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Risk Factors for False-Negative Interferon-γ Release Assay Results in Culture-Confirmed Childhood TB

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Abstract. A negative interferon-γ release assay (IGRA) result might inappropriately lower the clinical suspicion for childhood tuberculosis (TB) and result in delayed treatment initiation. However, the risk factors associated with false-negative IGRA results in children remain unclear. Between May 2012 and January 2018, 156 culture-confirmed childhood TB patients who had received T-SPOT.TB test were included. Data, including demographic information and clinicopathological variables, were collected via questionnaires. Univariate and multivariate logistic regression analyses were performed to estimate the odds ratio (OR) and corresponding 95% CI of risk factors associated with false-negative T-SPOT.TB results. The positive rate of T-SPOT.TB test was 85.9% in childhood TB patients. Multivariate analysis revealed that younger age (≤ 9 years; OR = 4.782; 95% CI: 1.689, 13.539), weight for age (*z*-score > 0.37; OR = 4.256; 95% CI: 1.458, 12.428), and hypoproteinemia (total protein ≤ 68.4 g/L; OR = 7.131; 95% CI: 1.864, 27.271) were risk factors for false-negative T-SPOT.TB results in childhood TB. Younger age, overweight, and hypoproteinemia were found to be associated with false-negative T-SPOT.TB results in childhood TB. Health care professionals should consider these risk factors when evaluating suspected childhood TB with negative T-SPOT.TB results.

INTRODUCTION

Presently, childhood tuberculosis (TB) remains poorly understood and presents diagnostic and therapeutic challenges. Moreover, the incidence of multidrug-resistant (MDR) TB among children is increasing, making the situation more complicated.[1] Interferon-γ release assays (IGRAs), that is, QuantiFERON-TB Gold In-Tube (QFT; Qiagen, Valencia, CA) and T-SPOT.TB (Oxford Immunotec, Inc., Abingdon, UK) assays, measure immune reactivity to *Mycobacterium tuberculosis*–specific antigens ESAT-6 and CFP-10 to assess latent TB infection (LTBI).[2] Latent TB infection has been defined in most studies by a positive response to the tuberculin skin test (TST) or the IGRA test in exposed individuals without signs of active disease. In children, LTBI may develop to severe active TB and can serve as a reservoir for future transmission of TB. Thus, accurate diagnosis of LTBI may help reduce these risks. Interferon-γ release assays are useful as an indicator of TB infection and can be used to support the diagnosis of TB. According to previous study results, frequent use of IGRAs in routine clinical practice suggests that a negative test result could rapidly exclude TB from a differential diagnosis.[3–6] Therefore, IGRAs are still the preferred tools used by health care professionals to evaluate suspected TB before microbiological examinations. However, in children, TB is paucibacillary in nature and usually smear negative, and a negative IGRA result might inappropriately lower the clinical suspicion of TB and result in delayed treatment initiation.

Interferon-γ release assays quantify cell-mediated immune responses. Therefore, sensitivity may be limited in subjects who may be anergic as a result of significant immunosuppression, malnutrition, or disseminated/severe TB. Several factors, such as older age, overweight, treatment delay, human leukocyte antigen (HLA)-DRB1*0701 allele, lymphocytes, malnutrition, and forms of TB, have been found to be associated with negative IGRA results.[7–16] However, the factor associated with childhood TB remains unclear. Therefore, a great deal of effort needs to be made to clarify these factors. The aim of this retrospective study was to identify the risk factors associated with false-negative IGRA results in children with culture-confirmed TB.

METHODS

The retrospective study was approved by the Ethics Committee of Shandong Provincial Chest Hospital. A written informed consent was waived because of the retrospective design of the study and anonymous nature of the data collection.

Between May 2012 and January 2018, 156 culture-confirmed childhood TB patients who had received T-SPOT.TB tests were included in the study. Mycobacterial culture was performed using Löwenstein–Jensen medium, and the drug susceptibility assay was performed as previously reported.[17] Data, including demographic information and clinicopathological variables, were collected via questionnaires. The following patient information was gathered for further analysis: time between symptom onset and the T-SPOT.TB test, demographics (gender, age, weight, TB contact history, transfer times, and comorbidities), and laboratory data (acid-fast bacilli [AFB] smear, biochemical analyses, hematological tests, and flow cytometry (FCM) analysis). The *z*-score for weight for age was calculated using the WHO–National Center for Health Statistics reference with Epi Info version 7.2 (CDC, Atlanta, GA). The T-SPOT.TB tests were performed according to the manufacturer's guidelines, and the result of the testing was categorized as "positive" or "negative" using a cut-off point of six spots or less.[18]

Statistical analyses were performed using SPSS software (version 16.0; SPSS Inc., Chicago, IL). Categorical variables are described as counts (percentages) and were compared with the chi-squared test. Continuous variables are presented as mean ± SD and were compared using the Mann–Whitney U test. Univariate logistic regression analysis was used to evaluate risk factors associated with false-negative T-SPOT.TB test results, and significant variables ($P < 0.1$) were included in the multivariate logistic regression model. Before conducting multivariate logistic regression, the continuous variables were transformed into categorical variables based on the cut-off points determined with the receiver operating characteristic curve (ROC) analysis. The

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multivariate logistic regression analysis was then performed, including significant variables estimated in the univariate analysis or variables suggested in the literature. A $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics of enrolled patients. A total of 156 culture-confirmed TB children were recruited for the present study. In total, the positive rate of the T-SPOT.TB test was 85.9% in culture-confirmed childhood TB patients. The mean ± SD age of the patients was 11.2 ± 4.8 years, and males accounted for 60.3% of the participants. One hundred forty-two (91.0%) patients were tested for HIV serology, and all were negative. The *z*-score for weight for age was –0.15 ± 1.47 for all enrolled subjects.

The time between symptom onset and the T-SPOT.TB test was 55.1 ± 76.6 days. Twenty-three patients (14.7%) had a contact of TB history. Thirty-two patients (22.5%) were positive for AFB smears. Fever and lung cavity were found in 101 (64.7%) and 23 (14.7%) patients, respectively. Among them, 103 (66.0%) participants had pulmonary TB, 104 (66.7%) had extrapulmonary TB, and 51 (32.7%) had both. In addition, 64 patients (41.0%) had pleural TB, 24 (15.4%) had tuberculous lymphadenitis, 10 (6.4%) had tuberculous meningitis, four (2.6%) had disseminated TB, and three (2.9%) had MDR-TB. Of these, 147 patients (94.2%) were new cases and the remaining nine patients (5.8%) were retreatment cases. With regard to the outcome, four patients relapsed after anti-TB therapy. Table 1 shows the characteristics of the enrolled patients.

The results of the biochemical analyses, FCM analysis, and hematological tests are also summarized in Table 1. Statistical analysis showed that there were significant differences in total protein ($P < 0.001$), red blood cell count (RBC, $P < 0.01$), hemoglobin ($P < 0.05$), hematocrit ($P < 0.01$), lymphocyte ($P < 0.001$), and eosinophil ($P < 0.01$) levels between the positive and false-negative T-SPOT.TB groups.

Risk factors for false-negative T-SPOT.TB results. Table 1 shows the univariate analysis of risk factors associated with false-negative T-SPOT.TB results in children with TB and the comparison between the positive and false-negative T-SPOT.TB result groups. The false-negative T-SPOT.TB results were associated with age, fever, pulmonary TB, tuberculous lymphadenitis, total protein, RBC, hemoglobin, hematocrits, lymphocytes, and eosinophils (all $P < 0.05$) (Table 1).

Furthermore, multivariate analysis revealed that younger age (age ≤ 9 years; OR = 4.782; 95% CI: 1.689, 13.539), weight for age (*z*-score > 0.37; OR = 4.256; 95% CI: 1.458, 12.428), and hypoproteinemia (total protein ≤ 68.4 g/L; OR = 7.131; 95% CI: 1.864, 27.271) were risk factors for false-negative T-SPOT.TB results in children with TB (Table 2).

DISCUSSION

The diagnosis of childhood TB remains difficult. A recent meta-analysis showed that the IGRAs have a moderate sensitivity (approximately 80%) and a high specificity in the diagnosis of childhood TB.[19] In this study, the positive rate of the T-SPOT.TB test was 85.9% in the culture-confirmed cases. The unsatisfactory accuracy of IGRAs gives rise to potential misinterpretation in the diagnosis of childhood TB. Thus, the investigation of risk factors responsible for the reduced sensitivity of IGRAs facilitates better interpretation of the IGRA results. Our analysis found that false-negative IGRA results were more likely to occur in children with younger age, overweight, and hypoproteinemia.

The T-SPOT.TB tests in this study had a positive rate of approximately 85%, which was higher than that reported in a previous meta-analysis.[19] This may contribute to regional disparities because most of the studies in the meta-analysis were conducted in low TB burden countries. In the univariate logistic regression analysis, several variables, such as fever, pulmonary TB, tuberculous lymphadenitis, RBC, hemoglobin, hematocrit, lymphocytes, and eosinophils, were found to be significantly related to the false-negative results. However, in the multivariate regression model, these variables did not remain significant.

Several factors associated with negative or indeterminate IGRA results, such as demographic characteristics, hemoglobin, lymphocyte counts, underlying diseases, and immunosuppressive treatment, have been evaluated and identified in adults before. For example, first, age was associated with the IGRA results.[20–22] In addition, body mass index < 16.0 kg/m^2), HIV infection, race, HLA allele, and smoking habit were also associated with negative IGRA results.[6,15,23,24] Second, Sharninghausen et al.[15] found that anemia (less hemoglobin) was an independent risk factor for an indeterminate IGRA result. Consistent results were obtained in the studies by Jung et al.,[25] Shu et al.,[26] and Kim et al.[27] Third, lymphopenia was also considered a risk factor for the indeterminate IGRA results.[25] Similar results were published by Kwon et al.,[12] Cho et al.,[28] Jeong et al.,[29] and Jung et al.[25] Fourth, Di et al.[7] found that tuberculous meningitis, a severe manifestation of extrapulmonary TB, was a risk factor for false-negative T-SPOT.TB results. Similarly, an association between the IGRA results and TB site was reported by Lee et al.[9] and Auld et al.[14] In addition, other underlying diseases, such as miliary TB, diabetes mellitus, helminth infection, systemic lupus erythematosus, and cancer, were also found to be associated with the negative or indeterminate IGRA results.[12,16,20,25,30–32] Fifth, immunosuppressive drugs, such as glucocorticoids and tumor necrosis factor-α inhibitors, could also influence the IGRA results.[25,33,34]

The risk factors for negative or indeterminate IGRA results have been widely evaluated. However, in childhood TB, information available on factors leading to false-negative T-SPOT.TB test results have been limited to date. In this study, younger age, overweight, and hypoproteinemia were included in the final multivariate logistic regression model, and the corresponding odds ratios (ORs) were assessed.

Banfield et al.[35] showed that younger age was associated with an indeterminate IGRA result, and similar results were reported by Critselis et al.,[36] Hang et al.,[23] and Jenum et al.[37] Our results are in agreement with their results. In addition, Kampmann et al.[38] found that IGRA responses were lower in children aged < 5 years than in children aged 5 to 15 years. Some authors have proposed that very young children produce, on average, less interferon-γ (IFN-γ) than older children.[39] This may explain the association.

Overweight is an unhealthy state of overnourishment and is associated with issues with immunity,[40,41] and we found that a high *z*-score for weight for age was a risk factor for a negative IGRA result. Overweight individuals may have dysfunctional T-cell responses, leading to negative results. Because few

TABLE 1
Univariate analysis of risk factors associated with false-negative T-SPOT.TB results in childhood TB

	N	Negative	Positive	*P*-value	Odds ratio (95% CI)
N	156	22 (14.1%)	134 (85.9%)	–	–
Gender (male)	94 (60.3%)	15 (68.2%)	79 (59.0%)	0.415	
Age (years)	11.2 ± 4.8	6.5 ± 6.2	12.0 ± 4.0	0.000	0.117 (0.043, 0.318)
History of a TB contact	23 (14.7%)	2 (9.1%)	21 (15.7%)	0.426	–
Time between symptom onset and T-SPOT.TB test (days)	55.1 ± 76.6	46.7 ± 49.8	56.5 ± 80.2	0.578	–
Acid-fast bacilli smear (+)	32/142 (22.5%)	2/14 (14.3%)	30/128 (22.4%)	0.443	–
Fever	101 (64.7%)	9 (40.9%)	92 (68.7%)	0.015	3.164 (1.255, 7.979)
Lung cavity	23 (14.7%)	1 (4.5%)	22 (16.4%)	0.177	–
Retreatment case	9 (5.8%)	2 (9.1%)	7 (5.2%)	0.477	–
Relapse	4 (2.6%)	0 (0%)	4 (100%)	0.999	–
Weight for age	–0.15 ± 1.47	0.39 ± 1.96	–0.24 ± 1.37	0.067	–
Comorbidity					
Pulmonary TB	103 (66.0%)	9 (40.9%)	94 (70.1%)	0.010	3.394 (1.343, 8.577)
Extrapulmonary TB	104 (66.7%)	18 (81.8%)	86 (64.2%)	0.113	–
Pulmonary and extrapulmonary TB	51 (32.7%)	5 (22.7%)	46 (34.3%)	0.287	–
Pleural TB	64 (41.0%)	5 (22.7%)	59 (44.0%)	0.067	–
Tuberculous lymphadenitis	24 (15.4%)	10 (45.5%)	14 (10.4%)	0.000	0.140 (0.051, 0.383)
Tuberculous meningitis	10 (6.4%)	2 (9.1%)	8 (6.0%)	0.583	–
Disseminated TB	4 (2.6%)	0 (0%)	4 (3.0%)	0.999	–
Multidrug-resistant tuberculosis	3/102 (2.9%)	0/11 (0%)	3/91 (3.3%)	0.999	–
Biochemical analysis					
Total protein (g/L)	68.3 ± 6.9	64.6 ± 5.9	69.0 ± 6.8	0.009	1.102 (1.025, 1.185)
Albumin (g/L)	40.6 ± 4.6	41.9 ± 4.9	40.4 ± 4.5	0.168	–
Flow cytometry analysis					
$CD19^+$ (%)	17.1 ± 7.7	16.2 ± 5.0	17.2 ± 7.9	0.732	–
$CD3^+$ (%)	67.1 ± 10.1	69.8 ± 6.9	66.9 ± 10.4	0.409	–
$CD3^+CD4^+$ (%)	35.4 ± 7.6	35.7 ± 10.5	35.4 ± 7.4	0.888	–
$CD3^+CD8^+$ (%)	27.4 ± 7.4	29.3 ± 7.7	27.2 ± 7.3	0.418	–
$CD3\text{-}CD16^+CD56^+$ (%)	11.3 ± 6.8	9.6 ± 5.5	11.4 ± 7.0	0.442	–
$CD4^+/CD8^+$	1.4 ± 0.5	1.4 ± 0.7	1.4 ± 0.5	0.792	–
Hematological tests					
White blood cell (109/L)	8.1 ± 3.5	9.1 ± 5.0	7.9 ± 3.2	0.171	–
Red blood cell (1,012/L)	4.5 ± 0.5	4.2 ± 0.5	4.5 ± 0.5	0.004	3.963 (1.551, 10.125)
Hemoglobin (g/L)	118.7 ± 16.7	111.0 ± 17.5	120.0 ± 16.3	0.020	1.035 (1.005, 1.065)
Hematocrit (%)	38.2 ± 28.9	33.3 ± 4.5	39.0 ± 31.0	0.003	1.192 (1.061, 1.340)
Mean corpuscular volume (fL)	80.8 ± 6.2	80.2 ± 5.2	80.9 ± 6.4	0.641	–
Mean corpuscular hemoglobin (pg)	26.7 ± 2.5	26.7 ± 2.3	26.6 ± 2.6	0.940	–
Mean corpuscular hemoglobin concentration (g/L)	329.7 ± 13.0	332.5 ± 13.5	329.3 ± 12.9	0.270	–
Platelet (109/L)	353 ± 111	361 ± 89	352 ± 115	0.715	–
Neutrophil (109/L)	4.9 ± 2.7	4.3 ± 2.8	5.0 ± 2.7	0.248	
Lymphocyte (109/L)	2.2 ± 1.6	3.5 ± 2.6	2.0 ± 1.3	0.000	0.660 (0.522, 0.834)
Monocyte (109/L)	0.8 ± 1.0	0.9 ± 0.9	0.8 ± 1.0	0.637	–
Eosinophil (109/L)	0.2 ± 0.3	0.4 ± 0.6	0.1 ± 0.1	0.003	0.010 (0.001, 0.213)
Basophil (109/L)	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.02	0.559	–
Mean platelet volume (fL)	9.4 ± 0.9	9.4 ± 0.9	9.4 ± 0.9	0.889	–
Plateletcrit (%)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.670	–
Coefficient of variation of red cell distribution width (%)	14.1 ± 2.0	13.8 ± 1.5	14.1 ± 2.1	0.497	–
Platelet distribution width (fL)	10.9 ± 2.0	10.3 ± 1.3	11.0 ± 2.1	0.148	–
Red cell distribution width (fL)	40.1 ± 5.3	39.4 ± 3.0	40.2 ± 5.6	0.530	–
Erythrocyte sedimentation rate (mm/hour)	32.2 ± 25.0	30.0 ± 29.4	32.6 ± 24.3	0.655	–

TB = tuberculosis.

patients (only six) in the false-negative group in our study had a *z*-score > 1 (defining overweight), a cut-off value (> 0.37) was established for the interpretation of the results using ROC analysis. Similar results were also reported. For example, Pan et al.[8] found that overweight was an independent risk factor for false-negative IGRA results in pulmonary TB patients. Reechaipichitkul et al.[42] found that overweight was significantly associated with the TST+/QFT- results. However, given the fact that only few patients were overweight (*z*-score > 1) in our study, the association between false-negative IGRA results and obesity (or overweight) should be further analyzed in a larger childhood TB population.

Interferon-γ release assay performance depends on effective cellular immune responses. However, malnutrition status is associated with the suppression of such responses.[43,44] Cell-mediated immune responses, specifically Th1 responses, are important for protection against TB infection.[45–47] The influence of malnutrition on cell-mediated immunity is relevant to TB and includes decreased IFN-γ production and an imbalance between the Th1 and Th2 responses. In a severely malnourished state, the absolute number and function of T lymphocytes are compromised,[48] which corresponds to a decreased production of IFN-γ production.[49,50] This phenomenon is potentially problematic

TABLE 2
Multivariate analysis of risk factors associated with false-negative T-SPOT.TB results in childhood tuberculosis

	P-value	Odds ratio (95% CI)
Age (≤ 9 years)	0.003	4.782 (1.689, 13.539)
Total protein (≤ 68.4 g/L)	0.004	7.131 (1.864, 27.271)
Weight for age (> 0.37)	0.008	4.256 (1.458, 12.428)

when tests rely on IFN-γ production. As demonstrated in our study, a malnourished status, such as hypoproteinemia, is considered a risk factor for a false-negative IGRA result. A vast majority of these negative results may be caused by anergic T-cell responses and reduced production of IFN-γ. In addition, as previously reported, hypoalbuminemia is also an independent risk factor for a false-negative IGRA outcome.[15,25,51] However, in our study, hypoalbuminemia was not associated with the outcome. There are two possibilities to explain this fact: 1) the serum levels of IgG (8.20 ± 4.46 versus 13.68 ± 4.87 g/L) and IgM (0.68 ± 0.59 versus 2.27 ± 1.17 g/L) in the negative group were higher than those in the positive group. This may be the first cause contributing to the lack of an association between hypoalbuminemia and the IGRA results. 2) Increased C-reactive protein levels were also reported to be associated with indeterminate results.[28]

The study has several limitations: 1) A qualitative method was used, and T-SPOT.TB test results were analyzed retrospectively. 2) The sample size was small, and there is a possibility of selection bias. 3) Although several risk factors have been investigated in children, the contribution of other factors, such as diabetes mellitus, rheumatoid arthritis, bacille Calmette-Guérin vaccination, HIV status, immunological mechanisms, and genetic variability, remains unclear and requires further investigations. In addition, the results should be interpreted with caution because a poor reproducibility was found in the T-SPOT.TB testing.[18]

CONCLUSION

In conclusion, younger age, overweight, and hypoproteinemia were found to be associated with false-negative T-SPOT.TB results in children with TB. Health care professionals should consider these risk factors when evaluating suspected TB in children with negative T-SPOT.TB results.

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