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ORIGINAL ARTICLE

The sensitivity of an interferon-γ release assay in microbiologically confirmed pediatric tuberculosis

Kurt Schopfer · Hans L. Rieder · Thomas Bodmer · Jacqueline F. Steinlin-Schopfer · Yay Chantana · Peter Studer · Denis Laurent · Beat Richner

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Abstract This study aimed at determining the sensitivity of a whole blood interferon-γ release assay (IGRA) among children with microbiologically confirmed tuberculosis in a high-burden country. Children with a diagnosis of tuberculosis based on clinical and radiographic assessment were tested with an IGRA in addition to microbiologic examination of appropriate specimens for acid-fast bacilli, mycobacterial rRNA, and observation for growth of *Mycobacterium tuberculosis* on appropriate culture media. Of the 405 children with a clinical diagnosis of tuberculosis, 91 (22.5 %) had microbiologically confirmed tuberculosis, of whom 81 were tested with an IGRA. A positive result was obtained in 43 (sensitivity 53.1 %, 95 % confidence interval 42.3 to 63.6 %), uninfluenced by age, sex, or disease manifestation. *Conclusions*: The sensitivity of a whole blood interferon-γ release assay in microbiologically confirmed pediatric tuberculosis was low. An IGRA cannot, thus, be used as rule-in test, but it might be useful to rule in tuberculosis among children in whom tuberculosis is notoriously difficult to confirm microbiologically.

K. Schopfer (✉) · T. Bodmer · J. F. Steinlin-Schopfer
Institute of Infectious Diseases, University of Bern,
Friedbühlstrasse 51, 3010 Bern, Switzerland
e-mail: kurt.schopfer@bluewin.ch

K. Schopfer
Scheuermattweg 43, 3043 Uettligen, Switzerland

H. L. Rieder
International Union Against Tuberculosis and Lung Disease,
68 blvd Saint-Michel, 75006 Paris, France

H. L. Rieder
Institute of Social and Preventive Medicine, University of Zurich,
Hirschenbraben 84, 8001 Zurich, Switzerland

Y. Chantana · P. Studer · D. Laurent · B. Richner
Kantha Bopha Foundation, Phnom Penh, Cambodia, c/o Intercontrol
AG, Seefeldstrasse 17, 8008 Zurich, Switzerland

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The diagnosis of pediatric tuberculosis proves difficult in any setting. In high-burden countries with constrained resources, the diagnosis of childhood tuberculosis is often reliant on clinical judgment alone. Even with resources available, the paucibacillary nature of childhood tuberculosis makes its microbiological confirmation a formidable challenge. Demonstration of bacilli by microscopy, culture, or nucleic acid amplification is feasible in a minority of cases only [4]. The yield is particularly poor in very young children [15].

We previously reported on the implementation of laboratory diagnostic services in a large pediatric referral hospital in Cambodia [15]. While the focus was on the feasibility of implementing microbiological services for tuberculosis, an interferon-γ release assay (IGRA) was also included in the evaluation of children with a clinical diagnosis of tuberculosis. The purpose here was to determine the potential contribution of an IGRA, evaluated against the standard of microbiologically confirmed tuberculosis.

Material and methods

Setting

The setting has been described [15]. The Jayavarman VII Hospital in Siem Reap is the largest of the five Kantha Bopha pediatric referral hospitals in Cambodia. Together, these hospitals took care of about 120,000 hospitalized and 800,000 ambulatory pediatric patients in 2011 [13]. During the period from 1 July 2005 through 31 March 2006, all children hospitalized in this hospital, who are with a clinical diagnosis of tuberculosis were systematically evaluated based on a protocol ensuring a standardized flow for examinations.

Procedures and ethics considerations

At admission to the hospital, the child's guardian is requested to provide informed and signed (fingerprint) consent for the necessary diagnostic and treatment procedures. This record is kept as a permanent document in the patient's file. Before project implementation, diagnosis of tuberculosis was based on clinical assessment, imaging techniques, and bright field microscopy. A key purpose of this project was upgrading diagnostic mycobacteriological techniques on the same specimen with rRNA amplification. The diagnostic procedure was supplemented by an IGRA in children suspected of having tuberculosis to establish the presence of tuberculous infection to enhance the probability to rule in tuberculosis [2].

Methods

Routine laboratory examinations upon admission included hematology, biochemistry, and urine examinations in accordance with locally established hospital standards. Laboratory tests, made after obtaining written consent from the child's guardian, included testing for human immunodeficiency virus (HIV) with an opt-out approach as recommended [17]. Imaging techniques were utilized at the discretion of the clinician subsequent to hospital admission once tuberculosis was clinically suspected. Among the children with a clinical diagnosis of tuberculosis supported by radiographic evidence, routine laboratory tests were supplemented by a whole blood IGRA (QuantiFERON®-TB Gold In Tube, Cellestis Ltd., Carnegie, Australia). The IGRAs were done on the same day as blood was collected. A positive IGRA result was defined according to the manufacturer's recommendation.

Tuberculosis-specific laboratory examinations included collections of clinical specimens: a first one on the spot, followed by additional ones to preferably obtain three specimens on three consecutive days. All the specimens were decontaminated and concentrated by centrifugation prior to splitting into aliquots for microscopy (Ziehl–Neelsen method), rRNA amplification and culture, respectively, as described [15]. A case of microbiologically confirmed (henceforth, shortened to "confirmed") tuberculosis was a patient with at least one positive result by any of the three methods on at least one specimen.

Electronic database and analysis

The data were captured in an EpiData relational database (EpiData Association, version 3.1) and analyzed with EpiData Analysis version 2.2 (freely available at http://www.epidata.dk) and OpenEpi (http://www.openepi.com/OE2.3/Menu/OpenEpiMenu.htm) as appropriate. The data were abstracted from the paper documents as recorded by health care staff. Up to three disease sites were electronically captured. Electronic recoding used an algorithm with a predetermined hierarchy of intrathoracic (including pulmonary, intrathoracic lymphatic, and pleural), followed by lymphatic (extrathoracic), soft tissue, osteoarticular, central nervous system (CNS), and finally other sites to obtain a uniform classification for major, second, and third disease sites. We used the classification "intrathoracic" rather than "pulmonary" because of the difficulties in childhood tuberculosis of the respiratory tract to clearly separate tuberculosis limited to the lung parenchyma (pulmonary) from other intrathoracic manifestations, primarily the intrathoracic lymphatic system. For categorical variables, we determined proportions with 95 % confidence intervals (CI) and odds ratios with 95 % CI for contrasting binomial outcomes. Where deemed appropriate, adjustments for potentially confounding factors were made by stratification using the Mantel–Haenszel procedure. For continuous variables, we used standard measures of central tendency such as means and percentiles.

Results

Four hundred and five children had a clinical diagnosis of tuberculosis in the period from 1 July 2005 through 31 March 2006. In 91 (22.5 %), the clinical diagnosis was confirmed (Table 1). Intrathoracic, lymphatic, soft tissue, and osteoarticular tuberculosis contributed 316 (89.1 %) of all cases, and 88 (96.7 %) of confirmed cases. The proportion of the confirmed cases among each of these forms was similar (odds ratio of the confirmed intrathoracic tuberculosis versus all other confirmed forms 1.5, 95 % CI 0.9–2.4). Among the 21 cases with tuberculosis of the CNS, only 1 was confirmed and only 2 of the 23 cases with other disease manifestations. However, six of an additional seven patients with CNS tuberculosis as hierarchically non-primary site had microbiological confirmation.

While 71 cases (17.5 %) were diagnosed among children less than 1 year old, only 4 of the 91 confirmed cases were from this age group. Except for soft tissue tuberculosis, more boys than girls were diagnosed with tuberculosis, reflecting the admission pattern [15]. Given a diagnosis of tuberculosis, confirmation was more often obtained among girls than among boys (odds ratio 1.64, 95 % CI 1.03–2.6).

Two or more disease sites were noted in 66 patients (16.3 %). The probability of obtaining confirmation increased with the number of sites affected (significant chi-square for trend). Among the children with intrathoracic tuberculosis, other forms were almost as frequently diagnosed (26 cases) as major second sites as were lymphatic, soft tissue, osteoarticular, and CNS tuberculosis combined (29 cases). Of the 405 children, the HIV test result was recorded in 23 (5.7 %) and was negative in all.

The IGRA results were available for 338 (83.5 %) children: 71 IGRA-positive children, 43 (60.6 %) had confirmed

Table 1 Clinical and bacteriological characteristics of tuberculosis patients from Jayavarman VII Hospital, Siem Reap (period of representative sampling is from 1 July 2005 through 31 March 2006)

Characteristic	Hierarchically first disease site																		Total		
	Intrathoracic			Lymphatic			Soft tissue			Osteoarticular			CNS			Other					
	Pos	Total	% pos	Pos	Total	% pos	Pos	Total	% pos	Pos	Total	% pos	Pos	Total	% pos	Pos	Total	% pos	Pos	Total	% pos
Total	56	219	25.6	9	41	22.0	11	46	23.9	12	55	21.8	1	21	4.8	2	23	8.7	91	405	22.5
Age group																					
Under 1	4	49	8.2	0	5	0.0	0	7	0.0	0	4	0.0	0	3	0.0	0	3	0.0	4	71	5.6
1 to <3	6	49	12.2	0	7	0.0	1	3	33.3	3	6	50.0	1	1	100.0	0	4	0.0	11	70	15.7
3 to <5	6	17	35.3	2	5	40.0	0	9	0.0	0	1	0.0	0	1	–	0	0	–	8	33	24.2
5 and older	40	104	38.5	7	24	29.2	10	27	37.0	9	44	20.5	0	16	0.0	2	16	12.5	68	231	29.4
Sex																					
Female	31	101	30.7	5	18	27.8	7	25	28.0	5	19	26.3	0	6	0.0	1	10	10.0	49	179	27.4
Male	25	118	21.2	4	23	17.4	4	20	20.0	7	36	19.4	1	15	6.7	1	13	7.7	42	225	18.7
Missing	0	0	–	0	0	–	0	1	0.0	0	0	–	0	0	–	0	0	–	0	1	0.0
Number of involved sites																					
1 site	27	161	16.8	9	40	22.5	5	39	12.8	12	55	21.8	1	21	4.8	2	0.0	–	56	339	16.5
2 sites	24	53	45.3	0	1	0.0	6	7	85.7	0	0	–	0	0	–	0	0	–	30	61	49.2
3 sites	5	5	100.0	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	5	5	100.0
Major second site																					
Any second site	29	58	50.0	0	1	0.0	6	7	85.7	0	0	–	0	0	–	0	0		35	66	53.0
Lymphatic	5	7	71.4	NA	NA	NA	0	0	–	0	0	–	0	0	–	0	0	–	5	7	71.4
Soft tissue	3	10	30.0	0	0	–	NA	NA	NA	0	0	–	0	0	–	0	0	–	3	10	30.0
Osteoarticular	5	6	83.3	0	0	–	6	7	85.7	NA	NA	NA	0	0	–	0	0	–	11	13	84.6
CNS	5	6	83.3	0	1	0.0	0	0	–	0	0	–	NA	NA	NA	0	0	–	5	7	71.4
Other	11	26	42.3	0	0	–	0	0	–	0	0	–	0	0	–	NA	NA	NA	11	26	42.3
IGRA result																					
Negative	23	138	16.7	2	28	7.1	5	34	14.7	6	35	17.1	0	15	0.0	2	17	11.8	38	267	14.2
Positive	27	39	69.2	6	8	75.0	5	8	62.5	4	9	44.4	1	4	25.0	0	3	0.0	43	71	60.6
Missing	6	42	14.3	1	5	20.0	1	4	25.0	2	11	18.2	0	2	0.0	0	3	0.0	10	67	14.9

CNS central nervous system, *NA* not applicable, *IGRA* interferon-γ release assay, *Pos* positive, % *pos* percentage of positive cases

tuberculosis. Conversely, 38 (14.2 %) of all children with a negative IGRA result had confirmed tuberculosis. To further examine the role of the IGRA, additional analyses were done among the 338 children with an IGRA result (Table 2). Stratifying by diagnostic type of tuberculosis (clinical diagnosis versus confirmed diagnosis), 43 of the 81 confirmed cases had a positive IGRA result (a sensitivity of 53.1 %, 95 % CI 42.3–63.6 %), as compared to 28 of 257 (10.9 %) children with a clinical diagnosis but without confirmation. The age-specific distribution of IGRA positive showed no predilection for any age group (Fig. 1).

Analyzing the IGRA test results by site of disease, we found overlapping confidence intervals for the proportion of a positive IGRA result among patients with intrathoracic tuberculosis only, intrathoracic plus extrathoracic, and extrathoracic tuberculosis only.

The odds ratio of finding a positive IGRA result among the microbiologically confirmed versus the clinically diagnosed only cases was 9.2 (Table 3). While the ratio varied somewhat by age group, sex, and number of disease sites (one versus two, three or more), these variations were small, and adjustment for all these variables did not make any significant difference (odds ratio 7.6, 95 % CI 3.7 to 15.7).

Discussion

The diagnosis of tuberculosis in children is fraught with problems in the operating characteristics, notably test sensitivity, of virtually any assay being used. While systems based on the identification of molecular methods [12, 15] hold great promise, the yield remains very low even under optimized conditions, and complex sample collection procedures are required (sputum induction and gastric lavage with subsequent concentration). The problem is compounded the younger the children are [15].

Table 2 Univariate analysis of interferon-γ release assay result by case definition (clinical only versus microbiologically confirmed), Jayavarman VII Hospital, Siem Reap (period of representative sampling is from 1 July 2005 through 31 March 2006)

Characteristic	IGRA result								Odds ratio[a]		
	Clinical cases only				Microbiologically confirmed cases				Point	Confidence interval	
	Neg	Pos	% pos	Total	Neg	Pos	% pos	Total		Low	High
Total	229	28	10.9	257	38	43	53.1	81	9.3	5.1	16.6
Age group (years)											
Under 1	42	2	4.5	44	2	2	50.0	4	21.0	1.9	236.0
1 to <3	44	5	10.2	49	3	8	72.7	11	23.5	4.7	118.3
3 to <5	22	2	8.3	24	4	2	33.3	6	5.5	0.6	51.2
5 and older	121	19	13.6	140	29	31	51.7	60	6.8	6.8	13.7
Sex											
Female	92	14	13.2	106	18	24	57.1	42	8.8	3.8	20.1
Male	136	14	9.3	150	20	19	48.7	39	9.2	4.0	21.3
Missing	1	0	0.0	1	0	0	NA	0	NA	NA	NA
Major site											
Intrathoracic	115	12	9.4	127	23	27	54.0	50	11.3	5.0	25.4
Lymphatic	26	2	7.1	28	2	6	75.0	8	39.0	4.5	335.3
Soft tissue	29	3	9.4	32	5	5	50.0	10	9.7	1.7	53.8
Osteoarticular	29	5	14.7	34	6	4	40.0	10	3.9	0.8	18.8
CNS	15	3	16.7	18	0	1	100.0	1	NA	NA	NA
Other	15	3	16.7	18	2	0	0.0	2	0.0	–	–
Number of sites											
1 site	209	24	10.3	233	22	27	55.1	49	10.7	5.3	21.6
2 sites	20	4	16.7	24	14	13	48.1	27	4.6	1.3	17.3
3 sites	0	0	NA	0	2	3	60.0	5	NA	NA	NA

Adjusted odds ratio of a positive IGRA result by case definition, adjusted for age group, sex (one case with missing information excluded), and number of sites: 7.6 (95 % confidence interval 3.7 to 15.7), suggesting no confounding by the variables considered for adjustment

Neg negative, *Pos* positive, % *pos* percentage of positive cases

[a] Univariate odds of a positive IGRA among microbiological cases divided by odds of a positive IGRA among clinical cases

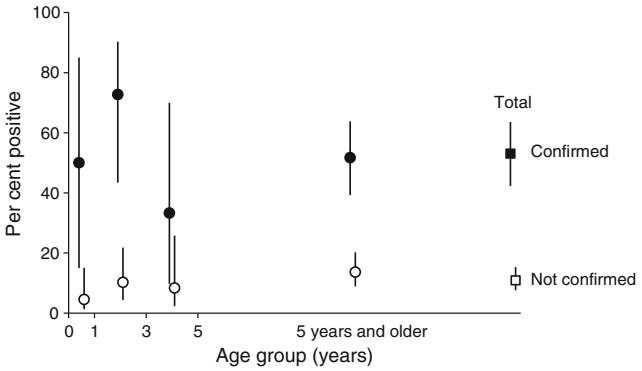

Fig. 1 Age-specific proportion of positive IGRA results, by case definition (clinical only versus microbiologically confirmed). *Filled symbols* denote cases with microbiological confirmation; *hollow symbols* denote clinically diagnosed cases without microbiological confirmation. Jayavarman VII Hospital, Siem Reap; period of representative sampling is from 1 July 2005 through 31 March 2006

The difficulty in obtaining microbiological confirmation of tuberculosis also affects the choice of the gold standard to evaluate any new diagnostic technique in childhood tuberculosis. As we have shown [15] even when using different techniques on multiple specimens, tuberculosis could not be confirmed in more than three quarters of children with a clinical diagnosis. This diagnostic shortcoming potentially introduces a selection bias in test evaluation. Conversely, a large number of children with microbiological confirmation were available in this study, providing a fairly sturdy estimate for the test sensitivity.

Thus, a high index of suspicion coupled with clinical acumen remains the most important prerequisite for the diagnosis of tuberculosis in children. The frequency with which extrathoracic tuberculosis as the sole presentation was identified in our setting indicates that tuberculosis was high on the list of the differential diagnosis. It is not surprising that the frequency of confirmation increased if more than one disease site was involved because the likelihood of tuberculosis increases with multi-system disease [16]. We were interested in learning to what extent an IGRA could be of assistance in the diagnosis of tuberculosis in children as demonstrated in this comparatively large series of 81 confirmed tuberculosis cases. This issue has been addressed by others [1, 3, 5, 6, 8, 9, 11]. A limitation to any study of tuberculosis in children is the gold standard of microbiological confirmation. It is not surprising that sample sizes in most studies have commonly been relatively small. Of the above seven studies, the one from South Africa [9] was the largest (involving 57 cases), the other six included fewer than 30 children. Small sample sizes result in relatively large reported variations in IGRA sensitivity, in these studies ranging from 56 to 93 %. The issue has also been a subject of a meta-analysis [10]. Unfortunately, that analysis mixed "confirmed and/or probable active" tuberculosis and thus essentially invalidated it. Among our 81 children with confirmed tuberculosis, only 43 had a positive IGRA, a disappointingly low sensitivity of 53 %. Therefore, an IGRA test result cannot be used as a rule-out test for tuberculosis and does not substitute microbiological investigation. Conversely, because of the great difficulty in obtaining microbiological confirmation among children, a positive result can be useful to rule in tuberculosis. While only 6 % of our patients had their HIV status recorded, we have no evidence that there was a selection bias for the transcription. All 23 of these patients had a negative HIV test result. This suggests that HIV infection cannot explain the limited sensitivity of the IGRA in our patient population. The low frequency of positivity was independent of age, sex, and multi-system disease. Our study was not designed to determine IGRA specificity, but specificity is of lesser concern as it is precisely this characteristic where the test offers a large advantage over tuberculin skin testing [10]. While research points to the future possibility to distinguish latent tuberculosis infection and active disease [7], none of the currently available immunological tests has that discriminatory ability [14].

In this setting, the sensitivity of the whole blood IGRA for diagnosing active tuberculosis was (remarkably) low and barely exceeded 50 % in microbiologically confirmed pediatric tuberculosis. This poor sensitivity was not related to age, sex, and disease manifestation or to HIV infection. Nevertheless, given the very low yield of microbiological investigations in the very young child with suspected tuberculosis, the addition of even a low-sensitivity test may substantially improve the accuracy of the diagnosis in children under the age of 3 years.

Table 3 Odds ratio of a positive IGRA result among microbiologically confirmed versus non-confirmed cases (crude and adjusted results)

Variable(s) adjusted for	Odds ratio		
	Point estimate	95 % confidence interval	
		Low	High
Crude, non-adjusted	9.2	5.1	16.6
Adjusted for age	8.3	4.6	15.0
Adjusted for sex	9.0	5.0	16.2
Adjusted for multiple sites	8.5	4.5	16.0
Adjusted for age and sex	8.2	4.5	14.9
Adjusted for age and multiple sites	7.4	3.9	14.3
Adjusted for sex and multiple sites	8.5	4.5	16.2
Adjusted for age, sex, and multiple sites	7.6	3.7	15.7

Jayavarman VII Hospital, Siem Reap; period of representative sampling is from 1 July 2005 through 31 March 2006

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Conflict of interest The authors declare that they have no conflict of interest.

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