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Comparative performance of QuantiFERON-TB Gold versus skin test with tuberculosis recombinant allergen (Diaskintest) among patients with suspected pulmonary tuberculosis in Russia

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ABSTRACT

Background: The early identification of *Mycobacterium tuberculosis* infection can prevent tuberculosis (TB) transmission. A skin test with a tuberculosis recombinant allergen (Diaskintest) is a new method for identification that has been implemented in Russia. This study was performed to compare the performances of Diaskintest and QuantiFERON-TB Gold (QFT) in adults and children with suspected TB in Moscow, Russia.

Methods: Adults ($n = 85$) and children ($n = 96$) were tested using Diaskintest and QFT. Concordance and comparative analyses were performed.

Results: Diaskintest and QFT were concordant in 84% of adults and 90% of children (overall concordance 87%, $\kappa > 0.6$, $K_c > 0.5$). The concordance between QFT, Diaskintest, and the final diagnosis was good in adults (86% and 81%, respectively) and moderate in children (77% and 79%, respectively). In adults, QFT had a higher sensitivity for detecting TB than Diaskintest (82% and 68%, respectively); in children, Diaskintest was more sensitive (73% and 65%, respectively). In patients with a confirmed TB diagnosis, negative Diaskintest/QFT results were associated with low disease activity. Combined Diaskintest/QFT results identified TB patients with higher sensitivity and specificity than each test separately.

Conclusions: Diaskintest is a low-cost diagnostic tool that shows a test positivity rate similar to QFT and can be used in combination with QFT as an adjunctive test for TB diagnosis.

Introduction

Tuberculosis (TB) is one of the top 10 causes of death worldwide and the primary cause of death due to any single infectious agent. In 2017, approximately 10 million people developed TB, and 1.3 million died from the disease (WHO, 2018). According to a recent estimate, approximately 1.7 billion people have a latent TB infection (LTBI) (Houben and Dodd, 2016), which can progress to active TB during the person's lifetime. As long as individuals with LTBI exist, TB elimination will not be feasible (ECDC, 2018). Thus, the identification and elimination of LTBI is critical to stop TB transmission in the community.

Currently, two methods are used for the detection of LTBI, i.e., the tuberculin skin test (TST) and interferon-gamma release assays (IGRAs). The TST measures a complex delayed-type hypersensitivity reaction to purified protein derivative (PPD). The diagnostic value of the TST is limited by its lack of specificity, due to the cross-reactivity of PPD with bacille Calmette–Guérin (BCG) and non-tuberculosis mycobacteria, and its insufficient sensitivity, especially in immunocompromised individuals (Pai et al., 2014). IGRAs are in vitro assays that measure the antigen-driven interferon-gamma (IFN-γ) response using an enzyme-linked immunosorbent assay (Quantiferon-TB Gold (QFT); Qiagen, Germany) or enzyme-linked immunospot assay (T-SPOT.TB (T-SPOT); Oxford Immunotec, UK). In both the QFT and T-SPOT tests, peptides derived from ESAT-6 (early secreted antigenic target-6) and CFP-10 (culture

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filtrate protein-10) proteins encoded in the genome of *Mycobacterium tuberculosis* and absent from BCG and most non-pathogenic mycobacteria are used as antigens. This allows antigenic cross-reactivity to be avoided and increases test specificity to *M. tuberculosis* (van Pinxteren et al., 2000; Diel et al., 2011). The limitations of IGRAs are that they require laboratory facilities and are more expensive compared to the TST.

To overcome the limitations of the TST and IGRAs, ESAT-6/CFP-10-based skin tests have been developed. Tuberculosis recombinant allergen (Diaskintest; Generium, Russia) utilizes recombinant fusion ESAT-6/CFP-10 protein and is currently used in Russia to screen children between 8 and 17 years of age for *M. tuberculosis* (Ministry of Health of Russian Federation, 2014). C-Tb (Statens Serum Institute, Denmark) is currently in phase III clinical trials (Ruhwald et al., 2017).

The performances of the TST and IGRAs have been compared in multiple studies and discussed in many reviews and meta-analyses (Menzies et al., 2007; Campbell et al., 2015). Diaskintest has been compared to the TST and demonstrated a significantly higher specificity, apparently due to the use of *M. tuberculosis*-specific antigens not reacting with BCG or non-tuberculous mycobacteria (Slogotskaya et al., 2011, 2017, 2018). However, publications on the comparative performances of Diaskintest and IGRAs are limited in number (Slogotskaya et al., 2012; Losovskaya et al., 2016; Starshinova et al., 2018).

This study was performed to compare the performances of Diaskintest and QFT by analyzing results obtained in adults and children with suspected pulmonary TB in Russia. It is known that the TST, IGRAs, and Diaskintest were developed for LTBI screening and are not intended to be used for TB diagnosis; this is first because they have suboptimal sensitivity and second because they do not distinguish between LTBI and active TB. However, there is currently no gold standard for LTBI. Therefore, to assess assay sensitivity for *M. tuberculosis* infection, active TB is routinely used as a surrogate for LTBI (Kasprowicz et al., 2011). Furthermore, some authorities believe that in certain clinical situations (extrapulmonary TB, TB in patients with negative microbiological results, etc.), IGRAs can contribute supplementary information as part of the diagnostic process (ECDC, 2018). Finally, the immunological test performance depends on the TB burden, indicating that the diagnostic values and the concordances of different immunological tests should be determined for each particular setting. Therefore, comparative and concordance analyses of the results of currently used immunological tests in TB patients in countries with different TB burden are important.

Methods

Study design and participants

The study included 238 participants who were examined in the Central Tuberculosis Research Institute (CTRI, Moscow) between 2011 and 2018 due to suspected TB (clinical symptoms and/or changes on chest X-ray/computed tomography (CT)) or LTBI (positive TST). Exclusion criteria were HIV-positive status, TB diagnosis in the past, pulmonary mycobacteriosis, severe or decompensated comorbidity, secondary immunodeficiency disorders (i.e., diabetes mellitus, organ transplantation, and malignancies), hormone therapy, pregnancy, lactation, and age <1 year.

After applying the exclusion criteria, 85 adults (median age 42 years, range 18–84 years; 48 women, 37 men) and 96 children (median age 10 years, range 3–16 years; 49 girls, 47 boys) were included in the study. All participants were tested using the QFT and Diaskintest tests. To avoid sensitization, blood was collected for the QFT before the performance of the Diaskintest for most participants ($n = 82$, 96% adults; $n = 56$, 58% children). Forty-three participants had been tested using Diaskintest before their admission to the CTRI. For these participants, the QFT was performed not earlier than 10 days after the Diaskintest (median 45 [30;75] days). The Diaskintest and QFT tests were performed in a blinded manner by independent clinicians and immunologists.

The TB diagnosis was made by clinicians based on the results of the following: microbiological examination (sputum smear, culture/BACTEC, and/or PCR analysis) of respiratory specimens (sputum, bronchoalveolar lavage (BAL), bronchial washing, nasopharyngeal aspirates), surgical material, and/or pleural effusion; conventional chest X-ray and/or CT; clinical examination (anamnesis, complaints, history of TB exposure).

The study design and the baseline patient information are presented in Figure 1.

QuantiFERON-TB Gold

The QFT was performed according to the manufacturer's protocol (Qiagen, Germany). Optical densities were measured using a Sunrise reader and Magellan software (Tecan Group Ltd, Switzerland). The concentration of IFN-γ in each tube and the results of the assay were determined using QuantiFERON-TB Gold Analysis Software and interpreted as specified by the manufacturer.

Skin test with tuberculosis recombinant allergen (Diaskintest)

The Diaskintest (Generium, Russia) was performed as recommended by the manufacturer. Briefly, Diaskintest was injected intradermally at a dose of 0.2 μg/0.1 ml by an experienced nurse. The induration was measured 48–72 h later by an experienced clinician unaware of the results of the QFT. The presence of infiltration of any size was considered as positive.

Statistical analysis

The statistical analysis was performed using GraphPad Prism 7.0 (GraphPad Software Inc.), SPSS Version 18, and program R (http://www.r-project.org). In the text, all numerical data are reported as the median with 25th and 75th percentiles. To determine the diagnostic accuracy, the sensitivity and the specificity of the tests were calculated. The prognostic significance was determined by calculating the positive (PPV) and negative (NPV) predictive values and the positive (LR+) and negative (LR−) likelihood ratios. The significance of the differences between categorical data was determined using the Chi-square test with Yates correction ($p < 0.05$). The concordance between the tests or between immunological test results and the final diagnosis was determined by calculating the contingency coefficient (K_c) and kappa index (κ). $K_c > 0.5$ was considered as an indication of strong contingency; $0.41 < \kappa < 0.6$ and $0.61 < \kappa < 0.8$ were considered moderate and good agreement, respectively (Kwiecien et al., 2011). The effectiveness of immunological tests was determined using the Cochran Q test and receiver operating characteristics (ROC) curve.

Results

QFT and Diaskintest results in participants with suspected TB

After applying the exclusion criteria, 181 patients were enrolled in the study and tested using the Diaskintest and QFT at diagnosis. All of the adults ($n = 85$) had determinate results for the Diaskintest and QFT tests. Among the 96 children, 95 had determinate results for both tests and one participant had an indeterminate QFT result.

Among the adults participants, 26 (31%) responded and 59 (69%) did not respond to the Diaskintest (Table 1, $p < 0.0001$). For

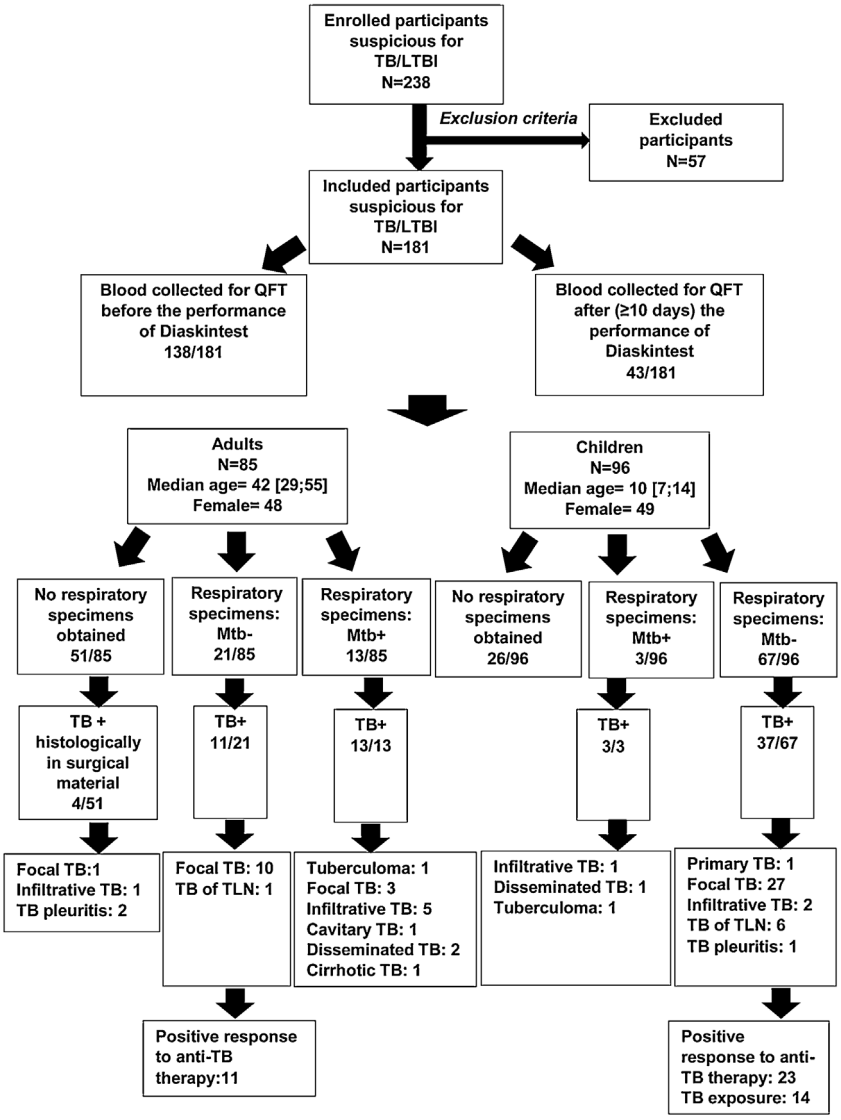

Figure 1. Study design and baseline patient information. Clinical and baseline characteristics of all participants. TB, tuberculosis; Mtb, *Mycobacterium tuberculosis*; LTBI, latent tuberculosis infection; QFT, QuantiFERON-TB Gold; TLN, thoracic lymph node.

Table 1
Results of Diaskintest and QuantiFERON-TB Gold (QFT) in adults and children with suspected *Mycobacterium tuberculosis* infection.

	Adults ($n = 85$)						Children ($n = 96$)					
	QFT											
Diaskintest	Positive	Negative	Indeterminate	Agreement	K_c	κ	Positive	Negative	Indeterminate	Agreement	K_c	κ
Positive	21/26 (81%)	5/26 (19%)	0/26 (0)	84%	0.52	0.63	31/38 (82%)	7/38 (18%)	0/38	90%	0.62	0.80
Negative	9/59 (15%)	50/59 (85%)	0/59 (0)				2/58 (3%)	55/58 (95%)	1/58 (2%)			

K_c, the coefficient of contingency; κ, the coefficient of the agreement strength.

QFT, a positive result was registered for 30 participants (36%) and a negative result for 55 participants (65%). Concordant results were registered for 71 participants (84% agreement): 50 did not respond and 21 responded to both tests. The concordance analysis showed a strong contingency ($K_c = 0.52$) and a good quality of agreement ($\kappa = 0.63$) between Diaskintest and QFT (Table 1).

Out of the 96 children tested using the Diaskintest, 38 (40%) were responders and 57 (60%) were non-responders (Table 1, $p < 0.0001$). Among the 95 children with determined QFT results, 33 were QFT-positive (35%) and 62 were QFT-negative (65%). Concordant results were registered for 86 children (90% agreement): 55 were non-responders and 31 were responders. Compared to adults, the concordance was higher ($K_c = 0.62$, $\kappa = 0.80$, Table 1).

Diagnostic accuracy of Diaskintest and QFT tests for active TB

To characterize the diagnostic accuracy of QFT and Diaskintest, their sensitivity, specificity, PPV, NPV, LR+, and LR – values were determined.

With regard to the adult participants, TB was diagnosed in 28 (33%) and excluded in 57 (67%). For the 28 adults diagnosed with active TB, the diagnosis was confirmed by culture or sputum smear/BAL positivity in 13 participants and by histological examination of surgically resected lung tissue in four participants, while the diagnosis was based on presenting symptoms, chest X-ray/CT, and response to TB treatment in 11 participants. The sensitivity was defined as the percentage of responders among TB patients and was determined separately for (1) participants with a microbiologically/histologically confirmed TB diagnosis; (2) participants with a microbiologically/histologically unconfirmed TB diagnosis; and (3) all TB patients. By performing this type of analysis, the inclusion of patients with negative or uncertain microbiological examination results was considered as critically important, because these patients represent a population in which TB diagnosis is most challenging and for which clinicians tend to apply immunological tests as part of the diagnostic process.

Among the 17 patients with a microbiologically/histologically confirmed TB, 12 patients responded to the Diaskintest and 14 patients responded to the QFT (sensitivity 71% and 82%, respectively; Table 2). Out of the 11 patients with negative microbiological results, seven were responders to the Diaskintest and nine responded to the QFT, i.e., the sensitivity was close to that observed in patients with microbiologically/histologically confirmed TB (64% and 82%, respectively; Table 2).

The specificity of the tests was defined as the percentage of non-responders among adults who had TB ruled out and was 88% for both Diaskintest and QFT. Overall, Diaskintest had a sensitivity of 68%, PPV of 0.73, and LR + of 5.53; QFT had a sensitivity of 82%, PPV of 0.77, and LR + of 6.69 (Table 3).

In children, TB was diagnosed in 40 participants (42%) and excluded in 56 participants (58%). Only three children had microbiologically confirmed TB, which is in line with other data showing the predominance of sputum smear/culture-negative TB in children (Nicol and Zar, 2011; Walters et al., 2018). For the 37 children with negative sputum smear/culture results, the TB diagnosis was based on presenting symptoms, chest X-ray/CT, and the response to TB treatment ($n = 23$), or a history of TB exposure ($n = 14$). Among the three children with microbiologically confirmed TB, all were Diaskintest responders and two children were QFT responders (with a sensitivity of 100% and 67%, respectively; Table 2). Out of the 37 children with a microbiologically unconfirmed TB, 26 responded to the Diaskintest and 24 responded to the QFT (with a sensitivity of 70% and 65%, respectively; Table 2). Overall, in children, Diaskintest had a sensitivity of 73%, a specificity of 84%, PPV of 0.76, and LR + of 4.51 (Table 3). For QFT, these parameters were 65%, 86%, 0.79, and 5.11, respectively (Table 3). Thus, in children, Diaskintest had a higher sensitivity, a lower LR+, and similar PPV compared to the QFT. Among the 56 children with a TB diagnosis excluded, 47 had negative Diaskintest results and 48 had negative QFT results (with a specificity of 84% and 86%, respectively; Tables 2 and 3, $p < 0.0001$).

When analyzing the clinical data, it was noticed that TB patients differed according to the activity of the TB process and we wondered whether there was an association between TB activity and Diaskintest/QFT results. To address this question, adults and children with a confirmed TB diagnosis were segregated into groups with low and high TB activity ($n = 37$ and $n = 31$, respectively). The first group included patients with primary TB ($n = 1$), cirrhotic TB ($n = 1$), Ghon focus ($n = 3$), residual TB lesions ($n = 3$), focal pulmonary TB with radiological signs of calcification and condensation ($n = 22$), or thoracic lymph node TB with calcification ($n = 7$). All patients from the second group had active TB characterized by radiological signs of infiltration and/or dissemination and were diagnosed with TB pleuritis ($n = 3$), focal TB ($n = 14$), infiltrative TB ($n = 9$), disseminated TB ($n = 2$), cavitary TB ($n = 1$), or tuberculoma ($n = 2$). For both adults and children, the rates of Diaskintest and QFT responders were higher in the second group, i.e., in patients with a high TB activity (Tables 2 and 3).

Table 3
Diagnostic accuracy of Diaskintest and QuantiFERON-TB Gold (QFT) tests for active TB.

		Adults		Children	
Parameter	Group	Diaskintest	QFT	Diaskintest	QFT
Sensitivity	All TB+	68%	82%	73%	65%
	TB+, *Mtb*+[a]	71%	82%	100%	67%
	TB+, *Mtb*−[b]	64%	82%	79%	73%
	TB+, low activity	55%	64%	58%	58%
	TB+, high activity	76%	94%	100%	79%
Specificity	All TB−	88%	88%	84%	86%
PPV+	All TB+	0.73	0.77	0.76	0.79
NPV−	All TB−	0.85	0.91	0.81	0.77
LR+	All TB+	5.53	6.69	4.51	5.11
LR−	All TB−	0.37	0.20	0.33	0.40
Agreement	All participants	81%	86%	79%	77%
κ	All participants	0.57	0.69	0.57	0.54
K_c	All participants	0.47	0.65	0.48	0.46

TB, tuberculosis; *Mtb*, *Mycobacterium tuberculosis*; +, positive; −, negative; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio; K_c, the coefficient of contingency; κ, the coefficient of the agreement strength.
[a] TB diagnosis confirmed microbiologically or histologically.
[b] TB diagnosis not confirmed microbiologically.

Table 2
Results of Diaskintest and QuantiFERON-TB Gold (QFT) in patients with different final diagnoses.

		Adults					Children				
		QFT			Diaskintest		QFT		Diaskintest		
Group	Readout	Positive	Negative	Indeterm.	Positive	Negative	Positive	Negative	Indeterm.	Positive	Negative
All TB+	68/181	23/28	5/28	0/28	19/28	9/28	26/40	14/40	0/40	29/40	11/40
	(38%)	(82%)	(18%)	(0)	(68%)	(18%)	(65%)	(35%)	(0)	(73%)	(28%)
TB+, *Mtb*+	20/68	14/17	3/17	0/17	12/17	5/17	2/3	1/3	0/3	3/3	0/3
	(29%)	(82%)	(18%)	(0)	(71%)	(29%)	(67%)	(33%)	(0)	(100%)	(0)
TB+, *Mtb*−	48/68	9/11	2/11	0/11	7/11	4/11	24/37	13/37	0/37	26/37	11/37
	(71%)	(82%)	(18%)	(0)	(64%)	(36%)	(65%)	(35%)	(0)	(70%)	(30%)
TB+, low activity	37/68	7/11	4/11	0/11	6/11	5/11	15/26	11/26	0/26	15/26	11/26
	(54%)	(64%)	(36%)	(0)	(55%)	(45%)	(58%)	(42%)	(0)	(58%)	(42%)
TB+, high activity	31/68	16/17	1/17	0/17	13/17	4/17	11/14	3/14	0/26	14/14	0/14
	(46%)	(94%)	(6%)	(0)	(76%)	(24%)	(79%)	(21%)	(0)	(100%)	(0)
All TB−	113/181	7/57	50/57	0/57	7/57	50/57	7/56	48/56	1/56	9/56	47/56
	(62%)	(12%)	(88%)	(0)	(12%)	(88%)	(12%)	(86%)	(2%)	(16%)	(84%)

Indeterm., indeterminate; TB, tuberculosis; *Mtb*, *Mycobacterium tuberculosis*; +, positive; −, negative.

The concordance analysis revealed a strong contingency ($K_c > 0.5$) and good quality of agreement ($\kappa > 0.6$) between the results of the QFT and the final diagnosis in adults (Table 3). For the other types of comparison (i.e., Diaskintest in adults and QFT and Diaskintest in children), the contingency and agreement between the test results and TB diagnosis were lower ($K_c < 0.5$, $\kappa < 0.6$; Table 3).

*Combined Diaskintest*QFT prognostic and diagnostic effectiveness in participants with suspected TB*

The results demonstrated an overall good agreement between Diaskintest and QFT results. Nevertheless, some participants had discordant results (16% of the adults, 10% of the children; Table 1). It was therefore asked whether a simultaneous performance of both assays would improve their accuracy. To address this, the sensitivity and specificity of the combined Diaskintest*QFT results were analyzed. Only concordant results were taken into account, whereas Diaskintest-negative QFT-positive and Diaskintest-positive QFT-negative results were interpreted as undetermined. Among the 21 adults with positive Diaskintest*QFT results, active TB was diagnosed in 18 (PPV 0.86, LR + 13.36; Table 4). Among the 50 adults with negative Diaskintest*QFT results, a TB diagnosis was confirmed in four (8%) and excluded in 46 patients (NPV 0.92, LR − 0.19; Table 4). Based on the results of Diaskintest*QFT for children, the PPV, NPV, LR+, and LR − were 0.77, 0.84, 5.51, and 0.31, respectively (Table 4).

The concordance analysis revealed a strong contingency ($K_c > 0.5$) and a good quality of agreement ($\kappa > 0.6$) between the results of Diaskintest*QFT and the final diagnosis in adults and children (Table 4).

Overall, the sensitivity and the specificity of combined Diaskintest*QFT were 82% and 94% in adults, and 73% and 87% in children, respectively (Table 4, $p < 0.0001$).

To compare the accuracy of Diaskintest, QFT, and combined Diaskintest*QFT, the Cochran Q test and ROC analysis were performed. The Cochran Q test revealed that QFT, Diaskintest, and Diaskintest*QFT can be successfully used for TB confirmation in children and in adults ($Q = 0.2$, $p = 0.978$ and $Q = 2$, $p = 0.572$, respectively). However, ROC analysis showed that in adults, the combined use of Diaskintest*QFT was characterized by an increased specificity (94%) compared with the separate use of Diaskintest and QFT (88%; Figure 2, Tables 3 and 4). In children, the combined use of Diaskintest*QFT was characterized by an increased sensitivity (73%) compared with the separate use of QFT (65%) and with an increased specificity (87%) compared with the separate use of Diaskintest (84%; Figure 2, Tables 3 and 4).

Table 4
Diagnostic accuracy of combined results of Diaskintest and QuantiFERON-TB Gold (Diaskintest*QFT) for active TB.

	Adults		Children	
	Diaskintest*QFT			
Final DS	Positive	Negative	Positive	Negative
TB	18/22 (82%)	4/22 (18%)	24/33 (73%)	9/33 (27%)
No TB	3/49 (6%)	46/49 (94%)	7/53 (13%)	46/53 (87%)
PPV+	0.86		0.77	
NPV−	0.92		0.84	
LR+	13.36		5.51	
LR−	0.19		0.31	
Sensitivity	82%		73%	
Specificity	94%		87%	
Agreement	90%		81%	
κ	0.77		0.60	
K_c	0.59		0.50	

TB, tuberculosis; DS, diagnosis; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio; K_c, the coefficient of contingency; κ, the coefficient of the agreement strength.

Discussion

The TST and IGRAs are methods that are currently used for the early identification of *M. tuberculosis* infection, including LTBI. However, the low specificity of the TST, high cost of IGRAs, and the inability of both tests to distinguish between LTBI and active TB, limit the use of these methods in countries with a low/medium income level and a medium/high level of bacterial load. A skin test with the tuberculosis recombinant allergen (Diaskintest) is an alternative method. This method was developed in Russia and has been recommended for the identification of *M. tuberculosis* infection during mass screening of children aged between 8 and 17 years for TB since 2009 (Ministry of Health of Russian Federation 2014). Compared to IGRAs, the advantages of the Diaskintest are its ease of use and low cost (€ 1 for one test) (Slogotskaya et al., 2012), which makes it ideal for countries with a low/medium per capita income. Therefore, studies aimed at comparing the diagnostic significance of Diaskintest and IGRAs for TB immunodiagnosis are currently particularly relevant and important. In the present study, a comparative analysis of Diaskintest and IGRA results in adults and children examined due to suspected TB was conducted.

Research studies performed worldwide to assess the sensitivity of the TST and IGRA immunological tests have typically included either patients who have been recently diagnosed with TB or individuals with a confirmed TB exposure as surrogates for LTBI (Kasprowicz et al., 2011), while for the assessment of the specificity of these tests, healthy BCG-vaccinated donors with an unconfirmed TB exposure have been included (Lalvani and Pareek, 2010). Apart from studies evaluating the ability of immunological tests to identify LTBI, some researchers have conducted an assessment of test effectiveness for the diagnosis of active TB, comparing the test results of healthy donors and TB patients (Diel et al., 2010; Syed Ahamed Kabeer et al., 2010). The present study included patients with suspected TB without immunodeficiency disorders (HIV etc.). It is believed that investigating the sensitivity and specificity of immunological tests for this group of patients allows an accurate assessment of their effectiveness. Of 181 patients tested, 68 were diagnosed with TB. For Diaskintest, positive results were registered for 26 adults and 38 children, with 73% of the adults and 76% of the children being diagnosed with TB. Therefore, the probability of identifying active TB in patients with positive Diaskintest results was over 70% (PPV), which is quite high. The data correspond to those reported in other studies, in which the PPV of Diaskintest for identifying active TB was between 63% and 84% in adults and children (Slogotskaya et al., 2011; Shovkun et al., 2015; Losovskaya et al., 2016; Starshinova et al., 2018). The present analysis of QFT results showed a comparable outcome, while according to the data of other authors, the PPV of QFT ranges from 65% to 86% (Shovkun et al., 2015; Losovskaya et al., 2016; Starshinova et al., 2018).

Overall, the sensitivity of the Diaskintest for active TB was 68% in adults and 73% in children, which is lower compared to that reported in other studies. According to a review study by Slogotskaya et al., the sensitivity of the Diaskintest was 97% in children and 84% in adults (Slogotskaya et al., 2017), yet there were data suggesting a low sensitivity of Diaskintest (61%) for identifying TB in individuals with a hyperergic reaction to the injection of the recombinant allergen (Slogotskaya et al., 2018). The sensitivity of QFT for active TB was 82% in adults and 65% in children. According to a meta-analysis of 21 research studies, which were conducted in 12 countries with a varying TB incidence, the pooled sensitivity of QFT in children and adults was 84% (Lu et al., 2016). The relatively low sensitivity of Diaskintest and QFT in

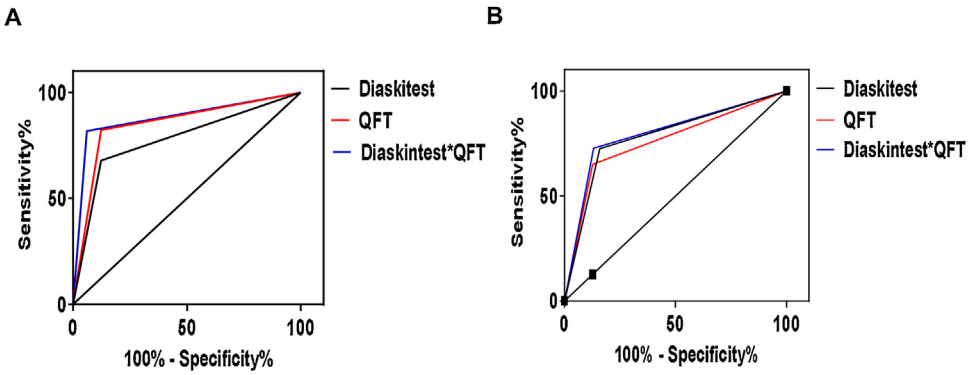

Figure 2. Receiver operating characteristics curve analysis. Receiver operating characteristic curves analysis for the diagnostic evaluation of Diaskintest, QuantiFERON-TB Gold, and combined Diaskintest*QuantiFERON-TB Gold in adults (A) and children (B). QFT, QuantiFERON-TB Gold.

the present study could be explained by the inclusion of patients with microbiologically unconfirmed TB (39% of adults and 93% of children); this differs from other studies, which have included patients with microbiologically confirmed active TB (Detjen et al., 2007; Syed Ahamed Kabeer et al., 2010). Indeed, patients with unconfirmed TB likely had lower disease activity. We have shown previously that the sensitivity of the QFT depends on the activity of the TB process: patients with active TB had a higher percentage of positive results compared to patients with residual TB (Nikitina et al., 2016). After excluding the latter patient group from our analysis, the sensitivity of Diaskintest and QFT in this study was 93% in adults and 77% in children. It is unlikely that the final TB diagnosis was not accurate in patients with a microbiologically unconfirmed diagnosis, as the diagnosis was verified by a positive patient response to anti-TB therapy (100% in adults, 62% in children) and a history of TB exposure (38% in children).

The relatively small patient sample in this study could also have had an effect on the sensitivity of the immunological tests.

In adults with unconfirmed TB, 12% responded to both tests; in children, 16% and 13% responded to the Diaskintest and QFT, respectively. Thus, the specificity of both tests was over 80%, which is quite high and corresponds to data obtained in a number of countries with low TB incidence (Bartu et al., 2008; Bianchi et al., 2009).

The World Health Organization does not recommend using the TST and IGRAs to identify active TB. However, the European Centre for Disease Prevention and Control (ECDC) has suggested that IGRAs could contribute supplementary information as part of the diagnostic process, particularly to diagnose extrapulmonary TB, TB in patients with negative microbiological results, and TB in children, as well as when making a differential diagnosis of TB and mycobacteriosis (ECDC, 2018).

The combined use of the two tests (Diaskintest* QFT) resulted in an increased sensitivity and specificity, significantly in the case of concordant results (Table 4). In individuals with discordant results, TB was registered in 54% of cases. Therefore, whenever additional tests for diagnosing TB are needed, we can recommend the combined use of both tests (Diaskintest/QFT); it is important to take into account concordant results and to examine patients with discordant results carefully using other available diagnostic approaches.

To conclude, the Diaskintest and QFT demonstrated good concordance. Provided that there are no contraindications to the use of the tests in vivo, the Diaskintest can be recommended as a low cost TB infection screening test in countries with a low/medium per capita income. The Diaskintest and QFT showed quite high specificity and an optimal sensitivity, which depended on the TB process activity. This means that positive test results should indicate the need to consider TB, while negative test results along with small changes in the lungs and/or lymph nodes should not be used as a criterion to exclude TB. The combined use of the two immunological tests will allow the sensitivity of TB immunodiagnosis to be increased.

Author contributions

Irina Yu Nikitina and Irina V. Lyadova conceived and designed the study. Natalya L. Karpina and Olga V. Kasimceva were involved in the data analysis and collection. Vladislav Y. Gergert and Atadzhan Ergeshov conducted the data analysis and interpretation. Irina Yu Nikitina and Irina V. Lyadova drafted and wrote the article and all authors provided critical revisions. All authors approved the final version of the article.

Ethical approval

The study protocol was submitted and approved by the Institutional Review Board #1 of the Central Tuberculosis Research Institute. Written informed consent was obtained from all participants (adults) or their legal representatives (children).

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Conflict of interest

The authors declare that the work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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