

Diagnosis of Tuberculosis of the Intrathoracic Lymph Nodes in Children with the Use of New Immunologic Tests

A. Starshinova^a, I. Dovgaliuk^a, N. Korneva^a, U. Ovchinnikova^a, P. Gavrilov^a

^aSt. Petersburg Research Institute of Phthisiopulmonology, St. Petersburg, Russian Federation

Abstract

This study was performed to evaluate the diagnostic opportunities provided by the use of new immunologic tests: Diaskintest (DST) and QuantiFERON-TB(QFT) in children with tuberculosis of the intrathoracic lymph nodes. These tests are more informative than tuberculin skin test (TST) in determining activity of tuberculosis infection. The evaluation parameters of diagnostic value (diagnostic sensitivity (DSS), the diagnostic specificity (DSC), positive predictive value (PPV) and negative predictive value (NPV), and diagnostic efficiency (DE)) with DST (DE = 77.5%, NPV= 72.4%, PPV = 91.1%) were significantly higher than with TST (DE = 57.2%, NPV = 47.6%, PPV = 53.2%). In a positive results of the tests in 80.4% lymph nodes diagnosed by computed tomography were in size larger than 0.5 cm (CI 95% 0.4% - 1.8%). Identification of these changes is an indication for prescribing a course of specific therapy.

KEYWORDS: tuberculosis, children, Diaskintest, QuantiFERON-TB test, computer tomography.

Introduction

The diagnosis of tuberculosis of the intrathoracic lymph nodes (TILN) is a little more difficult. Diagnosis of this form of TB in children includes the clinical symptoms, positive tuberculin skin test (TST) in all children after vaccination by Bacillus Calmette—Guérin (BCG) and the absence of large changes in X-ray examination. Russia is the country with a high burden of tuberculosis and routine BCG vaccination at birth. Screening of tuberculosis in children by tuberculin skin test (TST) provides low information, because almost all

children have positive results of TST. Diaskintest ® (DST) – modern immunological skin test with TB antigens ESAT-6 and CFP-10 have been used since 2009 year in Russia.

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 positive result of TST [3, 4, 5]. Currently, computed tomography (CT) is found to detect a specific process on the calcinations of children with positive TST [7,

8]. 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. To create new approaches for the diagnosis of tuberculosis of the intrathoracic lymph nodes in children and for the prompt use of adequate therapy, the results of employing new immunological tests (Diaskintest) necessitate a comparison with the disorders identified on the CT scan.

The aim diagnosis of intrathoracic lymph nodes in children during computed tomography with varying results of immunological tests.

Material and Methods

A prospective cohort study in the department of phthysiology of children we examined 120 patients aged from 3 to 14 years with positive TST, during 2009-2011. Diagnostic complex included: DIASKINTEST® (DST) (Generium), QuantiFERON-TB (QFT-G), multislice computer tomography (MCT). A total of 120 children were examined, of whom 50 (41.7%) were between 3 to 6 years of age ($m = 4.5 \pm 0.2$), and 70 (58.3%) were between 7 and 14 years of age ($m = 12.3 \pm 0.3$).

X-ray examination were done using a spiral CT (multislice spiral computed tomography (MSCT) and MSCT angiography

(MSCT-AG)) 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.).

Initially, a venous blood sample was drawn for QuantiFERON-TB test (QFT, ELISA), 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 using 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.

Before performing these tests, an analysis of the results of the Diaskintest® (DST) – recombinant tuberculosis allergen based on M.tuberculosis specific proteins: ESAT-6 and CFP-10)(Generium) – immunological skin-test) was conducted [11,12]. A positive result was then papule at more than 0.5 cm, doubtful papule at least 0.5 cm, negative in the absence of papules.

According to patient history, all the children had been

vaccinated with BCG in the maternity hospital, 65 of them effectively (54.2%), as evident from the presence of a vaccination scar (more than 4mm).

Manifestations of intoxication syndrome were absent in 17.5% (weight loss, fever, sweating), were moderate in 39.2% (loss of appetite, fatigue) and more pronounced in 43.3%.

Assessment of intrathoracic LNs in children using X-ray methods was performed 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 and the age of the child; however, that did not exclude the presence of pathological changes, including specific changes, in the smaller nodes [11]. According to Ya.V. Lazoreva [12], F. E. Gegeeva [13], and Ya. A. Dauletova [14], all LNs between 5 and 10 mm should be treated as manifestations of intrathoracic adenopathy, although, the standard CT imaging studies can be the limit for visualization of the LNs according to criteria of "imaging". In complex X-ray studies, single LNs (from 0.2 to 0.4 cm in transverse dimension, of conventional structure and density) were visualized in 16.7% of the cases, while single and multiple LNs (from 0.5 to 1.2 cm, of conventional structure and density) were visualized in 50.0% of the cases. Lime deposition in the intrathoracic LNs was

detected in 39 (32.5%) patients, which is twice as high as the standard study. Subpleural located foci (up to 0.3 cm in diameter) in the lung tissue were identified in 27.5% of the cases.

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. Definition of the confidence interval (CI 95%). 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

Clinical manifestations of the intoxication syndrome were mild in 39.2% (CI 95% 28.2%- 43.4%) of cases, pronounced in 43.3% (CI 95% 35.4%-

48.3%) and not significant in 17.5% (CI 95% 15.2%- 23.6%). At the same time, sensitivity to tuberculin in TST of 10-15 mm was detected in 62.5% (CI 95% 54.3%- 73.6%) of the cases, a low level of specific sensitization (<10mm) was marked in 17.5% (CI 95% 13.5%- 21.6%) of the cases, and hyperergic sensitivity (>15mm) was evident in 20% (CI 95% 17.4%- 22.4%).

DST was negative in 48.3% (CI 95% 43.6%- 52.4%), doubtful in 5% (CI 95% 3.2%- 6.4%) ($m=3.2\pm 0.1$ mm), and positive 46.7% (CI 95% 37.4%- 54.2%) ($m=18.0\pm 0.3$ mm). QFT showed a negative result in 57.5% (CI 95% 52.3%- 62.4%), a positive response in 41.7% (CI 95% 34.4%- 51.3%), and a doubtful one in 0.8% (CI 95% 0.5%- 0.9%), which confirms the positive results of DST in 87.5% (CI 95% 82.3%- 94.5%), and allows us to define a differentiated approach to the administration of QFT.

An analysis of the clinical manifestations, the results of the immunological tests and X-ray methods (MSCT and MSCT-AG), and changes in the intrathoracic LNs were done in the children examined. The direct dependence of the severity of the intoxication syndrome in both negative and positive DST/QFT was detected. Therefore, significantly, often negative DST (66.7% (CI 95% 54.6%- 68.5%)) and QFT (80.9% (CI 95% 76.4%- 85.3%)) were marked by the absence of clinical manifestations; however, the presence of positive DST (67.3% (CI 95% 61.3%- 72.4%)) and QFT (59.6% (CI 95% 57.4%- 62.5%)) was associated with significantly pronounced intoxication symptoms. At the same time, manifestations of the intoxication syndrome did not significantly differ in

children possessing a different type of sensitivity to TST.

Results of the QFT study confirmed the dependence obtained during DST. Clinical symptoms were often absent with negative QFT (80.9%, $p<0.001$, $\chi^2=10.8$), whereas pronounced intoxication syndrome was marked on positive testing (59.6%, $p<0.01$, $\chi^2=9.86$), which confirmed the presence of the activity of tuberculosis infection.

Significantly, often negative and positive DST results were detected in 75 (62.5%) children with moderate sensitivity in TST, which indicated the presence of the activity of tuberculosis infection in half the cases. Here, LNs (larger than 0.5 cm) were visualized in 52.0% (CI 95% 43.6%- 58.3%) of the cases. Patients with low sensitivity to TST frequently showed significantly negative results of DST (66.7%, $p<0.01$, $\chi^2=9.6$) with the absence of intrathoracic LNs in 71.4% ($p<0.001$, $\chi^2=10.5$) of cases (Table 1).

The positive results of DST in children (42.7%) with moderate sensitivity confirmed the presence of frequent changes in the intrathoracic LNs determined by MSCT, which already exist, but were not detected by TST.

A comparison of the results of the X-ray examinations (MSCT and MSCT-AG) revealed the frequent absence of changes in the intrathoracic LNs at low sensitivity (71.4%, $p<0.001$, $\chi^2=13.2$), the presence of the LNs (larger than 0.5 cm) at moderate sensitivity (in 52.0%, $p<0.001$, $\chi^2=11.5$), and changes in the intrathoracic LNs (often significantly, in 75.0%, $p<0.001$, $\chi^2=12.2$) at high sensitivity, in 85.5% with lime deposition.

Analysis of the results of QFT in patients with different parameters of DST showed the predominance of negative results (98.3%, $p < 0.001$, $\chi^2 = 88.5$) in QFT at a negative DST and positive results (87.5%) in QFT at a positive DST.

Thus, QFTs confirm the results of DST in 85% of the cases, which makes it possible to limit applying the QFT and recommend it only in the case of contraindications to the use of DST.

Changes of the intrathoracic LNs during X-ray examination (MSCT and MSCT-AG) were not marked in 43.1% ($p < 0.01$, $\chi^2 = 8.53$) patients with no activity of tuberculosis infection according to the results of DST (negative), and LNs (smaller than 0.5 cm) were visualized in 31.1% cases, which is the norm. LNs larger than 0.5 cm were found significantly frequently (80.4%, $p < 0.001$, $\chi^2 = 33.93$) with positive DST, with lime deposition 73.9% ($p < 0.001$) of cases (Table 2).

The results obtained imply that LNs larger than 0.5 cm, against the background of a positive DST result (QFT) are typical of a specific process.

During a standard phthisiatric complex examination with MSCT and MSCT-AG, the data for specific changes was not received in 51 (42.5%) children - infected by *M. tuberculosis*, and the tuberculosis of intrathoracic lymph nodes were diagnosed in 69 (57.5%) children.

The calculated data of the diagnostic value of TST (DSS-84.1%, DSC-19.6%, PPV- 53.2%, NPV- 47.6%, DE-57.2%) gives evidence of its low degree of information and the pressing need for the introduction of new

methods to determine tuberculosis activity.

The data of the diagnostic value of DST (DSS-77.3%, DSC-89.4%, PPV- 91.1%, NPV- 72.4%, DE-77.5%) and the QFT (DSS-69.6%, DSC-96.0%, PPV- 96.0%, NPV- 69.6%, DE-80.0%) do not exhibit significant differences among themselves. However, the degree of information provided by DST is twice as high as the data from TST, which are confirmed during QFT.

Children with a positive DST have pronounced symptoms of intoxication in 67.3% of the cases, and LNs (larger than 0.5 cm on MSCT and MSCT-AG) are visualized in 80.4%, and in 73.9% of the cases in the reverse phases of development. Thus, the data obtained requires mandatory testing using DST in the complex of the phthisiatric survey to determine the activity of tuberculosis infection, and conducting MSCT and MSCT-AG to verify the specific changes in the intrathoracic LNs and lung tissue to administer adequate and timely treatment.

Conclusion

Thus, this study demonstrates highly informative data with DST vs TST in determining the activity of tuberculosis of intrathoracic lymph nodes in children. The data of the diagnostic values of DST (DSS-77.3%, DSC-89.4%, PPV- 91.1%, NPV- 72.4%, DE-77.5%), the QFT (DSS-69.6%, DSC-96.0%, PPV- 96.0%, NPV- 69.6%, DE-80.0%) are significantly higher than the data of the diagnostic value of TST (DSS-84.1%, DSC-19.6%, PPV- 53.2%, NPV- 47.6%, DE-57.2%). With a positive DST the intrathoracic LNs (larger than 0.5 cm) are determined in

80.4%, which against background of high activity of tuberculosis infection may be regarded as evidence of small manifestations of tuberculosis of intrathoracic lymph nodes. Currently, positive result of DST, QFT, and size of lymph nodes more 0.5 cm at carrying out X-ray examinations (CT and MSCT-AG) must be considered as tuberculosis of intrathoracic lymph nodes that requires timely administration of specific therapy.

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Table №1

Results of immunological tests

TST			DST			QF-test		
<10mm	10-15 mm	>15mm	negative	doubtful result	positive	negative	doubtful result	positive
17.5 (21)	62.5 (75)	20.0 (24)	48.3 (58)	5.0 (6)	46.7 (56)	57,5 (69)	0.8 (1)	41.7 (50)
120			120			120		

Table №2

Lymph Nodes (LN) in the computer-tomography examination in children with different results of immunologic tests

TST	DCT (n/%)			Lymph Nodes (LN) in the computer-tomography examination (n/%)			
	negative	doubtful result	positive	Not lymph nodes	LN 0,2-0,5mm	LN < 0,5mm	calcifications in the lymph nodes
<10mm (A) (n=21)	14* (66.7) p<0,01	2 (9.5)	5 (23.8)	15* (71.4) p<0.001	3 (14.3)	3 (14.3)	0
					6		

10-15mm (B) (n=75)	39** (52.0) p<0.01	4 (5.3)	32** (42.7) p<0.01	21 (28.0)	15 (20.0)	39* (52.0) p<0.001	22 (40.7)
					54		
>15mm (C) (n=24)	5 (20.8)	0	19*** (79.2) p<0.01	4 (16.7)	2 (8.3)	18*** (75.0) p<0.001	17** (85,0) p<0.01
					20		
Total	58 (48.3)	6 (5.0)	56 (46.7)	40 (33.3)	20 (16.7)	60 (50.0)	39 (32.5)
					80		

* p<0.001 compared with A and B

** p<0.01 compared with B and C

*** p<0.001 compared with A and C