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Wykrywanie latentnej gruźlicy u dzieci szczepionych szczepionką BCG

Detection of latent tuberculosis infection in BCG vaccinated children

Streszczenie

Wstęp. Gruźlica u dzieci stanowi ok. 11% ogółu przypadków tej choroby. Trudności diagnostyczne gruźlicy u dzieci wynikają z powodów klinicznych i laboratoryjnych, do których należy brak dostatecznie czułych i swoistych metod diagnostycznych. Testem umożliwiającym identyfikację osób z latentną gruźlicą (LTBI), w grupach zwiększonego ryzyka rozwoju gruźlicy, był do tej pory tuberkulinowy test skórny. W chwili obecnej test tuberkulinowy jest zastępowany przez nowoczesną generację testów opartych na detekcji IFN- γ , jak QuantiFERON-TB Gold In-Tube.

Cel. Celem pracy była ocena częstości występowania latentnej gruźlicy u dzieci oraz ocena przydatności testu QFT-IT do wykrywania latentnej gruźlicy u dzieci szczepionych BCG.

Materiał i metody. Badaniem objęto 156 pacjentów w wieku 0-18 lat. Dzieci zostały włączone do grupy badanej z powodu podejrzenia aktywnej gruźlicy (płucnej lub pozapłucnej), lub ze względu na rozważaną możliwość leczenia inhibitorami TNF.

Obecność zakażenia latentnego *M. tuberculosis* określono na podstawie analizy wywiadu medycznego oraz testów screeningowych: prześwietlenie klatki piersiowej oraz test QFT-IT. Wywiad medyczny obejmował następujące punkty: obecność symptomów gruźlicy, leczenie przeciwgruźlicze w przeszłości oraz bliski kontakt z chorym na gruźlicę w przeciągu ostatniego roku.

Wyniki. Odsetek dzieci z pozytywnym wynikiem testu (5.1%) był najwyższy w grupie wiekowej $>3\leq 12$ lat. Ujemny wynik testu miało 127 (81,4%) dzieci. Wśród nich u jednej osoby zanotowano wynik podprogowy (0.31UI/ml), a 21 (13.5%) spośród 156 dzieci miało nieokreślony wynik QFT-IT. Liczba wyników nieokreślonych była najwyższa wśród najmłodszych dzieci.

Na podstawie wykonanych badań stwierdzono niską częstotliwość występowania latentnych zakażeń *M. tuberculosis* u badanych dzieci.

Abstract

Introduction. Children with tuberculosis represent about 11% of all notified incidences of the disease. Especially young children are at high risk of infection because of the decreased ability of their immunes to combat the pathogens and children <5 years of age are the most vulnerable.

In Poland tuberculin skin test has been used as Latent Tuberculosis Infection (LTBI) detection method since 1966. Because of the low specificity of TST, the global actions were undertaken aimed at introducing to use more specific test for LTBI detection. Recently, QuantiFERON-TB Gold In-Tube assay has been used as an alternative to TST for the diagnosis of LTBI. There are only few reports about the precision of the assay in pediatric cases.

Aim. The purpose of the study was to assess LTBI rate in children as well as to determine usefulness of QFT-IT assay for LTBI detection in BCG vaccinated children.

Material and Methods. One hundred fifty six patients aged 0-18 years were enrolled into the study. The children were selected due to suspected active tuberculosis (pulmonary or extrapulmonary) or because of the considered treatment with TNF inhibitors.

The presence of LTBI was assessed by medical history and screening tests: chest radiography and QFT-IT. The evaluation of the medical history included: current symptoms, prior history of treatment for tuberculosis and close contact with active pulmonary tuberculosis within the last year.

Results. Eight children (5.1%) had positive QFT-IT results. The percentage of children with positive QFT-IT results was the highest in the age group $>3\leq 12$. In the analyzed group of patients, 127 (81.4%) had negative tests results. Among these, one QFT-IT result was subliminal (0.31UI/ml). Twenty one (13.5%) of the 156 studied children had indeterminate QFT-IT results. The proportion of indeterminate results was the highest in the youngest age group.

Conclusions. Our results indicate the low rate of LTBI among the studied children. The infection rate detected with QFT-IT is more likely than identifying with TST owing to small rate of childhood TB in Poland.

Słowa kluczowe: *Mycobacterium tuberculosis*, QuantiFERON-TB Gold In-Tube, gruźlica latentna.

Key words: *Mycobacterium tuberculosis*, QuantiFERON-TB Gold In-Tube, latent tuberculosis.

INTRODUCTION

Tuberculosis (TB) is still the leading cause of death worldwide despite the availability of effective antituberculosis chemotherapy for over half-century and widespread Bacilli Calmette-Guerin (BCG) vaccination. Of the estimated 9.4 mlnt cases of TB in 2008, nearly 11 % occurred in children [1]. In Poland, children with TB represent 0.9% of all 8 642 notified incidences of the disease and the notification rate of pediatric tuberculosis is 1,2 per 100.000 population, at the same time [2].

As infection is a necessary prerequisite for active disease, persons with latent tuberculosis infection (LTBI) are considered to be at the highest risk for developing active disease in their lifetime [3]. Most children are infected with *M. tuberculosis* from the adults. Especially young children are at high risk of infection because of the decreased ability of their immunes to combat the pathogens and children <5 years of age are the most vulnerable. *M. tuberculosis* infected children are more likely than adults to develop active tuberculosis, very often without signs or symptoms [4,5]. It is well accepted that identification and treatment of children with LTBI, to prevent future disease, is an important component of TB control efforts.

In Poland tuberculin skin test (TST) has been used as LTBI detection method since 1966. The skin test based on immunological mechanism of delayed type hypersensitivity (DTH) occurs in sensitized individuals and indicates their contact with tubercle bacilli. Although the TST has proved to be useful in clinical practice, it has well known limitations including variable specificity, cross-reactivity with BCG and nontuberculous mycobacterial (NTM) infection and problems with reproducibility [6,7]. The positive TST result can mean tuberculosis infection or cross-reactions with BCG vaccination and NTM infection. It is also possible that the test is false negative because of the recent vaccination, immune deficiency or malnutrition. Additionally, reading the skin test is associated with subjectivity. Because of the low specificity of TST, the global actions were undertaken aimed at introducing to use modern, more specific test for LTBI detection [6].

Recently, whole blood interferon gamma released assays (IGRAs) have been used as an alternative to TST for the diagnosis of LTBI, based on measuring responses to ESAT-6, CFP-10 and TB7.7 peptide antigens. It has been demonstrated that QuantiFERON-TB Gold (QFT-IT) is less influenced by BCG vaccination and infection with NTM. Moreover, the quality control assessment has been performed by QFT software and provides a test result for each subject individually [7,8]. The QuantiFERON-TB Gold test has been applied in Poland since 2008 year. The current studies of interferon- γ tests have shown good diagnostic accuracy for LTBI in adults. However, there are only few reports about their precision in pediatric cases.

Early diagnosis of *M. tuberculosis* infection is important especially among hospitalized children with inflammatory pulmonary and connective tissue diseases (CTD). In the first case differential diagnosis between pulmonary TB and other pulmonary diseases is usually required. Secondly, treatment of CTD, in particular juvenile rheumatoid arthritis (JRA),

needs the careful screening for TB before initiating Disease-Modifying Anti-Rheumatic Drugs (DMARDs) treatment. Use of innovative anti-TNF therapy leads to modification of immune response and consequently leads to reactivation of TB infection [7,9]. Therefore, specific LTBI detection is the necessary first step in obtaining the definitive diagnosis of TB and starting the proper treatment.

AIM

The purpose of the study was to assess LTBI rate in children as well as to determine usefulness of QFT-IT assay for LTBI detection in BCG vaccinated children.

MATERIAL AND METHODS

Patients

For 17 months, between July 2007 and December 2008, 156 patients aged 0-18 years (mean 10.2 years) hospitalized at Children's Hospital, Lublin, Poland were enrolled into the study. Of these patients 115 were examined at the Clinic of Pulmonology and Rheumatology, 17 were admitted to the Clinic of Orthopedics, 9 were hospitalized at the Pediatric Surgery and Traumatology Clinic, 8 - at Neonatal Pathology Department and the remaining 7 were hospitalized at other clinics. The children were selected due to suspected active tuberculosis (pulmonary or extrapulmonary) or because of the considered treatment with TNF inhibitors.

For each child enrolled in the study, data on clinical history, physical examination, data on prior treatment for TB and contact history with active TB patients, was recorded. All children were at least once BCG vaccinated as newborns. Chest radiograph findings were recorded and reviewed by an experienced pediatric pulmonologist. Detection of *M. tuberculosis* infection was performed using QFT-IT assay in all testing children.

Specimens for bacteriologic analysis were collected when appropriate, and sent to a bacteriology laboratory. Staining and microscopy, culture and PCR tests were performed following standard procedures. Cases of active tuberculosis were diagnosed on the basis of culture, molecular, clinical and radiological findings.

As a comparative data for the study, the results of TST examination among children and youth, collected by National Institute of Health in Poland, were used [10].

The data of active tuberculosis incidence in children were used according to Polish National Tuberculosis and Lung Diseases Research Institute in Poland.

Diagnosis of latent tuberculosis infection

The presence of LTBI was assessed by medical history and screening tests: chest radiography and QFT-IT. The evaluation of the medical history included: current symptoms, prior history of treatment for tuberculosis and close contact with active pulmonary tuberculosis within the last year.

QuantiFERON-TB Gold In-Tube test: The test was performed according to the manufacturer recommendations. Briefly, the test was performed in two stages. In the first

stage, the whole heparinized blood was collected into tubes with ESAT-6, CFP-10 and TB7.7 antigens as well as T-cell mitogen phytohaemagglutinin and negative control and incubated for 16 to 24 h. After centrifugation, the plasma samples were harvested.

In the second stage, plasma samples and conjugate were loaded into QFT ELISA plate and the detection of IFN- γ levels was performed simultaneously with duplicate kit standards. Substrate reagent was added after 2h incubation, Stop Solution was added $\frac{1}{2}$ an hour later, then the OD values of all samples were read on a ELISA Multiscan reader (Lab-system, Finland). The results were interpreted using QFT Analysis Software. The positive result was considered when the concentration of IFN- γ was greater than or equal to 0.35 IU/ml and less than 0.35 IU/ml for the negative result after the subtraction of the respective nil value. Apart from this, the indeterminate result were generated when the IFN- γ concentration in the TB antigen was less than 0.35 IU/ml and less than 0.5 IU/ml in the mitogen tube after the subtraction of the respective nil value.

Data were analyzed by using STATISTICA version 9.0. For statistical analysis the χ^2 tests for categorical variables, and nonparametric tests for continuous variables with skewed distributions was used.

For statistical analysis results were expressed as the mean 95% confidence intervals (95%CI).

TABLE 1. Characteristics of study group.

Features	Study group N=156(%)
Gender	
Male	77 (49.4%)
Female	79 (51.6%)
Age groups	
≤ 3	26 (16.7%)
$>3 \leq 12$	86 (55.1%)
$>12 \leq 18$	44 (28.2%)
Mean age	10.2 years (0-18 years)
Clinics	
Clinic of Pulmonology and Rheumatology	115(73.7%)
Clinic of Orthopedics	17 (10.9%)
Pediatric Surgery and Traumatology Clinic	9 (5.7%)
Neonatal Pathology Clinic	8 (5.1%)
Other Clinics	7 (4.4%)
BCG vaccination	
Yes	156 (100%)
No	0 (0%)
Previous TB treatment	
Yes	0 (0%)
No	156 (100%)
Close TB contact	
Yes	2 (1.3%)
No	154 (98.7%)
Chest x-ray	
Positive	0 (0%)
Negative	156 (100%)
Active Tuberculosis	3 (1.9%)

RESULTS

A total of 156 children were enrolled in the study. There were 77 (49.4%) males and 79 (51.6%) females. The mean age was 10.2 years (0-18 years). All children were divided into 3 age groups: 27 (17.3%) subjects younger than 3 years, 86 (55.1%) children $>3 \leq 12$ years and 43 (27.5%) patients $>12 \leq 18$ years. All 156 children included to the study were at least once BCG vaccinated as neonates. None of the children were previously treated for active tuberculosis. One child had the history of LTBI treatment 7 years ago. Two children had documented household, prolonged exposure to someone with active TB. Three patients had symptoms and laboratory findings suggestive of current active tuberculosis. Table 1 summarizes the main features of patients group.

The QFT-IT test was performed in all cases. The relationship of the QFT result with age is shown in Table 2. Eight (5.1%) children had positive QFT-IT results. The percentage of children with positive QFT-IT results was the highest in the age group $>3 \leq 12$.

In the analyzes group of patients 127 (81.4%) had negative tests results. Among these, one QFT-IT result was subliminal (0.31UI/ml).

Twenty one (13.5%) of the 156 studied children had indeterminate QFT-IT results. The proportion of indeterminate results was the highest in the youngest age group. Generally, the indeterminate responds were noted in children who were suffering from medical conditions that could be associated with impaired immune function (SLE, sarcoidosis, cystic fibrosis), rheumatic diseases (arthritis, JRA) or respiratory tract diseases (pneumonia, pleuritis) at the time of testing. The characteristics of the patients with indeterminate QFT results are presented in Table 3.

During the study 3 children were clinically and microbiologically diagnosed with active tuberculosis. The first child (9 months old) was diagnosed with BCG osteomyelitis as a complication of BCG vaccination. For this child, QFT-IT gave indeterminate results, but the infection with *Mycobacterium tuberculosis* BCG was confirmed by positive acid fast bacilli (AFB), conventional culture on Loevenstein-Jensen medium and PCR (INNOLipa Rif TB).

TABLE 2. The relationship of the QFT results with patients' age.

	Gender		QFT results		
	Male	Female	Positive	Negative	Indeterminate
Study group N=156 (100%)	77 (49.4%)	79 (51.6%)	8 (5.1%)	127 (81.4%)	21 (13.5%)
≤ 3 N=27 (100%)	19 (73.1%)	8 (29.6%)	1 (3.8%) 1F	20 (74%) 4F/16M	6 (23.1%) 3F/3M
$>3 \leq 12$ N=86 (100%)	47 (54.7%)	39 (45.3%)	6 (7.0%) 1F/5M	72 (83.7%) 34F/38M	8 (9.3%) 4F/4M
$>12 \leq 18$ N=43 (100%)	13 (29.5%)	30 (69.8%)	1 (2.3%) 1F	35 (81.4%) 24F/11M	7 (16.3%) 5F/2M

TABLE 3. Characteristics of the patients with indeterminate QFT results.

Age group N=21 (%)	Age min. - max.	Diagnosis	Treatment
≤3 N=6 (23.1%)	3 weeks-2 years	Pneumonia Pleuritis, Arthritis	Steroids Antibiotics
>3 ≤12 N=8 (9.3%)	4-8 years	Pneumonia Pleuritis, Cellulitis	Steroids Antibiotics
>12 ≤18 N=7 (15.9%)	13-18 years	Systemic lupus erythematosus, Pneumonia, Sarcoidosis, Juvenile rheu- matoid arthritis Cystic fibrosis	Steroids Antibiotics

The second child (15 years old) was diagnosed with active TB pleurisy, confirmed by chest radiography and positive culture on Loewenstein-Jensen medium. QFT-IT was positive.

The third child (aged 15 years) with active pulmonary TB was positive by AFB and culture, but the result of QFT-IT was subliminal (0.31 UI/ml).

The rates of positive TST results were high among Polish children ranging from 49.4% to 54.6% and the differences were not statistically significant in given age groups (Table 4).

Pediatric tuberculosis cases represented less than 2% of total TB cases in Poland while, about 5.0% in young people at the age between 15 and 18 years (Table 5).

TABLE 4. The results of tuberculin skin test examination in children and young people (according to National Institute of Health, Poland).

Year	Age (years)	TST results		
		mln general	Positive %	Negative %
2003	12	540483	49.5	50.5
	13-18	30817	54.6	45.4
2004	12	415946	49.4	50.6
	13-18	32645	54.3	45.7
2005	12	410509	51.7	48.3
	13-18*			

*No data due to discontinuance of BCG vaccination.

TABLE 5. Incidence of active tuberculosis in children, Poland (according to National Tuberculosis and Lung Diseases Research Institute).

Year	Age (years)	Number of cases	Incidence rate (%)
2003	0-14	100	1.5
	15-18	184	5.8
2004	0-14	120	1.9
	15-18	129	4.2
2005	0-14	99	1.1
	15-18	156	5.3

DISCUSSION

It is commonly accepted that eradication of TB depends on early detection of tubercle bacilli in LTBI phase [3,5,8]. Previously, TST have been used as an only source of infor-

mation to identify children who were probably infected with *M. tuberculosis*. Long-standing practice of TST revealed that skin test is not reliable tool of LTBI diagnosis, especially in immune compromised individuals [3,5,8,11]. Thus, CDC has been recommending QuantiFERON-TB Gold test as replacement of TST since 2005.

In this study we aimed to determine usefulness of QFT-IT for detection of LTBI among BCG vaccinated children who were hospitalized because of different clinical disorders with suspicion of tuberculosis infection. Due to lack of the gold standard for LTBI diagnosis, in the study we analyzed results obtained from QFT-IT in comparison with TST results collected by National Institute of Health.

In Poland TST was applied to all children before booster BCG vaccination until 2005 [2]. According to Polish National Institute of Health nearly 50% of children at the age of 18 years have had positive TST reaction (Table 4) [10]. It must be noted that nearly all of them were already BCG vaccinated at ages 0, 11-13 months, 7 years, 12 years and 18 years, and before each vaccination TST were always performed. Multiple TST and BCG vaccinations are factors known to affect the specificity of TST [6]. The assumption that factors other than tuberculosis infection are widely contributing to positive TST response is supported by this study, as only 5% of tested children aged 0-18 years had positive QFT results. The highest rate of LTBI equal to 7% was noted in the age group 3-12 years. Several other surveys in large American cities have consistently shown tuberculosis infection rates of 2 to 5% in elementary schoolchildren [12].

In literature, pediatric data about identification of LTBI with QFT-IT are limited (especially for those aged <5 years). In infants tuberculosis frequently progresses rapidly from infection to disease. The higher rate of active tuberculosis and severe forms of the disease in infants and children aged <5 years compared with older children suggests that the immune response to *M. tuberculosis* infection differs in these groups [13].

Manuel et al. indicates that among children with immunosuppressive therapy the QFT-IT better correlates with the risk of tuberculosis than the TST [8]. To our knowledge, such factors as corticosteroids and other immunosuppressive drugs inhibit reaction to the tuberculin therefore there are contra-indications against TST. The QFT-IT test can be performed irrespectively of therapy and in 80% gives determinate result. But, importantly in this case, corticosteroids and other immunosuppressive drugs are risk factors for progression of LTBI to active TB. Thus correct diagnosis of LTBI in that group of children is very important. In our study QFT-IT was performed for all children enrolled to the study. The negative results were obtained for 81.4% children, 5.1% of them had positive and 13.5% indeterminate results.

In high risk situations (e.g. contact investigations, infancy, immunosuppressive therapy or clinical suspicion of disease), neither a negative TST nor a negative QFT-IT excludes the presence of TB infection or disease. The results should be interpreted in the context of the individual case and with expert consultation when appropriate [4].

In our study indeterminate QFT-IT results were observed in 13.5% of children, largely in the youngest age group (23.1%). Generally, the indeterminate responses were noted

in children who were suffering from medical conditions that could be associated with impaired immune function (SLE, sarcoidosis, cystic fibrosis), rheumatic diseases (arthritis, JRA) or respiratory tract diseases (pneumonia, pleuritis) at the time of testing. These clinical conditions are consistent with previously reported risk factors for indeterminate QFT-IT results [4,11]. The rate of indeterminate response in our patient group is similar to that observed in other studies where patients with immunosuppression were tested, which ranged from 1-21% [6,7]. The relatively high proportion of indeterminate results in the group of children <3 years of age compared with older children could be explained by age-related immunologic differences including weaker cell mediated immunity in response to mycobacterial antigens and mitogen observed in infants and young children [12].

Early diagnosis of the active tuberculosis disease is especially needed in children under immune suppression, since there is an accelerated progression of TB and higher mortality [5,11,13]. During the study 3 children were clinically and microbiologically diagnosed with active tuberculosis and QFT-IT assay results for them were: positive, indeterminate and subliminal, respectively.

Indeterminate test result was noted in 9 months old child diagnosed with BCG osteomyelitis as a complication of BCG vaccination. In fact, indeterminate results frequently occur in immunocompromised subjects and have also been observed in children under the age of 5 years, as mentioned before [4,12].

In one child (15 years old) with active pulmonary tuberculosis, confirmed clinically and microbiologically, the result of QFT-IT was subliminal (0.31 UI/ml). The cutoff value for QFT-IT, that is 0.35 UI/ml, is established for adults. This cutoff value has not been validated for children, particularly very young children and children under immune suppression who produce, on average, less IFN- γ than adults. Therefore, the assay cutoff value may need to be adjusted for younger children. This may improve the sensitivity of the QFT-IT to identify children exposed to *M. tuberculosis* antigenic stimulation [5].

CONCLUSIONS

In conclusion, our results indicate the low rate of LTBI among tested children. The infection rate detected with QFT-IT is more likely than identifying with TST owing to small rate of childhood TB in Poland.

QuantiFERON might prove to be useful in BCG-vaccinated individuals, particularly in settings where TST specificity is compromised by BCG vaccination after infancy, by multiple BCG vaccinations or immune suppression. The data presented highlight that care should be considered when interpreting indeterminate QFT-IT results in young children with immunosuppressive conditions. QFT-IT assay better than TST screens children for TB infection and may facilitate the selection of patients who are most likely to benefit from prophylaxis or at least the identification of persons who need close monitoring and follow-up care.

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