three specific peptides to *M. tuberculosis* (ESAT-6, CFP-10, TB7.7), and determines IFN-γ concentration. T-SPOT.TB, an enzyme-linked immunosorbent spot, uses purified peripheral blood mononuclear cells and only two (ESAT-6, CFP-10) specific peptides to *M. tuberculosis*, and determines the percentage IFN-γ releasing lymphocyte.

The very important advantage of IGRAs over TST lies in the possibility of performance quality control. Negative control offers an insight into the nonspecific IFN-γ sinthesis or indicates the presence of heterophil antibodies in the sample. Positive control determines IFN-γ released from T-cells upon stimulation with the mitogen phytohemagglutinin (PHA). Positive control is used to assess the patient's

immune status (lymphocyte count and activity) and preanalytical sample manipulation, thus reducing the rate of false-negative results and increasing the negative predictive value of IGRA [31]. A reduced mitogen response, indeterminate result, is the uninterpretable result of IGRAs, and whole assay should be repeated in a new blood sample [32].

The advantage and disadvantage of IGRAs and TST are sumarized in Table 2.

Table 2 Comparison of interferon-gamma release assays and tuberculin skin test

	IGRAs	**TST**
Sample	Peripheral blood	Skin
Antigen	Specific *M. tuberculosis* antigens	Mixture of mycobacterial antigens
Reaction cells	Effector T-lymphocytes	Effector and memory T-lymphocytes
Cytokine	IFN-γ	IFN-γ, IL4, IL10, IL12, TNF-α, G-CSF
Result	*ex vivo* IFN-γ	*in vivo* induration
Effect of BCG vaccination	None	May give a false-positive result
Effect of non-tuberculous mycobacteria	None, except *M. kansasii*, *M. szulgai*, *M. marinum*, *M. flavescens*, *M. gastrii*	May give a false-positive result
„Boosting“	No	Yes
False-negative result	No, if use positive mitogen control	Possible (immunosuppressed subject)
Time to obtain result	At least 24 hours	At least 48 (72) hours
Subjectivity	No	Yes
Second visit	No	Yes
Required equipment	Yes	No
Distinguish active/latent infection	No	No

IGRA, interferon-gamma release assay; TST, tuberculin skin test

The sensitivity and specificity of IGRAs for diagnosis LTBI can not be reliably estimated because there is no gold standard method for the diagnosis of LTBI. Therefore, the sensitivity has been evaluated in patients with TB and the specificity in individuals with no known risk or low-risk factors of tuberculosis infection. In the

context of mentioned limitation in assessment of the sensitivity of IGRAs the European centre for disease prevention and control (ECDC) did not assess the sensitivity of IGRAs for LTBI diagnosis [33]. The pooled specificity of IGRAs in the diagnosis of LTBI was about 99% [34]. Evaluation of positive predictive value (PPV) and negative predictive value (NPV) of IGRAs to predict risk for reactivation of LTBI is not possible at this moment and further studies are indispensable.

The utility of IGRAs varies between different types of immunocompromised individuals.

Although the high sensitivity of IGRAs allows for accurate diagnosis of LTBI in high risk individuals, indeterminate IGRAs results have been more frequently reported in immunosuppressed subjects [35,36]. Based on the available results, IGRAs may be used as part of a combined approach based on IGRAs and TST in order to maximise the accuracy of diagnosis in high-risk groups but IGRAs should not be used to exclude diagnosis of LTBI in the immunocompromised individuals [37].

Numerous considerations on the application and cost effectiveness of screening with IGRAs have been published [37-39]. Although they are based on different models, their conclusion is corresponded. A dual screening strategy (TST followed by IGRAs) or IGRAs alone strategy are more cost effective than TST single screening. However, this conclusion may be dependent on the population being screened. Therefore, each national tuberculose control program should evaluate cost effectiveness of IGRAs for their own setting.

Recommendations and guidelines for the diagnosis of LTBI are not consistent between different European countries, because there are different TB-incidence countries with characteristic TB epidemiology. Therefore, ECDC developed guidance document [33] based on the up-to-date scientific evidence.

In conclusion, the introduction of IGRAs to routine clinical practice has improved the diagnosis of LTBI. However, still better diagnostic tests are needed. Therefore, novel concepts of IGRAs and new studies should be designed so as to provide answers to all open questions.

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Sažetak

Latentna tuberkulozna infekcija

Latentna tuberkulozna infekcija (LTBI) definira se kao supklinička infekcija bakterijom *Mycobacterium (M.) tuberculosis*. Kod djece i osoba oslabljenog imunološkog sustava, zbog pojedinih patoloških stanja ili terapijskih postupaka, postoji veći rizik za aktivaciju LTBI-ja u aktivnu tuberkulozu u odnosu na opću populaciju, te je takvu infekciju potrebno pravovremeno otkriti.

Dijagnostika LTBI-ja temelji se na određivanju imunološke reakcije usmjerene protiv *M. tuberculosis*. Primjena tuberkulinskog testa otežana je brojnim čimbenicima koji mogu uzrokovati lažno pozitivne i lažno negativne rezultate testa. Novi dijagnostički pristup temelji se na *ex vivo* testovima iz pune krvi kojima se određuje IFN-γ oslobođen iz T-limfocita (*interferon-gamma release assays*, IGRAs) nakon podražaja specifičnim antigenima za *M. tuberculosis*.

Primjena IGRAs-a u rutinskoj kliničkoj praksi unaprijedila je dijagnostiku LTBI-ja, ali preporuke i smjernice za dijagnostiku LTBI-ja nisu podudarne u pojedinim europskim zemljama. Zbog toga je Europski centar za prevenciju bolesti i kontrolu pripremio smjernice utemeljene na najnovijim znanstvenim podatcima.

Unaprjeđenja IGRAs-a i nova istraživanja treba kreirati tako da se mogu dati odgovori na sva otvorena pitanja.

Ključne riječi: IGRA; latentna tuberkulozna infekcija; tuberkulinski test