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Review

THE ROLE OF INTERFERON-GAMMA RELEASE ASSAYS IN DIAGNOSIS OF LATENT TUBERCULOSIS INFECTION IN CHILDREN

Mateja Šegović[1], Iva Mihatov Štefanović[2,3] and Ivan Pavić[4,5]

[1]Health Center of Varaždin County, Varaždin, Croatia;
[2]Department of Pediatrics, Sestre milosrdnice University Hospital Center, Zagreb, Croatia;
[3]School of Dental Medicine, University of Zagreb, Zagreb, Croatia;
[4]Department of Pulmonology, Allergology, Rheumatology and Clinical Immunology, Zagreb Children's Hospital, Zagreb, Croatia;
[5]School of Medicine, University of Split, Split, Croatia

SUMMARY – Despite numerous published papers, diagnosis of latent tuberculosis infection (LTBI) in children is still an undefined area. The importance of this topic lies in the fact that one third of the world's population is infected with *Mycobacterium (M.) tuberculosis*. The majority of infected individuals are LTBI cases which make a reservoir for future active tuberculosis (TB) patients. The gold standard for LTBI detection is still undetermined and this is due to the effect of various confounding factors on existing diagnostic tests. Until a decade or so ago, throughout the last century, tuberculin skin test (TST) was the only diagnostic test for LTBI. Due to scientific advances, new *in vitro* assays, interferon-gamma release assays (IGRAs) were discovered recently. The sensitivities of IGRAs are a bit better than those of TST, while great progress has been made in increasing the specificity of IGRA relative to TST. Nevertheless, in the diagnosis of LTBI in children, TST still has some advantages. However, generations of IGRAs have brought many diagnostic advantages that are emphasized in this review. In a difficult procedure of diagnosing LTBI in children, performance of IGRA could be the key factor in making decision whether to use preventive therapy or not.

Key words: *Children; Interferon-gamma release assay; Tuberculin skin test; Latent tuberculosis*

Introduction

Latent tuberculosis infection (LTBI) is defined as infection with *Mycobacterium (M.) tuberculosis* without clinical, radiological, or bacteriological evidence for active tuberculosis (TB), but with the risk of progression from infection to active TB disease[1]. Overall, a lifetime risk of progression to TB disease is 10%, which increases to 10% *per annum* in persons with compromised immune systems[2]. However, the risk of progression in children younger than 5 years is 20%-40% and it decreases to 10%-15% in older children and adolescents[3]. It is estimated that one third of the world's population is infected with *M. tuberculosis*, most of them as LTBI, representing a reservoir of future active TB patients, especially in low-TB incidence settings. Therefore, correct identification and treatment of individuals with LTBI is an important tool for TB control worldwide. The main limitation in detection of LTBI is the lack of a gold standard for LTBI, which is the main drawback in determination of sensitivity and specificity for each particular LTBI test. Therefore, defined contact with active TB has been accepted as surrogate gold standard

Correspondence to: *Mateja Šegović, MD*, Health Center of Varaždin County, Kolodvorska 20, HR-42000 Varaždin, Croatia
E-mail: mateja.segovic@gmail.com

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for the evaluation of available tests for the detection of LTBI[4].

Until recently, tuberculin skin test (TST) was the only diagnostic tool for detection of *M. tuberculosis* infection despite its known limitations. It is well known that TST is a mixture of more than 200 antigens, causing lower specificity due to cross-reactivity with antigens present in other mycobacteria[5]. This can lead to false-positive responses in case of infection with nontuberculous mycobacteria (NTM) or vaccination with Bacille Calmette-Guérin (BCG) vaccine. TST has other downsides, such as low sensitivity due to false negatives in a proportion of patients with *M. tuberculosis* infection, especially in small children, the elderly, immunocompromised and malnourished individuals[6]. Moreover, recently, there has been a shortage of TST in many European countries, which has led to changes in TB screening capabilities and practices[7].

Advances in scientific knowledge of *M. tuberculosis* immunology have led to the development of a new generation of *in vitro* assays that measure interferon-gamma (IFN-γ) release by sensitized T-lymphocytes after M. *tuberculosis* specific antigen stimulation[8]. These tests are known as IFN-γ release assays (IGRAs). Until recently, there were 2 commercially available IGRAs, i.e., Quantiferon TB-Gold Plus assay (Qiagen, Hilden, Germany), an enzyme-linked immunosorbent assay (ELISA) that uses whole blood, and T-SPOT. TB test (Oxford Immunotec Ltd., Abingdon, United Kingdom), an enzyme-linked immunosorbent spot (ELISPOT) that uses peripheral blood mononuclear cells. In 2019, a novel IGRA called LIOFeron®TB/LTBI (Lionex GmbH, Braunschweig, Germany) was introduced. The antigens used in these assays are not shared with BCG vaccine strains or the majority of NTM species, thus eliminating BCG vaccination and most NTM as confounding factors and causing higher specificity of these assays. The great advantage of IGRAs is the performance of quality control by using the patient's internal positive and negative control, reducing the rate of false-positive and false-negative results of IGRA. As with the TST, the IGRAs cannot distinguish between TB infection and TB disease.

The sensitivities of IGRAs are a bit better than those of the TST, although both tests have reduced sensitivity in immunocompromised patients who are at the greatest risk of progressing from LTBI to active TB disease[9-11]. However, the improved specificity of IGRAs may help reduce the number of individuals requiring preventive treatment, which is more important in children. Numerous studies on the use of IGRAs in children have been published and clinical experiences with IGRAs are accumulating.

The aim of this paper is to review current evidence for the use of IGRAs in detection of LTBI in children.

Material and Methods

A review of the literature using defined search criteria was performed. A PubMed search, up to March 2020, was conducted. The following key terms were used: ("children" OR "pediatric" OR "infants") AND ("latent tuberculosis infection" OR "latent tuberculosis") AND ("interferon-gamma release assays" OR "IGRA"). The searches were limited to human studies, manuscripts in the English language, and only published data were taken into consideration.

Chronology of interferon-gamma release assay development

For the last twenty years, the focus of LTBI diagnostics has been on laboratory blood tests that detect infection with *M. tuberculosis ex vivo* under controlled conditions, known as the IGRAs. These are diagnostic tests that detect sensitization to *M. tuberculosis* by measuring IFN-γ release in response to antigens representing *M. tuberculosis*.

Since the beginning of this century, these tests have been developing and improving. In short, since 2005, QuantiFERON-TB Gold test (QFT-G) (commercialized by Cellestis Ltd., Carnegie, Victoria, Australia) has been used to evaluate the host immune response to ESAT-6 and CFP-10 peptides present in all *M. tuberculosis* strains and absent in the BCG vaccine and most NTM[12].

However, since rare mycobacteria such as *M. kansasii*, *M. szulgai*, and *M. marinum* contain these peptides, the possibility of cross-reactions and false positives should be mentioned[13].

Two years later, QuantiFERON-TB Gold In-Tube (QFT-GIT) (commercialized by Cellestis/Qiagen, Carnegie, Australia) was developed, to which another antigen, a part of TB7.7 antigen, was added in addition to the amino acid sequences of ESAT-6 and CFP-10[11]. These two tests are based on measurement of IFN-γ levels in whole blood. The whole blood sample is subdivided in three tubes, one of which contains all antigens, the second one is negative control which

contains heparin, and the last one represents positive control which contains heparin, dextrose and phytohemagglutinin. IFN-γ levels are measured by ELISA and the results are calculated by subtracting IFN-γ concentration in plasma of stimulated sample from the IFN-γ concentration in unstimulated sample, i.e., negative control value.

After only one year, in 2008, the next generation of IGRA was approved by the US Food and Drug Administration (FDA), under the name T-SPOT.TB test (T-SPOT) (commercialized by Oxford Immunotec Ltd., Abingdon, UK). This test includes incubation period of peripheral blood mononuclear cells (PBMCs) with control materials, both positive and negative, and two mixtures of peptides, entire amino acid sequence of ESAT-6 and CFP-10[14]. The test uses an enzyme-linked immunospot assay (ELISpot) to detect increases in the number of cells that produce IFN-γ after stimulation with antigen[14]. The results are presented as the number of IFN-γ producing T cells (spot-forming cells) and interpreted by subtracting the number of spots after incubation with antigen from the spots in negative control. In 2015, the fourth generation of IGRAs, Quantiferon TB-Gold Plus assay (commercialized by Qiagen, Hilden, Germany) was developed, and in 2019, Lionex GmbH (commercialized by Braunschweig, Germany) introduced a novel IGRA called LIOFeron®TB/LTBI[15]. Table 1 shows the main differences between TST and novel generations of IGRAs.

Novel generations of ex vivo interferon-gamma release assays

In all the IGRAs mentioned above, CD4+ T-lymphocytes which are activated as part of immune defense against *M. tuberculosis*, play a major role in producing cytokines such as IFN-γ and TNF-α that further trigger the immune response of macrophage activation[16]. However, studies have shown the importance of an immune specific CD8+ T-cell response, which is primarily stimulated by the presence of higher concentrations of ESAT-6 and CFP-10 antigens. This observation is corroborated by the fact that CD8+ T-lymphocytes are found in a greater number in children with active TB relative to LTBI, whereas the response of CD4+ lymphocytes was similar in both groups[17]. Furthermore, it was shown that latently infected individuals had lower frequencies of antigen-specific CD8+ IFN-γ + compared to CD4+ IFN-γ + T cells[18]. In the context of these findings, a new version of the QFT-GIT test was gradually developed, i.e., Quantiferon TB-Gold Plus (QFT-Plus) assay, which differs from the previous assay by antigens and test performance technique[19]. The fundamental difference of this assay compared to the previous ones is the activation of CD8+ T-lymphocytes[20]. It is well established that these subtypes of T-lymphocytes have the main role in defense against *M. tuberculosis* by producing IFN-γ, stimulating macrophages to suppress the growth of *M. tuberculosis*, killing infected cells, and by direct lysis of intracellular *M. tuberculosis*[21]. Numerous studies in adults have been conducted to date, but there are still not enough data in children to evaluate the accuracy of QFT-Plus assay in children with suspected active TB or LTBI. A systematic review and meta-analysis on QFT-Plus, published in 2019, compared its diagnostic performance with previous immunological tests and showed greater sensitivity of QFT-Plus in children with active TB disease, but also in children with recent *M. tuberculosis* exposure[19].

In a recent prospective cross-sectional study, Buonsenso *et al.* showed that QFT-Plus assay had good sensitivity for active TB and was particularly useful for the evaluation of children with suspected LTBI[22]. Although it was shown that active TB group had a slightly higher CD8+ T cell responses and LTBI group had a slightly higher CD4+ T cell responses, these differences were not statistically significant to conclude that QFT-Plus is able to distinguish active TB from LTBI[22].

Despite the fact that the QFT-Plus has been shown to have high concordance with previous generation of QFT assay, it seems that it is more strongly associated with time of exposure[23,24]. Given these results, QFT-Plus has proven to be a useful test in assessing children with suspected LTBI. Moreover, the QFT-Plus assay has the potential to be very useful in immunocomprising condition due to CD4+ T-cell impairments[25].

It is important to point out the new IGRA that Lionex GmbH (Braunschweig, Germany) presented in 2019, called LIOFeron®TB/LTBI[15]. The advantage of this test is precisely the new LTBI-specific antigen which has recently been reported that in adult patients, it may have diagnostic potential to differentiate active TB from LTBI[26]. In their new study, Della Bella *et al.* compared LIOFeronTB/LTBI assay with QFT-Plus assay and showed a higher sensitivity of LIOFeronTB/LTBI assay in LTBI detection[27]. QFT-Plus showed

Table 1. Comparison of TST and novel generations of IGRAs for diagnosis of LTBI in children

Test	TST	LIOFeron TB/LTBI	QuantiFERON TB-Gold Plus	T-Spot.TB Assay	Comments
Method	*In vivo* (intradermal)	*Ex vivo* ELISA-based	*Ex vivo* ELISA-based	*Ex vivo* Elispot-based	IGRAs are more objective methods
Sample	Skin	Peripheral blood	Peripheral blood	Peripheral blood	
M. tuberculosis antigens	Mixture of mycobacterial antigens RT-23 or PPD-S	ESAT-6, CFP-10, TB 7.7 and Ala-DH	ESAT-6 and CFP-10	ESAT-6 and CFP-10	Diminished specificity and sensitivity of TST
Positive control	No	Yes	Yes	Yes	TST may produce false-negative results due to immunosuppressive conditions; increase in the negative predictive value of IGRA
Subjectivity	Yes	No	No	No	Subjective measurement of skin induration; inter-reader variability
Number of patient visits	2	1	1	1	In case of low raes of return, IGRA is preferred
Cytokine involved	IFN-γ, IL4, IL10, IL12, TNF-α, G-CSF	IFN-γ	IFN-γ	IFN-γ	
Measurement	Induration after intradermal injection	IFN-γ concentration after stimulation to TB antigens	IFN-γ concentration after stimulation to TB antigens	Number of IFN-γ producing cells after stimulation to TB antigens	Subjective measurement of skin induration; inter-reader variability
Units of measurement	Millimeters of induration	International units of IFN-γ	International units of IFN-γ	IFN-γ SFC	
Cross-reactivity with BCG	Yes	No	No	No	IGRAs are preferred in BCG-vaccinated children
Cross-reactivity with NTM	Yes	Less likely	Less likely	Less likely	IGRA can be positive in case of *M. kansasii*, *M. szulgai*, and *M. marinum*
Laboratory required	No	Yes	Yes	Yes	Lower rate of false-positive and false-negative IGRA results due to good control of preanalytical and analytical procedures (i.e., good clinical practice and good clinical laboratory practice)
Time to result	48-72 hours	16-24 hours	16-24 hours	16-14 hours	
Definition of positive test	5 or 10 mm	IFN-γ ≥0.35 IU/mL	IFN-γ ≥0.35 IU/mL	≥8 SFC	Specificity and sensitivity of the TST are diminished by the variable cut off values used on positive TST determination
Indeterminate results	In case of anergy	IFN-γ <0.5 IU/mL in positive control OR IFN-γ >8 IU/mL in negative control	IFN-γ <0.5 IU/mL in positive control OR IFN-γ >8 IU/mL in negative control	<20 SFC in positive control OR >10 SFC in negative control	Standardization of preanalytical and analytical procedure and performance after resolution of acute inflammation reduce the risk of indeterminate results of IGRA

TST = tuberculin skin test; IGRA = interferon-gamma release assay; LTBI = latent tuberculosis infection; SFC = spot-forming cells; *M. tuberculosis* = *Mycobacterium tuberculosis*; NTM = nontuberculous mycobacteria; BCG = Bacille Calmette-Guérin

sensitivity and specificity of 98% and 97% in diagnosing active TB patients, and 85% and 94% in diagnosing LTBI subjects, respectively. LIOFeronTB/LTBI assay showed sensitivity and specificity of 90% and 98% in diagnosing active TB patients, and 94% and 97% in diagnosing LTBI subjects, respectively. Therefore, the authors demonstrated the same high accuracy of the LIOFeron® TB/LTBI assay and the QFT-Plus test in LTBI detection; however, the former had higher sensitivity[27].

Contact investigation

Contacts of TB cases are persons who share the same indoor environment over a period of at least 8 hours with a person who has smear-positive or culture-positive TB[28]. The time of contact is considered to last until the person with active TB is isolated from others or the diseased person's sputum smears are negative after at least 2 weeks of treatment[29]. Children with TB who are less than 10 years old are less frequently contagious because their pulmonary lesions are usually small and paucibacillary, and their cough is often unproductive[6,30,31].

In contacts, it is always important to examine a detailed epidemiological history of the disease, in particular the place of residence and migration because of differences in the prevalence of the disease in individual areas. The US Centers for Disease Control (CDC) instructions are to terminate contact with a diseased person as soon as possible and to have screening by IGRA (or TST if IGRA is unavailable) within 2 weeks of exposure. If the disease is clinically, microbiologically and radiologically excluded and the person is not immunocompromised and IGRA is positive, then LTBI is diagnosed and chemoprophylaxis is introduced according to the guidelines[29].

The previously mentioned epidemiological history of the disease is important in the diagnosis of TB[32]. People with suspected TB in low-burden countries are mostly members of an ethnic group with a high prevalence of LTBI[33]. In addition to foreign origin, other known risk factors for TB transmission are severe cough, cavitary lung lesions, closer and longer contacts, and delayed diagnosis of tuberculosis[34-36]. Multiple studies have shown a link between a positive IGRA and risk factors, including the time of exposure with active TB, then acid-fast bacillus (AFB) smear positivity, sputum AFB grade, and extent of chest x-ray disease in an index case[37,38]. The possibility of transmission of TB infection is almost four times higher in contacts of smear-positive patients than in contacts of smear-negative patients[39]. Exposure to *M. tuberculosis* associated with positive IGRA, TST or both has become an accepted rule, a kind of 'gold standard' to consider a child infected[40]. Prolonged close contact of children with an adult case of active TB is one of the highest risk factors for young children to become infected with *M. tuberculosis*[40,41].

Considerable ongoing risk of developing TB is observed during a period of 5 years after the contact, particularly within the first year, and therefore Fox e*t al.* underlined the potential importance of serial screening for TB in contacts that do not undergo treatment for LTBI[42].

In populations where the sensitivity and specificity of the TST is thought to be high relative to IGRAs, e.g., in children not vaccinated with BCG, TST is a superior test to IGRA[43]. An IGRA is recommended for patients who have been vaccinated with BCG in order to confirm/exclude the presence of *M. tuberculosis* infection in subjects with a positive TST, and also in HIV-infected subjects[28].

The most comprehensive Bayesian latent class analysis of published data on the performance of IGRAs and TST for the diagnosis of LTBI conducted by Doan *et al*. has confirmed that IGRAs appear to be a more favorable choice in settings where BCG vaccination is widely administered but in non-BCG-vaccinated populations IGRA may be inferior to TST for diagnosing LTBI because of lower sensitivity than TST in immunocompetent populations[44].

The importance of time interval between exposure to *M. tuberculosis* and IGRA conversion should also be emphasized, especially because of close contacts with an initially negative IGRA result[45]. Lee *et al*. estimated that it generally occurred 4-7 weeks after exposure to patients with active pulmonary TB, although it could occur as late as 14-22 weeks after exposure[46]. The latter supports the importance of serial testing in children at a higher risk of acquiring *M. tuberculosis* infection and those at an increased risk of progression from LTBI to active TB.

Based on the studies published so far, Lancella *et al*. have singled out some aforementioned risk factors for TB infection or disease and they include direct contact with TB patients, especially those with a positive microbiological finding or x-ray verified cavitary lesions, individuals with malignancies and diseases of the im-

mune system, low socioeconomic status, and residence in the country that is highly epidemic for *M. tuberculosis*[28].

It is important to note that in addition to the mentioned laboratory tests, the overall clinical picture and broader diagnostic processing are important in making the diagnosis because the gold standard for LTBI diagnosis is still not determined, and the choice of diagnostic method to distinguish active TB from LTBI is still a subject of research. In addition to laboratory screening or TST, assessment of TB contacts also requires other diagnostic procedures that include medical history, physical examination, and chest radiography[47,48]. However, if active TB is excluded by other diagnostic methods, positive results from commercial laboratory diagnostic tests indicate LTBI.

There are several indications when chest radiography should be performed with the initial TST and/or IGRA. They include the presence of TB symptoms in contact person or immune deficiency of contact person; age <5 years; and the initial IGRA is positive or the TST reaction size exceeds 5 mm[45].

Immunocompromised patients

Immunocompromised children are one of the most important targets for the screening of LTBI because of the increased risk of progression to active TB. Therefore, special attention must be paid to children with HIV infection, those treated with anti-tumor necrosis factor-alpha (TNF-α) drugs, patients on pre-organ transplatation, those with end-stage renal failure on dialysis, etc.[49].

Hence, those with HIV co-infection have an increased risk of LTBI progression to active TB. All HIV-infected subjects with CD4+ count >200 cells/mcL should be tested for LTBI, either TST or IGRA can be used[50]. Cases with active HIV disease and CD4+ cell count ≤200 cells/mcL should be assessed for active TB including chest x-ray and sputum examination. In immunocompromised children, LTBI should be considered if there is no evidence for active disease, and it is advised to perform both IGRA and TST tests[51].

To date, studies on immunocompromised adults have compared the performance of TST and IGRAs for LTBI diagnosis. A recent large meta-analysis found optimal specificity and suboptimal sensitivity of both TST and QFT-IT in this group of patients[44]. However, another study found that ELISPOT test performance appeared to be independent of HIV-associated immunosuppression[52]. Data on the performance and choice of diagnostic tests for LTBI in immunocompromised patients are still limited and mutually contradictory[50,53]. In HIV-infected individuals who are exposed to TB (active infectious TB case) in the household or other indoor space, the introduction of chemoprophylaxis should be considered, regardless of performance and results of IGRA and TST because of high suspicion of possible infection transmission with a high risk of disease progression[51].

There is a significant number of children with immune-mediated inflammatory diseases (IMID), e.g., ulcerative colitis, Crohn's disease, rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis, who are receiving anti-TNF-α therapy (infliximab, adalimumab, etanercept, etc.). These children are at a greater risk of LTBI progression to active TB disease due to receiving immunosuppressive and biological drugs. It should be emphasized that the risk of developing active TB is higher in children receiving immunosuppressive therapy in addition to corticosteroids, methotrexate or azathioprine as compared with those on monotherapy regimen[10]. In individuals with a history of BCG vaccination after infancy or with repeated BCG vaccinations, it is important to prefer IGRA over TST, but it should also be remembered that both IGRA and TST can be false-negative in those patients.

Screening for TB disease and LTBI is mandatory prior to the initiation of TNF-α inhibitor therapy[54,55]. In a large cohort observational study in children and adolescents receiving anti-TNF-α in a TB low-incidence country, Calzada-Hernández *et al*. showed that only 1.4% of patients were diagnosed with LTBI, they all received chemoprophylaxis and did well upon anti-TNF-α resumption. Moreover, during follow-up period, there was not a single incident case of TB disease[56]. However, there were reported cases of TB activation both in adults and children after the initiation of treatment with infliximab[57-60].

It consequently makes initial screening for TB mandatory prior to initiation of novel immunosuppressive drug treatment. Screening should include TST and an IGRA. It should be emphasized that there is no need for systematic repetition of immunodiagnostic tests as long as there is absence of symptoms or known TB contact[56].

The IGRA is considered to be very useful in evaluating LTBI, in particular T-SPOT.TB which is not

affected by immunosuppression therapy and may be slightly more sensitive than the enzyme-linked immunoassays[61].

According to the CDC guidelines, children under the age of 4 who have impaired immunity and have been in contact with TB patients should start chemoprophylaxis immediately, regardless of IGRA results. In cases when there is no multidrug-resistant TB disease or isoniazid resistance, isoniazid is used as therapy of choice. Therapy is administered for 8 weeks after the end of exposure, when the IGRA is repeated and, if the result is negative, there is no need to continue therapy[29].

Limitations of IGRAs

Due to the question of the maturity of the immune system of children less than 5 years of age and the possibility of a valid response to *ex vivo* antigen stimulation, the adequacy of IGRAs in children has been the topic of several studies to date[62-64]. The results of the studies so far are contradictory and IGRAs are still not widely accepted as a routine screening of children for LTBI, therefore the lack of a gold standard remains a serious problem in diagnosing and determining accuracy when developing new laboratory tests.

Indeterminate results are one of the possible problems in interpreting the results of diagnostic IGRAs. Therefore, the question of consequently indeterminate/invalid results of the IGRAs in young children has been raised[64,65]. Ten years ago, a retrospective study found that the risk of indeterminate IGRA results in immunocompetent children correlated linearly with age, averaging 25%[62]. Moreover, conditions associated with impaired immunity increase this risk to 66%, independently of age[62]. However, several studies later showed the opposite. In one retrospective analysis of TB screening data from refugee and asylum seeker children, who attended a refugee clinic in Australia during 2014 and 2015, it was shown that only 1 in 68 results of the examined children was indetermined[66]. Another study found that 0.5% of children aged 2-14 and 1.4% of children younger than 5 years had indetermined IGRA results[67]. On the other hand, it should be emphasized that a recent study conducted in children younger than 5 years found no evidence for impaired performance on QFT-IT results and identified only 1 (0.7%) indeterminate response from the 142 children tested and the latter explained as a low response to mitogen due to acute infection[40]. Anyway, it should also be highlighted that different rates of indeterminate results could be explained by using different types of IGRAs[68,69]. In their retrospective study, Zrinski Topić *et al.* showed the rate of indeterminate QFT-IT results in nonimmunosuppressed children of all age groups to be very low (0.46%)[70]. They explained the occurrence of indeterminate results by the presence of risk factors and those are combinations of acute bacterial infection, elevated body temperature, therapy with beta-lactam antibiotics, and atopy[70]. Children with co-infection undergoing antibiotic therapy for a disease other than TB (not TB) had higher probability of having indeterminate results[22]. Therefore, IGRA should be delayed in acute inflammation because of the possibility of producing indeterminate results on initial testing during acute bacterial inflammation[70]. In previous studies, indeterminate IGRA results were also associated with immunosuppression, cancer chemotherapy, or HIV infection with CD4 lymphocyte count <100/microL[71,72]. A recent meta-analysis performed by Meier *et al.*, which included 133 studies in final analysis, found that 4% of IGRA results were indeterminate[73]. According to that systematic review, the main factor associated with indeterminate results in children was the presence of an immunocompromising condition other than HIV infection. Furthermore, they did not find difference in the proportion of indeterminate results between two commercial IGRAs (T-SPOT.TB *vs*. QFT). Moreover, younger age was not associated with indeterminate results[73].

Previously, it was shown that the production of IFN-γ, stimulated by phytohemagglutinin in the positive control of IGRAs, was lowest in newborns and increased during early childhood, reaching adult levels as early as around the age of 3 years[74,75]. Recently published data show that the age of children does not have any significant impact on IFN-γ values in response to mitogen, suggesting that the immune system of children is not impaired in its ability to mount an immune response[40,76-78]. Therefore, the sensitivity of IGRA should not be compromised by age in immunocompetent children, supporting the use of IGRA as a complementary test for the diagnosis of TB infection even in infants. It has also been suggested that serial IGRA testing may improve the accuracy of LTBI diagnosis in children[79]. However, because of the inconsistency in the results, the use of IGRAs alternatively to TST is still not widely recommended,

and various national guidelines have recommended the use of IGRA as a supplement to TST in LTBI screening algorithms[80].

The inability to distinguish between TB infection and TB disease is often highlighted as one of the limitations of IGRAs. However, through a large retrospective analysis, Lombardi *et al*. investigated the quantitative value of the QFT-IT test, which showed that children under 5 years of age with active TB disease responded with significantly higher IFN-γ production to *M. tuberculosis* antigen stimulation than those with LTBI[77]. The specific response of IGRA to *M. tuberculosis* antigen to differentiate TB infection and TB disease depending on age within the pediatric population has not yet been sufficiently investigated.

On the other hand, new generations of IGRAs, that are gradually reducing the limitations of diagnostic tests to date, are being developed.

Longitudinal studies with novel generations of IGRAs are warranted to see if there is any potential in identifying those with active TB, those with a recent exposure in TB contacts, and those with potential progression to active disease.

Who should be tested?

The review of the literature supports that testing for LTBI should be directed to children at an increased risk of acquiring *M. tuberculosis* infection and those at an increased risk of progression from LTBI to active TB. This includes children with known contacts of an active TB, immunocompromised children including HIV infection and other immunodeficiency disorders, and prior to initiation of novel immunosuppressive treatment.

The previously cited study conducted in pediatric refugee clinic in Australia identified 12 children with LTBI who would have been missed using current New South Wales Health Department screening practices. Consequently, it was concluded that these children were at a risk of progression to active disease[66]. The latter supports testing for LTBI in children from communities or countries with a significant incidence of TB.

Thus, screening for *M. tuberculosis* infection among immigrants from high-risk countries, as well as identifying LTBI among children because of a higher risk of disease progression, is an important part of controlling TB in the general population.

Conclusion

In a difficult procedure of diagnosing LTBI in children, performance of IGRA could be the key factor in making decision whether to use preventive therapy or not. This could be more pronounced in BCG-vaccinated children.

If both tests (TST and IGRA) are performed, IGRA may contribute to more precise diagnosis of LTBI in children, especially in children with discordant TST and IGRA results. Without a gold standard for LTBI, we cannot determine if one test is more accurate than the other. Therefore, setting the gold standard can have important role in strategies for ending the global TB epidemic.

However, in a high-risk population of children, both IGRA and TST testing should be performed and the child should be considered infected if either or both tests are positive.

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Sažetak

ULOGA TESTOVA KOJI OTPUŠTAJU INTERFERON GAMA U DIJAGNOSTICI LATENTNE TUBERKULOZE U DJECE

M. Šegović, I. Mihatov Štefanović i *I. Pavić*

Usprkos brojnim objavljenim radovima dijagnostika latentne tuberkulozne infekcije (LTBI) u djece je i dalje nedovoljno istraženo područje. Važnost ove teme leži u činjenici da je jedna trećina svjetske populacije zaražena bakterijom *Mycobacterium (M.) tuberculosis*. Većina zaraženih pojedinaca ima latentni oblik tuberkuloze (LTBI) koji čini rezervoar budućih bolesnika s aktivnom tuberkulozom. Zlatni standard za LTBI još nije utvrđen, čemu doprinosi utjecaj raznih zbunjujućih čimbenika na postojeće dijagnostičke testove. Do unazad desetak godina, a kroz cijelo prošlo stoljeće, tuberkulinski kožni test (TST) je bio jedini dijagnostički test za LTBI. Zahvaljujući znanstvenom napretku nedavno su otkriveni novi *in vitro* testovi otpuštanja interferona gama (*interferon-gamma release assays*, IGRAs). Osjetljivost ovih testova je nešto bolja u odnosu na TST, a velik je napredak postignut u povećanju specifičnosti IGRA u odnosu na TST. Međutim, TST i dalje ima određene prednosti u dijagnostici LTBI. Unatoč tome, testovi IGRA su donijeli mnoge prednosti koje su naglašene u ovom radu. U zahtjevnom postupku dijagnosticiranja LTBI u djece izvođenje testova IGRA može biti ključno u odluci uvođenja preventivne terapije.

Ključne riječi: *Djeca; Testovi otpuštanja interferona gama; Tuberkulinski kožni test; Latentna tuberkuloza*