Efficiency of Immunological Methods in the Diagnosis of Active Tuberculosis in Children

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Abstract

Nowadays in children there are no clear diagnostic criteria of tuberculosis due to absence of bacterial excretion unlike adults who have bacterial excretion quite often. This fact leads to necessity to implement immunological methods in diagnostic complex in children. Tuberculin test is a routine assessment that is used for diagnosis of tuberculosis. However, there are a number of factors that interfere with diagnostic value of tuberculin test. This study helped to ascertain the superiority of Diaskintest (DST) and QuantiFERON®-TB Gold IT (QFT-G) with respect to the wealth of information it provides when compared with the Tuberculin screening test (TST) in determining the activity of tuberculosis infection in children. The evaluation parameters of diagnostic value with DST (DSS-84.3%, DSC-59.1%, PPV- 61.9%, NPV- 82.7%, DE-71.7%), with QFT (DSS-78.4%, DSC-68.2%, PPV-57.7%, NPV-85.1%, DE-73.3%) were significantly higher than with TST (DSS- 91.5%, DSC-15.7%, PPV-74.4%, NPV- 45.8%, DE-53.6%). New immunologic methods (Diaskintest and QuantiFERON®-TB Gold IT) have higher specificity and diagnostic value in comparison with TST that makes their implementation in diagnosis of TB in children essential.

KEYWORDS: tuberculosis, children, Diaskintest, QuantiFERON-TB test, immunology.

Introduction

In Russian Federation tuberculosis of intrathoracic lymph nodes (TILN) prevails in children, commonly found in 74% of all cases [1, 2]. Today, in children there are no clear criteria in the diagnosis of tuberculosis at the absence of bacterial excretion unlike adults who have bacterial excretion quite often. This fact leads to necessity to implement immunological methods in diagnostic complex in children targeted to identify MBT-negative forms.

Tuberculosis is diagnosed by a data set: clinical symptoms, positive TST and radiologic data. However, the frequent absence of the intoxication symptoms of tuberculosis in children along with the increasing comorbidity in modern scenarios significantly impedes the assessment of the intoxication symptoms and nature of sensitivity to tuberculin, which has a normoergic response in 60% of the patients with tuberculosis disease [3, 4, and 5]. Often TST provides incorrect result, because many factors affect diagnostic value of tuberculin test. BCG vaccination, growth of allergy disorders and concomitant diseases in children lead to falsely positive or sometimes to falsely negative results of the test [6]. Due to this new immunologic methods and criteria are required for diagnosis of tuberculosis infection in children. This indicates late

detection of the disease; therefore, there is an urgent need for the introduction of new immunological tests for early detection and determination of the activity of the tuberculosis infection.

The aim objective of presented research was to identify immunologic features in children with tuberculosis of intrathoracic lymph nodes.

Material and Methods

Within 2010-2013 prospective study of complex diagnosis of tuberculosis in children was conducted at the Department of Pediatric Phthisiopulmonology. 213 children of 3 to 14 years old with positive tuberculin test were examined. Among them 102 (47.8%) were between 3 to 6 years of age and 111 (52.2%) were between 7 and 14 years of age. The patients were assessed by clinical symptoms and results of computer tomography (CT). Assessment of intrathoracic LNs was performed by CT in line with the recommendations according to which the transverse dimension of the lymph nodes in children (from 3 to 14 years) should not exceed 10 cm depending on the group of nodes and the age of a child. However the presence of pathological changes, including specific changes, in the smaller nodes could not be excluded [13]. According to Ya.V. Lazoreva [14], F. E. Gegeeva [15], and Ya. A. Dauletova [16], all LNs between 5 and 10 mm should be considered as manifestations of tuberculosis in intrathoracic LN. Since CT without contrast enhancement is limited in visualization of LNs therefore contrast media administration is recommended.

All children were divided into two groups. The first group includes 70 children who were infected by MBT, but have no tuberculosis disease – it was taken in the research as control group; the second group includes 143 children with tuberculosis of intrathoracic lymph nodes. Both groups were tested by Diaskintest® (Diaskintest (Generium). (DST) uses recombinant tuberculosis allergen based on M. tuberculosis specific proteins: ESAT-6 and CFP-10) – and by its nature represents immunologic skin-test. This test was designed in Russia and is used for diagnostic of tuberculosis since 2009. An analysis of the results of the tuberculin skin test (administration of the purified tuberculin in standard dilution (ready form) and DST (tests with recombinant TB allergens in standard dilution) was conducted [11,12].

In addition QuantiFERON test has been used. Before performing these tests, a venous blood sample was drawn for QuantiFERON-TB test (QFT), which is the reference method and allows a qualitative assessment of the information content. The QuantiFERON ®-TB Gold In-Tube is a diagnostic tool designed for the diagnosis of tuberculosis *in vitro*. This method is based on usage a peptide cocktail simulating ESAT-6, CFP-10 and TB7.7 (p4) proteins to stimulate the cells in heparinized whole blood. Determination of the quantification of IFN-γ by ELISA was performed to identify *in vitro* the cellular response to the stimulation of these peptide antigens associated with *Mycobacterium tuberculosis* infection. Since tuberculosis in literature described as the disease with immunosuppressive pattern the following immunologic parameters were chosen for assessment: leucocytes' subsets identification ($CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD3^+$, $CD8^+$, $CD16^+$, $CD20^+$, $CD25^+$, $CD95^+$, HLAII), induced cytokines levels (TNF-ά, IL-2, IL-4, INF-

γ), and assessment of antibodies IgA, IgG, IgM (anda-tb ELISA) [9,10].

Apart from these X-ray method diagnosis (multislice spiral computed tomography (MSCT) and MSCT angiography (MSCT-AG)) and X-ray examination were done using a spiral CT scanner with a multirow detector (multislice) «Aquilion-32» (Toshiba Medical Systems Corporation, Japan), with intravenous bolus contrast administration using an automatic injector "CT 9000 ADV» (Liebel-Flarshein (Mallincrodt Inc.).

In clinical example №1 (fig.1), child P.S., 5 years old, was vaccinated by BCG. Tuberculin test in 2007 – p 13 mm, 2008 – p 15mm, 2009 г – p 16mm, 2010 – p 15mm. In anamnesis she has – allergy. In examination - CD+ 16 is low, DST-negative, QFT- negative, Ig G is low, no symptoms of intoxication. In CT – in lungs and intrathoracic lymph nodes tuberculosis changes are not identified. In this example high result of TT is influenced by allergy growth.

In clinical example №2 (fig.2), child P.M., 3 years old. She has a contact with her father, who is ill since 2008 with lung tuberculosis MBT (+) MDR. BCG vaccination, preventive course of therapy in 2007, 2008. Tuberculin test : 2009 – p 6mm, 2010 – p 18mm; CD+ 16 is high, DST-p20mm, QFT- positive, Ig G is high, symptoms of intoxication. CT and CT-Ag – lymph nodes > 1.0 cm paratracheal, bifurcation, bronchopulmonal groups. In this example child had tuberculosis of intrathoracic lymph nodes and course of therapy has been administered.

All the data was processed employing the variation statistics methods using the software Microsoft Office Word Excel 2007, Statistica 8. The difference was considered reliable when $p < 0.05$. The Mann-Whitney (U Test) was used to compare the differences between two independent groups (for nonparametric data). The mean (M) and standard error of the mean (m) were deduced. Pearson's Correlation Coefficient (r) was used to determine the strength of the relationship between two continuous variables. P value less than 0.05 was considered significant. Spearman's rank correlation coefficient was also used. The diagnostic accuracy of the tests employed was analyzed as well as the method used to calculate the operating characteristics: diagnostic sensitivity (DSS), the diagnostic specificity (DSC), positive predictive value (PPV) and negative predictive value (NPV), and diagnostic efficiency (DE).

Results and discussion

No clinical manifestations of the intoxication syndrome were significantly higher in the I group **(**78.6% vs. 13.3%, x^2=74.9; p<0.001) in comparison with the II group and manifestation intoxication syndrome in the II group were significantly higher in comparison with the I group **(**61.5% vs. 4.3%, x^2=53.1; p<0.001) (table 1).

As one can see at the fig. 3, in the second group high sensitivity to tuberculin was observed in only 42% cases - that was higher in comparison with the control group (42.0% vs. 20.0%, χ^2 = 9.99, p<0.01), however results of Diaskintest and QuantiFERON tests being significantly higher in the

group with the disease versus control group were at the same time twice more frequently positive if compared with the results of tuberculin test. Positive DST was marked in 84.4% in the II group, which was significantly higher compared with the I group (41.5%, χ^2 = 40.36, p<0.001). At the same time, negative DST was detected significantly more frequently among healthy children in the I group (50.7% vs. 12.7%, χ^2 = 52.16, p<0.001).

Results of DST and of QFT were comparable. Negative results of DST in the I group (50.7%) were comparable to negative results of QFT in the I group (68.1%). Positive results in II group were comparable too (84.4% (DST) and 76.9% (QFT). As QFT is the reference method for DST, it confirms the results in 95% of the cases.

Analysis of leucocytes' subsets (fig.4) being compared to normal ranges revealed significant decrease in CD4+ (57.7 vs. 37.7, χ^2 = 3.99, p<0.05) level in children infected by MBT that may be considered as a sign of immunosuppression. In the second group (in children with tuberculosis of intrathoracic lymph nodes) the level of CD16+ (58.5 vs. 2.4, χ^2 = 38.74, p<0.001) was increased that may be assumed as a sign of activation. In both groups elevation of CD 25+ and CD 95+ levels were observed with the same frequency of occurrence.

No significant differences were identified between groups in the levels of induced cytokines, nevertheless tendency for elevation of TNF-α and IL-4 in children with TB of intrathoracic lymph nodes was marked (second group) (fig.5).

Comparison of the levels of specific immunoglobulins between groups revealed significant elevation of Ig M (45.8% vs. 25.6%, χ^2 = 4.07, p<0.05) and Ig G (22.9 vs. 7.7, χ^2 = 6.31, p<0.05) in children with TB of intrathoracic lymph nodes (fig.6).

Collected in the research data allowed us to figure out diagnostic values of the most relevant immunologic methods. The calculated data of the diagnostic value of TTS (DSS- 91.5%, DSC-15.7%, PPV- 74.4%, NPV- 45.8%, DE-53.6%) gives evidence of its low degree of information and the pressing need for the introduction of new methods to determine tuberculosis activity. New immunologic methods have higher specificity and diagnostic value in comparison with TST that makes their implementation in diagnosis of TB in children essential. The data of the diagnostic value of DST (DSS-84.3%, DSC-59.1%, PPV- 61.9%, NPV-82.7%, DE-71.7%) and the QFT (DSS-78.4%, DSC-68.2%, PPV- 57.7%, NPV-85.1%, DE-73.3%) do not exhibit significant differences among themselves. However, the degree of information provided by DST is twice as high as the data from TTS, which are confirmed during QFT.

Thus, TTS does not provide sufficient information to determine the activity of tuberculosis infection in children infected with MBT, which leads to a late diagnosis of the disease and identification of the specific process involved in the phase of reverse development.

Conclusion

Conclusions of the presented research are as following:

1. In children with tuberculosis no significant immunologic differences vs. normal ranges were identified, except elevated rate of $CD16^{+}$;

2. The most informative signs of active tuberculosis in children are positive QFT-G, Diaskintest, and higher levels of specific immunoglobulins M and G;

3. QFT-G and Diaskintest are very informative tests in diagnosis of tuberculosis in children and this tests are comparable.

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Fig.1. Child P.S., 5 years old

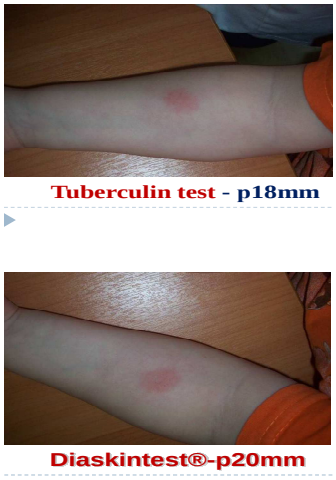

Fig.2. Child P.M., 3 years old

Tabl.1

Clinical manifestations of the intoxication syndrome in the groups

Groups	no intoxication syndrome	moderate intoxication syndrome	manifestation intoxication syndrome
	(%, n)		
I group (n=70)	**78.6% (55) ***	17.1% (12)	4.3% (3)
II group (n=143)	13.3% (19)	25.2% (36)	**61.5% (88)***

* p<0.001

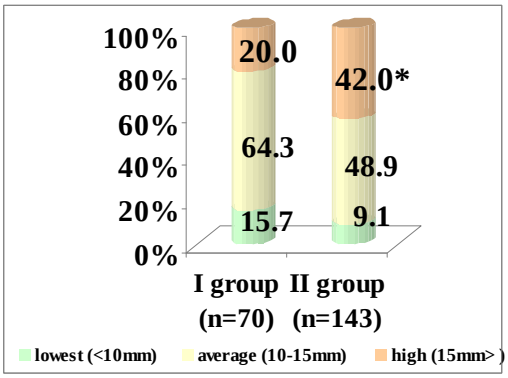

Fig.3. Results of TST

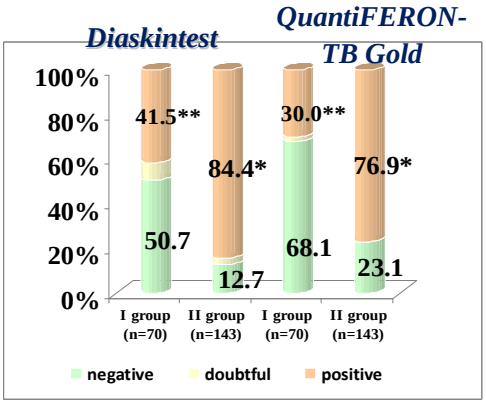

Fig.4. Results of DST and QFT

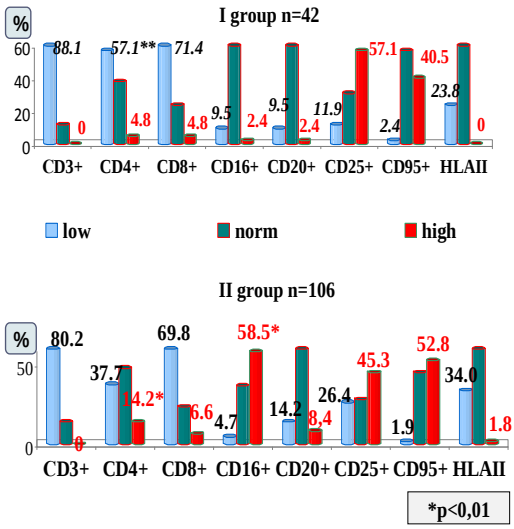

Fig.5. Leucocytes' subsets in the groups.

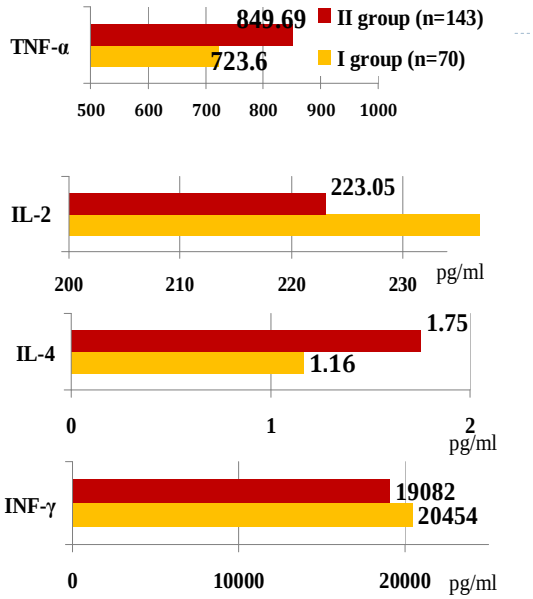

Fig.5. Levels of cytokine-induced

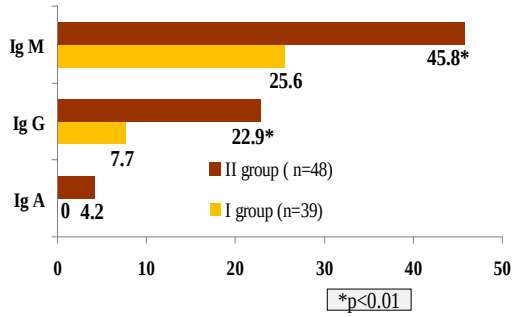

Fig.6. Levels of specific antibodies in immunological reaction