DE GRUYTER OPEN

Research article

DOI: 10.1515/rrlm-2015-0016

Performance of an interferon-gamma release assay in the diagnosis of tuberculous meningitis in children

Performanţa testului bazat pe eliberarea interferonului gamma în diagnosticul meningitei tuberculoase la copil

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Abstract

The new immunodiagnostic tests based on the Mycobacterium tuberculosis specific antigen, early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), showed promising results in the diagnosis of tuberculosis infection. However, there are only few studies in the published literature on performance tests in cerebrospinal fluid. We investigated whether a rapid diagnosis of tuberculous meningitis (TBM) could be established by interferon-γ blood and cerebrospinal fluid (CSF) tests in children.

We used the QuantiFERON-TB Gold in Tube test (QFT-IT) on blood and the QuantiFERON-TB Gold test (QFT-G) on the CSF of 63 subjects with TBM (including 25 case of definite TBM and 38 cases of probable TBM) and 62 controls.

The CSF analyses indicated possible TBM in 63.4% of cases. The sensitivity of the CSF culture for Mycobacterium tuberculosis was only 39.6%. The sensitivity of the tuberculin skin test (TST) was 49.2% and the specificity was 88.6%. The estimated sensitivities of the QFT-G for the CSF and QFT-IT for the blood in culture confirmed TBM cases (gold standard) were 84% and 80%, respectively. The estimated specificities were 98.2% for the CSF and 87.9% for the blood. This study showed that the sensitivity of QFT for the CSF could be higher than TST and culture and slightly higher in CSF than in blood. The specificity of QFT-G for the CSF was higher those of the TST, but the specificity of QFT-IT is lower.

QFT-G of the CSF is a useful diagnostic marker of tuberculosis that may improve the management of TBM, but the test results must be correlated with clinical, radiological and characteristics of CSF. New researches are needed to investigate the performance of QFT-G in the CSF compared with ELISPOT and PCR.

Keywords*: interferon gamma release assay, QuantiFERON-TB Gold, tuberculous meningitis, children, tuberculosis*

Rezumat

Noile teste imunologice bazate pe antigene specifice ale Mycobacterium tuberculosis, ESAT-6 şi CFP-10, au arătat rezultate promiţătoare în diagnosticul tuberculozei. Totuşi, există numai câteva studii in literatură asupra

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performanţei testelor în lichidul cefalorahidian. În studiul de faţă am investigat posibilitatea unui diagnostic rapid al meningitei tuberculoase (MTB) la copil prin efectuarea testelor bazate pe eliberarea interferonului gamma în sânge şi în lichidul cefalorahidian (LCR).

Am folosit testul QuantiFERON-TB Gold in Tube (QFT-IT) în sânge şi testul QuantiFERON-TB Gold (QFT-G) în LCR la 63 de subiecţi diagnosticaţi cu MTB (incluzând 25 cazuri definite de MTB şi 38 cazuri de menigită probabil TB) şi 62 cazuri control.

Analiza LCR a fost sugestivă pentru o MTB în 63.4% din cazuri. Sensibilitatea culturilor pentru Mycobacterium tuberculosis în LCR a fost de numai 39.6%. Sensibilitatea intradermoreacţiei la tuberculină (IDR) a fost de 49.2% si specificitatea de 88.6%. Sensibiltatea estimată a QFT-G in LCR şi QFT-IT în sânge în cazurile de MTB confirmate prin culturi (standardul de aur) a fost de 84% si respectiv 80%. Specificitatea estimată a fost de 98.2% in LCR şi 87.9% in sânge. Acest studiu a arătat că sensibilitatea QFT in LCR ar putea fi mai mare decât a IDR şi a culturilor pentru micobacterii şi uşor mai mare în LCR decât în sânge. Specificitatea QFT-G în LCR a fost mai mare decât a IDR, dar specificitatea QFT-IT în sânge a fost mai mică faţă de a IDR.

Testul QFT-G în LCR este un marker util pentru diagnosticul tuberculozei care ar putea îmbunătăţi managementul MTB, dar rezultatele testului trebuie corelate cu aspectele clinice, radiologice si caracteristicile LCR. Noi cercetări sunt necesare pentru a investiga performanţele QFT-G în LCR comparativ cu testul ELISPOT şi PCR.

***Cuvinte cheie**: teste bazate pe eliberarea interferonului gamma, QuantiFERON-TB Gold, meningita tuberculoasă, copii, tuberculoza*

Received: 23rd March 2014; Accepted: 29th April 2015; Published: 1st June 2015

Tuberculous meningitis (TBM) is a serious illness that, if not diagnosed and managed early, leads to a high rate of mortality and permanent disabilities. Despite the advances that have been achieved in the diagnosis of TBM and anti-tuberculosis and pathogenic therapies, the prognosis remains poor in many cases, and the mortality remains at approximately 20-30% [1]. In 2012 WHO reported 8.6 million new cases of tuberculosis and 1.3 millions of deaths [2]. In Romania, more than 30000 new tuberculosis (TB) cases or reinfections arise annually, and the incidence of new cases of children who develop serious forms of TB is high.

Studies have clearly demonstrated that the timing of TB meningitis (TBM) treatment is the most critical factor in determining the ultimate outcome, which underscores the importance of early diagnosis [3,4,5]. Clinical features and cerebrospinal fluid (CSF) findings are helpful in the diagnosis of TBM, but these features cannot be used to differentiate TBM from other infectious and non-infectious disorders.

The diagnosis of TBM is based on the analysis of the CSF collected by lumbar puncture. Diagnostic markers of TBM in the CSF typically include elevated protein levels, low glucose levels and predominant lymphocytic pleocytosis [6, 7]. More than half of TBM cases cannot be confirmed microbiologically, and these patients are treated based only on clinical suspicion. Culturing Mycobacterium tuberculosis from the CSF requires a minimum of 2-3 weeks; therefore, the majority of patients with TBM begin treatment before their diagnoses are confirmed. Polymerase chain reaction analyses of CSF samples are not available in our hospital. Brain CT scans and MRIs may show features that are strongly suggestive of TBM, but these techniques cannot diagnose the condition.

In recent years, important progress has been made in the development of in vitro T-cell-based IFN-gamma release assays (IGRAs). The QuantiFERON-TB Gold (QFT-G) and ELISPOT assays are examples. These assays measure the amount of IFN-gamma that is released after

blood is incubated with TB-specific antigens, early secretory antigenic target 6 (ESAT 6) and culture filtrate protein 10 (CFP 10), that stimulate IFN-gamma release from the sensitised T cells of the patients [8-13].

A small number of studies have investigated the use of ELISPOT assays of mononuclear cells from the site of infection (including CSF) compared to PBMCs to aid in the diagnosis of active TB with conflicting results. The performance of the QFT-G test for CSF has not yet been studied. Further studies are also needed to establish the sensitivities and specificities of interferon-gamma release assays in the detection of active TB, particularly in children and immunocompromised patients.

We investigated whether rapid diagnoses of tuberculous meningitis in children could be established with the interferon-gamma blood and cerebrospinal fluid test QuantFERON-TB Gold in the context of routine clinical practice and assessed the agreement of the results of this test with those of classical laboratory methods.

Materials and methods

Patients

We enrolled 125 children <17 years of age (range 8 months - 17 years) who were admitted with a diagnosis of meningoencephalitis between January 1, 2006 and January 31, 2010 in the New St. John Emergency County Hospital Suceava and Clinic of Infections Disease Iasi in this study.

The current study was approved by the Ethics Committee of the Emergency County Hospital of Suceava and the University of Medicine and Pharmacy Gr. T. Popa Iasi. Informed written consent was obtained from all of the parents of the participants or legal guardians. Verbal consent was also obtained from the children aged >8 years.

The patients were separated into two groups: 1) 25 cases of definite TBM and 38 cases of probable TBM; 2) 62 non-TB patients (controls).

Patients were classified as having confirmed TBM (definite cases) if Mycobacterium tuberculosis was cultured from CSF and/or was detected in acid fast staining (AFS).

Probable cases were not confirmed by culture and/or AFS, but with clinical, radiological or laboratory features that were suggestive of TBM and with clinical response to antituberculous treatment. The clinical features include fever, headache, vomiting, seizures, altered mental status or neurological deficit, neck stiffness and a history of illness longer than six days. Typical CSF findings in TBM are pleocytosis > 20 cells/mm^3, more than 60% lymphocytes, elevated protein level (more than 100mg/dL), low glucose concentration (less than 45 mg/dL or a CSF: plasma ratio <0.5) and low chloride level (less than 680 mg/dL). Radiological evaluation may support the diagnosis of TBM in cases that are suspected active pulmonary TB due to chest X ray or changes in brain computed tomography (CT) such as hydrocephalus, basal meningeal enhancement, tuberculoma, cerebral infarcts and edema.

The group with no TB cases was defined by evidence of others diseases and clinical improvement without antituberculous treatment, respectively on antimicrobial, antifungal or antiviral treatment. In the second group (i.e., control group), we enrolled patients with non-TB meningitis, partially treated pyogenic meningitis (diagnosed by CSF-positive for Gram staining or bacterial culture and bacteria-positive latex agglutination test), viral meningitis (i.e., lymphocytic predominant pleocytosis with normal glucose and proteins level in the CSF), cerebral toxoplasmosis (typical scan findings, IgG serology positive finding and a response to empiric treatment), cryptococcal meningitis (positive culture and the presence of cryptococcal antigens) and HIV

aseptic meningitis. Neurological manifestations in the control group were present in cases of cerebral toxoplasmosis, cryptococcal meningitis or HIV aseptic meningitis and were clinically improved without antituberculous therapy.

Complete physical examinations of the patients were performed on admission. Patient demographic information, clinical, microbiological and radiological data have been collected and correlated to the QFT-G results. Background information, including past history of tuberculosis or tuberculin skin test or BCG vaccination, contact grading, immune suppressed status were investigated.

Laboratory assays

Children admitted with suspected meningoencephalitis were investigated with blood counts, CSF cell count examinations, differential counts, protein, glucose, chloride, Gram, Ziehl-Nielsen and India ink stains (Biocenter Ltd, Szeged, Hungary), bacterial, fungal and mycobacterium cultures on liquid culture medium (Bactec 460 TB; Becton Dickinson Diagnostic Systems, Shannon, County Clare, Ireland), and latex agglutination tests (Pastorex Meningitis, Bio-Rad Laboratories, Marries-la-Coquette, France). We performed chest radiographies and brain CT scans on all patients. All children were tested for HIV. When possible, we obtained sputum specimens if we suspected pulmonary TB.

Tuberculin skin testing

For the tuberculin skin test (TST), two units of purified protein derivate (PPD, BB-NCIPD Ltd, Sofia, Bulgaria) were injected intradermal into the anterior region of the forearm, and the transverse induration diameters were measured 72 h later. Skin responses were definite as induration ≥10mm, were considered to be positive results. In HIV positive patients, TST result of ≥ 5 mm was considered positive and for active TB the cut-off remained 10 mm.

Interferon gamma release assay

We performed QuantiFERON-TB Gold In-Tube (QFT-IT, Cellestis, Carnegie, Australia) tests of the blood and QuantiFERON-TB (QFT-G, Cellestis, Carnegie, Australia) tests with separated packages of antigens on the CSF from all patients (n=125). QFT-IT (blood test) contains three antigens (ESAT-6, CFP-10 and TB7.7) and QFT-G (CSF) contains two antigens (ESAT-6 and CFP-10). Peripheral blood and CSF samples were obtained on the same day for the QFT-G tests as per the manufacturer's recommendations [13, 14].

For the QFT-IT test, blood was collected in specified tubes (test tube, mitogen and negative control) and incubated according to manufacturer's instructions (Cellestis, Carnegie, Australia). Samples were processed for ELISA measurement of IFN-gamma levels per manufacturer's instructions (Cellestis, Carnegie, Australia). Positive tests were defined using a standard cut-off of 0.35 IU/mL. Indeterminate results were possible with a lake of control mitogen response or nil control response of > 8.0 IU/mL.

CSF testing was performed with the QuantiFERON-TB Gold Tube technique. We collected 3 mL of CSF into a sterile tube and placed 1 mL of CSF into a three-plate well with a sterile pipette for reaction with ESAT-6, CFP-10 and negative control.

We added the stimulating antigens to each well and shook the covered plate for 60 seconds and the incubated the plates at 37°C. Interferon gamma release was measured by ELISA according to the manufacturer's protocol, for calculations and test interpretation using the QuantiFERON TB Gold analysis software. Qualitative results (yes, no or indeterminate) were obtained. We used the same cut-offs (positive, negative and indeterminate) for QFT-IT tests of the serum and the QFT-G tests the in CSF. A positive result was defined by a difference in the IFN-gamma

levels between the test tube and negative control that was greater than or equal to 0.35 IU/mL and no less than 25% of the nil value. The test results were considered to be indeterminate for TB antigen responsiveness if the IFN-γ level was < 0.35 IU/mL or if the nil value was >8.0 IU/mL. The absolute levels of antigen-specific IFN-γ were calculated by subtracting the value of the negative control from the antigen–specific value.

Statistical analyses

Statistical analyses were performed using SPSS software version 17.0 (SPSS, Inc. Chicago, IL, USA). The positive result rates were compared using the x^2 test, and the levels of IFN-gamma release were analyzed using the non-parametric Mann-Whitney test. $P<0.05$ was considered to indicate a statistically significant difference.

Student's t-tests were used for comparisons between two groups, and comparisons between more than two groups were performed with ANOVAs. The chi-square test was used to compare discrete variables (with Yates corrections when appropriate). ROC curves were analyzed to assess the optimal cut-off values of the QuantiFERON-TB Gold tests for mortality. The sensitivity and the specificity were calculated for the chosen cut-off value. Indeterminate assay results were excluded from statistical analyses of the sensitivity and specificity.

Results

Of the total of 125 subjects who were enrolled in the study, 63 children were diagnosed with TB meningitis (25 cases fulfill the criteria for definite TBM and 38 fulfill the criteria for possible TBM), and 62 were in the control group.

Characteristic of the study patients

The demographic characteristics of the patients are shown in table I. The male/female ratios were 1.33 in the TB meningitis group and 1.58 in the control group. The mean age of the first group was 10.41 years, and the range was eight months to 17 years. The majority of the TBM patients had lived in areas of poor socioeconomic status (76.2% versus 56.5%) and had experienced malnutrition (66.1% vs. 37.1%). In the TBM group, 14.9% of the patients were immunocompromised: seven patients had AIDS, one had acute lymphatic leukemia and was receiving chemotherapy and one had diabetes. In controls group, nine subjects had AIDS and one had leukemia. All HIV patients included in this study had CD4 <200/mm³.

In the TBM group, 35.5% of the children had a known household contact (p=0.239) and 12.7% of the children had histories of TB (p=0.319). Only 58.7% of the children with TB meningitis had received BCG vaccinations (p=0.213). In the control group 71% of cases have been BCG-vaccinated.

Signs and symptoms

The symptoms observed in the physical examination upon admission that suggested TB meningitis were anorexia (95.2%), low fever or prolonged fever syndrome (71.4% of subjects with TBM), neck stiffness (88%), headache (76.2%), altered mental status (69.8%), coma (61.9%), cranial nerve palsies (12.7%) and hemiparesis (12.7%) (table II).

Classic diagnosis methods

High numbers of leukocytes in the blood were present upon admission in 41.3 % of the patients with TBM and 54% of the TBM patients exhibited increased neutrophils. In the non-TB subjects group, 50% had leukocytosis in blood and 66.1% had increased neutrophils. All subjects with TBM had high erythrocyte sedimentation rates (p=0.001) and only 48.7% of non TBM.

Chest radiographies were suggestive of pulmonary TB in 60.3% of the children (miliary

Table I. Demographic details of the patients involved in the study

Parameter	TBM	Probable TBM	Control group	p* / **
Subjects	25	38	62	-
Age (mean ± SD yrs.) **Range between (months)**	11.25±5.65 8-206	9.78±5.85 8-204	10.32±5.16 14-206	0.278 / 0.929
Sex (Male)	13 (52.0%)	23 (60.5%)	38 (61.3%)	0.682 / 0.578
Residing in rural areas	16 (64.0%)	32 (84.2%)	35 (56.5%)	0.123 / 0.684
Malnutrition	16 (64.0%)	25 (65.8%)	23 (37.1%)	0.901 / 0.003
Household TB contact	8 (32.0%)	14 (36.8%)	15 (24.2%)	0.901 / 0.632
History of TB	4 (16.0%)	4 (10.5%)	13 (21%)	0.801 / 0.818
Immunocompromised	4 (16.0%)	5 (13.2%)	10 (16.1%)	0.958 / 0.988
BCG vaccinated	12 (48.0%)	25 (65.8%)	44 (71%)	0.253 / 0.044

* Student's tests were used for comparisons between mean age and the Chi-squared test (with Yates correction) was used to compare discrete variables between TBM and Probable TBM groups.
** Student's tests were used for comparisons between mean age and the Chi-squared test (with Yates correction) was used to compare discrete variables between TBM and Control groups.
TBM – tuberculous meningitis; TB - tuberculosis

or lobar forms or pleurisy) (p=0.001) in TBM group and normal of all cases of non TBM.

Brain CTs revealed hydrocephalus in 25.8% of subjects (p=0.001) and tuberculomas in 14.5% (p=0.006) in the first group. In the second group, CT scan showed multiple ring lesions and cerebral edema in 4.8% toxoplasmosis cases, hydrocephalus in 6.9% cases of cryptococcal

Table II. Signs and symptoms of patients involved in the study

Symptoms	TBM (n=25)	Probable TBM (n=38)	Control group (n=62)	p* / **
Anorexia	24 (96.0%)	36 (94.7%)	42 (67.7%)	0.708 / 0.012
Confusion	17 (68.0%)	27 (71.1%)	21 (33.9%)	0.982 / 0.008
Coma	13 (52.0%)	26 (68.4%)	15 (24.2%)	0.294 / 0.024
Low grade fever	18 (72.0%)	27 (71.1%)	44 (71.0%)	0.839 / 0.801
Headache	19 (76.0%)	29 (76.3%)	55 (88.7%)	0.784 / 0.241
Photophobia	6 (24.0%)	10 (26.3%)	17 (27.4%)	0.929 / 0.953
Neck stiffness	22 (88.0%)	34 (89.5%)	50 (80.6%)	0.820 / 0.314
CN palsies	3 (12.0%)	5 (13.2%)	5 (8.1%)	0.801 / 0.567
Hemi/Para paresis	3 (12.0%)	5 (13.2%)	5 (8.1%)	0.801 / 0.567
Cough	10 (40.0%)	14 (36.8%)	29 (46.8%)	0.990 / 0.736
Abdominal pain	5 (20.0%)	7 (18.4%)	10 (16.1%)	0.863 / 0.905
Vomiting	17 (68.0%)	30 (78.9%)	49 (79.0%)	0.500 / 0.417

* Chi-squared test (with Yates correction) was used to compare discrete variables between TBM and Probable TBM groups.
** Chi-squared test (with Yates correction) was used to compare discrete variables between TBM and Control groups.
TBM - tuberculous meningitis; CN – cranial nerve

meningitis and cerebral atrophy in 3.2% subjects.

We performed TSTs for all patients (n=125). The TSTs were positive in 49.2% of the patients with TBM and in 19.9% of controls (p=0.001).

According to the defined criteria, the CSF analyses indicated possible TBM in 63.4% cases as suggested by moderate pleocytosis (i.e. >20 cells/mm³), high CSF protein levels (i.e., >0.5 g/dL) and low CSF glucose levels (i.e., <45 mg/dL) (table III). The chloride levels in the CSF were decreased to 44.4% of TBM patients and 77.7% had elevated lymphocyte counts in their CSF. All TBM patients were negative for acid-fast staining, only 25 (39.6%) had positive Mycobacterium tuberculosis cultures in CSF and two subjects had positive sputum culture. In one patient, we isolated a MDR strain. The mean time interval necessary for the M. tuberculosis cultures was 25.6 days (range 18 – 33 days). In the controls group, all subjects had moderate CSF protein levels (0.8 – 2 g/dL), normal glucose and chloride levels. All controls had negative AFS and cultures from CSF.

QuantiFERON-TB Gold results

Of the 25 cases of culture-confirmed TBM (i.e., the gold standard), 21 had QFT-G positive results, and four had indeterminate results. In group of probable TBM, out of 38 cases, 24 had positive, 13 negative and one indeterminate QFT-G results (table IV). The percentage of QFT-G positive results for CSF and QFT-IT for blood was similar in TBM patients. The 11.3%

Table III. Cerebrospinal fluid findings

Protein levels	**QFT-IT positive in blood**		**QFT-G positive in CSF**	
Expected value < 0.45 g/dL	TBM	Controls	TBM	Controls
0.8-1 g/dL	26.7%	71.4%	26.7%	100.0%
1.1-2 g/dL	46.7%	28.6%	42.2%	-
2.1-5 g/dL	13.3%	-	15.6%	-
> 5 g/dL	13.3%	-	15.6%	-
P	0.028		0.001	
Chloride levels	QFT-IT positive in blood		QFT-G positive in CSF	
Expected value < 680 mg/dL	TBM	Controls	TBM	Controls
Expected value	55.6%	100.0%	55.6%	100.0%
< 680 mg/dL	44.4%	-	44.4%	-
P	0.024		0.024	
Glucose levels	**QFT-IT positive in CSF**		**QFT-G positive in CSF**	
Expected value 45-76 mg/dL	TBM	Controls	TBM	Controls
> 45 mg/dL	35.6%	100.0%	37.8%	100.0%
< 45 mg/dL	64.4%	-	62.2%	-
P	0.001		0.001	
Cells count	**QFT-IT positive in blood**		**QFT-G positive in CSF**	
Expected number 0-5 leukocytes/mm³	TBM	Controls	TBM	Controls
Lymphocytic pleocytosis	42.2%	42.9%	77.8%	-
Polymorph nuclear reaction	4.4%	-	22.2%	100.0%
P	0.849		0.001	

CSF – cerebrospinal fluid; QFT-IT – QuantiFERON TB Gold in Tube; QFT-G – QuantiFERON TB Gold; TBM – tuberculous meningitis

of patients with false positive QFT-IT results for blood had a history of active tuberculosis and one child with false positive QFT-G result for CSF had previously TBM.

We obtained 68.25% concordant positive QFT-G results (positive QFT for blood and CSF) of the first group and 80.64% concordant negative results (negative QFT for blood and CSF) in the group of controls (table IV). In the TBM group, we observed 9.52% discordant results (positive for CSF/negative for blood or negative for CSF/positive for blood), and we observed 12.90% discordant results in controls.

In TBM patients, we observed 12.5% indeterminate or false negative QFT-G results in patients with low number of cells in CSF and 7, 5% in group of controls.

The time interval required to obtain QFT-G results was 48 hours. The mean time interval necessary for the diagnosis of TB meningitis by QFT-G positive results was 4.31 days.

All 63 patients with definite or probable TB meningitis received antituberculous therapy with four first line drugs (rifampicin, isoniazid, pyrazinamide, ethambutol), and 90% of the patients received dexamethasone therapy. One patient with MDR strain received treatment with moxifloxacin, clarithromycin, rifampicin and pyrazinamide. Prognosis was unfavorable in 12.7% of patients with TB meningitis and 3.2% in the non-TB group and severe disabilities were observed significantly more frequently in patients with MTB than in the control group (30.9% vs. 11.7%) (p =0.02).

In the control group, 40.3% of the patients had viral meningoencephalitis; 45.2% had bacterial meningitis, 4.8% had toxoplasma encephalitis, 6.4% had cryptococcal meningitis and 3.2% had HIV aseptic meningitis. All of the controls recovered without antituberculous therapy.

Discussion

The present report evaluates the performance of the QuantiFERON-TB Gold in Tube using whole blood and the performance of the QuantiFERON-TB Gold assay using CSF for the diagnosis of tuberculous meningitis among children in Romania. The diagnosis of TBM remains a challenge in children, as bacteriological confirmation is the exception, because of the paucibacillary nature of the disease.

In our study, anorexia, altered mental status and coma were the clinical manifestations suggestive of the TBM diagnosis (p = 0.001). However, the initial presentation of the patients

Table IV – QuantiFERON-TB Gold test for 63 TB meningitis patients and 62 controls

Results	TB Meningitis		Probable TBM		Non-TB controls	
	No. cases	%	No. cases	%	No. cases	%
QFT-IT in blood (p values for F Test < 0.001)						
Positive	21	84.0	24	63.2	7	11.3
Negative	0	0	13	34.2	51	82.3
Indeterminate	4	16.0	1	2.6	4	6.4
Total	25	100	38	100	62	100
QFT-G in CSF (p values for F Test < 0.001)						
Positive	21	84.0	24	63.2	1	1.6
Negative	1	4.0	10	26.3	55	88.7
Indeterminate	3	12.0	4	10.5	6	9.7
Total	25	100	38	100	62	100

CSF – cerebrospinal fluid; QFT-IT – QuantiFERON TB Gold in Tube; QFT-G – QuantiFERON TB Gold; QFT-G and QFT-IT was manufactured by Cellestis Ltd. (Carnegie, Australia)

with TBM that could have been mistaken for other illnesses included cough and fever (38% of the children; these children received antibiotics for respiratory infection) and abdominal pain, vomiting, fever with suspected gastroenteritis or acute abdominal surgery (19%). On admission 11.1% of the patients exhibited no meningeal signs, and these signs appeared later in the evolution of the clinical courses of these patients.

Tuberculous meningitis is a severe illness in children, and 61.9% of the TBM group was admitted to the hospital with coma, 69.8% with confusion and 25.4% with neurological signs (cranial nerve palsies, hemi or paraparesis). In other studies, coma has been found in 30-60% of TMB cases, seizures in 50% of children and cranial nerves palsies in 30-50% of patients [15-17]. Kumar et al. in 1999, Youssef et al. in 2006, Thwaites et al. in 2002 and Moghtaderi et al. in 2009 analyzed the clinical and laboratory features that were predictive of tuberculous meningitis and found that history of illness >5-6 days, focal neurological deficits, abnormal movements were significantly more common in TBM patients than other meningoencephalites [16-19]. However, in our study, the onsets were frequently atypical (57% of patients) and manifested as digestive disorders or prolonged fever, and it was too late to seek care from infectious disease specialist in these cases.

More than 60% of the cases of TBM in this study had active pulmonary tuberculosis on chest X-ray, and 17.7% of the children had miliary tuberculosis, these results are similar to those of Donald et al. who reported a strong association between TBM and disseminated tuberculosis [20]. According to literature, TST has a low sensitivity in children with disseminated tuberculosis or TBM, but QFT may be an alternative.

In this report, brain CTs were highly suggestive of TBM (basal enhancement, hydrocephalus or tuberculoma) in 40% of the cases, similar results to those obtained by Andronikou et al. (2004) who reported 41% sensitivity and 100% specificity for brain CTs in the detection of childhood TBM [21]. Another study, of Kumar et al., reported 89% sensitivity and 100% specificity for CT findings in the diagnosis of TBM [16]. However, other conditions, such as fungal meningitis, toxoplasmosis, neurosarcoidosis and carcinomatous infiltration, can result in similar CT images.

Abnormal CSF profile (including lymphocytic pleocytosis, decreased glucose, increased protein and low chloride levels in the CSF) may suggest TBM, and some studies have found sensitivities and specificities that are comparable to those of PCR and superior to those of the culture [22].

A portion of the TBM patients (22.2%) exhibited polymorph nuclear reaction in the CSF and blood at admission, and these patients were initially treated with antibiotics for pyogenic meningitis. The outcomes were unfavorable, and the patients' conditions worsened. Some studies indicated that cell-mediated immune response may decrease in cases of active TB, when untreated. The results of QFT-G from CSF were more frequently false negative in this group and probably will be better to repeat the test after few days.

The AFS smears were negative in all of the cases and did not help us make rapid diagnoses. The CSF cultures were positive for *M. tuberculosis* in a small number of cases (39.6%) and because of the long time required for cultivation, were not useful for the rapid diagnosis of TBM. The CSF specimens used for the culture Mycobacterium were obtained upon the admissions of the patients prior to the initiation of antituberculous treatment.

In the culture-confirmed TBM cases (i.e., the definite cases), the sensitivity of the QFT-G tests of the CSF was 84% (the sensitivity was 100% when we excluded the indeterminate results), and the sensitivity of the QFT-IT tests of

the blood was 80% (95.2% when indeterminate results were excluded). The estimated sensitivity and specificity of QFT-G in CSF were higher than QFT-IT in blood (table V). In previous study, it has been reported that the sensitivity of QFT-IT with whole blood was 59-89% and specificity 70-98% [8,11,24]. We found only one false positive QFT-G results with CSF and this was correlated with history of TB. The false negative and indeterminate QFT-G results (18.6%) for CSF occurred frequently in patients with low numbers of cells in CSF. TB-specific interferon–gamma–producing T–cells might be present in lower numbers in children comparative with adults, resulting in lower levels of IFN-gamma, the target cytokine measured with QFT-G [25-27].

Due to its clinical, epidemiological and CSF features, the QFT-G test of the CSF enabled the rapid diagnosis of TBM in 71.4% of patients, and 34.9% of these cases were later confirmed by positive cultures. A positive QFT-IT blood test cannot distinguish between latent and active TB, but a positive QFT-G of the CSF may explain the etiology of tuberculous meningitis. Jafari et al. showed that IGRA response assayed in mononuclear cells from the site of infection can provide superior discrimination between active TB and latent TB [28]. In the remaining patients (28.6%), the diagnoses of TB meningitis were supported either by positive sputum cultures for M. tuberculosis (three cases) or clinical manifestations, exposure to TB, biochemical characteristics and the cytologies of the CSF, chest XR and CT scan results and favorable responses to antituberculous treatment. All of the cases were also negative for Gram and India ink stains and bacterial and fungal cultures.

The estimated sensitivity and specificity of the QFT-G test of the CSF were superior to those of the TST (80.4% vs. 49.2% and 98.2% vs. 88.6%, respectively) (table 5), and the sensitivity and specificity were higher for the tests of the CSF than those of the serum (figure 1).

The QFT-G might represent a cost-effective alternative to the TST, particularly for BCG-vaccinated persons born in countries in which TB is prevalent, such as Romania. In our study, we had children those parents refused BCG vaccination, especially in rural areas. The national promotion of BCG vaccination has made the interpretation of TST results difficult. The TST has a low specificity in the BCG-vaccinated population due to the cross-reactivity of the diagnostic antigens that are used in the TST for BCG vaccine strains. The greater specificity of the QFT-G and the requirement of only a single visit are compelling advantages, as TST requires a second visit for reading [29, 30].

The sensitivities of the QFT-G test of the CSF (84%) and the QFT-IT test of the blood (80%) for the culture confirmed TBM cases were similar to the sensitivity of the QFT-IT of the blood (80%) that was reported by Kampmann et al. for 209 children with active or latent TB, but the sensitivities of the TST estimated in our study were lower (56% vs. 83%) [31].

Table V. Sensitivity and specificity of utilizing methods

Method	Sensitivity (%)	Specificity (%)	Accuracy (%)	Area under the curve	Asymptotic 95% CI
Culture from CSF	39.6	100	64.8	0.360	0.152-0.569
TST	49.2	88.6	66.9	0.436	0.258-0.678
QFT-IT in blood	77.6	87.9	80.0	0.788	0.522-0.854
QFT- G in CSF	80.4	98.2	84.8	0.821	0.473-0.861

CSF – cerebrospinal fluid; QFT-IT – QuantiFERON TB Gold in Tube; QFT-G – QuantiFERON TB Gold; TST – tuberculin skin test

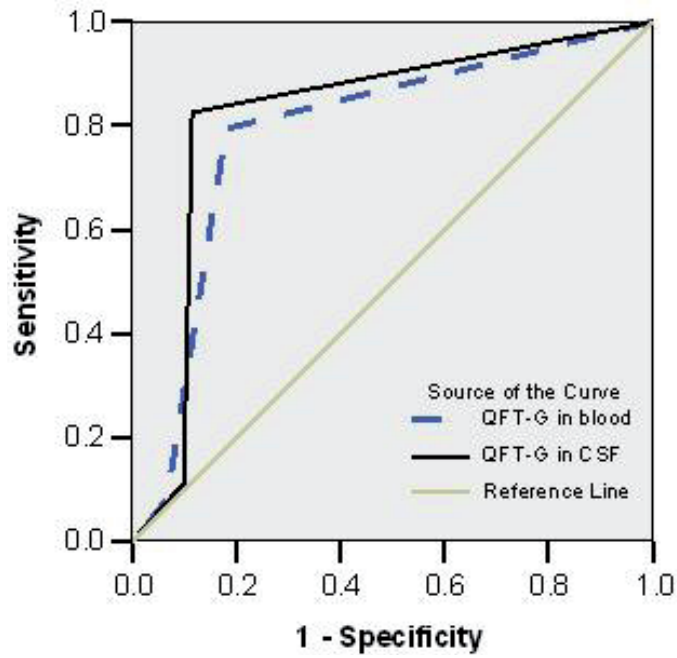

Figure 1. Sensitivity and specificity of QuantiFERON in blood and CSF

The indeterminate QFT-G results from CSF and blood were a little higher in group of TBM than in controls (11.1% vs. 9.7% and 8% vs.6.4%) and were lower indicated for QFT-IT in blood by Kobashi [32].

The negative and indeterminate QFT-G results from the CSF were more frequent among infants and children <5 years old (16.66%) than in children >5 years (9.8%). Kampmann et al. [31] suggested that IFN-γ producing T-cells might be present in lower numbers in children and those IFN-γ release assays of young children are more likely to yield false-negatives. Thirty per cent of the QFT-G indeterminate results in the CSF (n=4/13) and 33% of the QFT-IT indeterminate results in the blood (n=3/9) exhibited inadequate responses to mitogen, and these patients were immunocompromised (HIV positive with CD4 cell counts < 100/mm³). The lymphocytopenia that appeared in HIV patients caused a decrease in the production of IFN-gamma and induced indeterminate QFT results in blood and CSF due to a lower mitogen level. These results are similar to those of the report of Ferrara et al. regarding QFT of the blood [33]. Kobashi et al. also noted higher percentages of indeterminate QFT results when the blood of immunocompromised patients was tested [32]. Results from some studies indicated that frequencies of positive immune responses to IGRA are higher in immunosuppressed patients (i.e. with HIV infection) than to the TST [29,30]. The present report was indicated that 71.4% of patients with co-infection TB-HIV had positive QFT-G results with CSF, so this test may be used for diagnosis of TBM even in immunocompromised persons.

In our study, as in other studies [25, 27], negative or indeterminate QFT-G in the CSF were also influenced by previous TB treatment testing. In the 22.2% of patients who had undergone antituberculous therapy for longer than 14 days prior to the QFT-G tests, the numbers of

negative or indeterminate results were higher (85.7%). In 77.8% patients without antituberculous treatment prior to the QFT-G tests, we observed 12.2% indeterminate results. Therefore, it is better that QFT-G test to be performed before treatment anti TB.

In 2006, Quan et al. [34] assessed TBM diagnoses with different assays and found a sensitivity of 84% and a specificity of 91.8% for the enzyme-linked immunospot assay (ELISPOT) of the CSF; these values were higher than those of culture (sensitivity 16%) and PCR. Our study revealed that the sensitivity and specificity of the QFT-G of the CSF were similar to those of the ELISPOT in Quan's study. In other studies in 2010, Patel et al. [35] found only a 58% sensitivity and 94% specificity for the ELISPOT of the CSF and Kim et al. reported 59% sensitivity and 89% specificity of the ELISPOT assay in mononuclear cells of the CSF [36]. So, in our report the sensitivity of the QFT-G for the CSF seems to be similar or higher than that of the ELISPOT in some studies (80.4% vs. 84%, 58% and 59%, respectively) [34-36].

However, the diagnosis of TBM requires that a combination of epidemiological, medical history, clinical, radiological and laboratory findings be taken into account when interpreting the QuantiFERON TB Gold test results [37].

The present study has some limitations. First, we did not compare the diagnostic value of QFT-G from CSF to PCR for Mycobacterium tuberculosis in CSF because that technique was not available in our hospital. Future investigations are necessary to compare the sensitivity and specificity of QFT to those of PCR and ELISPOT for CSF are of significant interest. Secondly, we included in our report a limited number of infants and immunocompromised subjects. Furthers studies are needed to investigate if QFT-G for CSF is dependent of the very young age (< 2 years) or immunocompromised status of patients. In immunocompromised patients with higher likelihood of indeterminate results, the ELISPOT is an alternative to the QFT-G for CSF.

Other studies are also requires to estimate the period between exposure to TB, the onset of TBM and the occurrence of positive QFT test in CSF. An initially negative QFT-G result in CSF does not exclude TBM and will be useful to repeat the test in cases of presumed TBM. Making dynamics QFT tests could be useful for determining the effectiveness of anti TB drugs.

In **conclusion**, although cultures for M. tuberculosis remain the gold standard in the diagnosis of TB the new immunological interferon gamma releasing tests seem to be promising for the management of TB meningitis. In present study, the estimated sensitivity of QFT-G for CSF was comparable to that of the QFT-IT for whole blood and higher than TST and culture and the estimated specificity of QFT-G for CSF seems to be higher than other methods. A positive QFT-G test in the CSF may be a solid argument for a diagnosis of TBM, at least in cases with negative cultures.

Even in a paucibacillary population like children, the determination of gamma interferon in the serum and CSF are useful diagnostic markers of tuberculosis that may improve the management of TB meningitis by enabling rapid diagnosis and early initiation of treatment.

List of abbreviations

AFS – acid fast staining
BCG – Bacillus Calmette – Guerin
CN – cranial nerve
CNS – central nervous system
CFP – Culture filtrate protein
CSF – cerebrospinal fluid
ESAT – Early secretory antigen target
IFN-gamma – interferon-gamma
IGRA – interferon gamma release assay
LTBI – Latent tuberculosis infection

M. tuberculosis – Mycobacterium tuberculosis
PPD – purified protein derivate
QFT - QuantiFERON
QFT-IT – QuantiFERON-TB Gold In-Tube
QFT-G – QuantiFERON-TB Gold
TBM – tuberculous meningitis
TB – tuberculosis
TST – tuberculin skin test

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