

Gamma Interferon Release Assays for Diagnosis of Tuberculosis Infection in Immune-Compromised Children in a Country in Which the Prevalence of Tuberculosis Is Low[▼]

Children have a higher risk than adults of developing severe active tuberculosis (TB), and this risk further increases in patients undergoing long-term immune-suppressive therapies, including treatment with tumor necrosis factor alpha (TNF- α) blockers (12). In this setting, detection of TB infection may be difficult due to high rates of falsely negative results of tuberculin skin testing (TST) (2). Blood tests detecting gamma interferon (IFN- γ) release by effector memory T cells stimulated with *Mycobacterium tuberculosis* antigens are currently available (8, 13). They rely on the use of two region-of-difference (RD-1)-encoded genes, namely, ESAT-6 and CFP-10. To date, RD-1 has been found to be restricted to the *M. tuberculosis* genome, whereas it is absent from the *M. bovis* BCG strains and most environmental mycobacteria. Overall, IFN- γ release assays (IGRAs) are more specific than TST, correlate better with TB exposure, and are more sensitive in detecting active TB, at least in settings in which the prevalence of the disease is low (11). They also perform better with immune-compromised adults, and encouraging results have been reported in studies of children (4, 5, 6, 14, 17). Three Food and Drug Administration-approved IGRAs are commercially available: the enzyme-linked immunospot (ELISPOT)-based T-Spot.TB (TS-TB) assay (Oxford Immunotech, Abingdon, United Kingdom) and two whole-blood enzyme-linked immunosorbent assay (ELISA)-based tests, the QuantiFERON TB Gold assay and the QFT in-tube (QFT-IT) assay (Cellestis, Carnegie, Australia). QFT-IT is the latest improvement in this technology and includes a third antigen, namely, TB7.7. The aim of this prospective study was to perform head-to-head comparisons of TS-TB and QFT-IT performance to TST performance for TB infection-screening purposes for immune-compromised children, potentially at high TB risk, in a setting in which the prevalence of the disease is low.

A total of 80 consecutive Italian human immunodeficiency virus-negative immune-compromised children were enrolled. Demographics and clinical characteristics of the study population are reported in Table 1. The diagnostic preliminaries included a physical examination, routine blood tests, a chest X-ray, TST, and IGRAs. A cutoff value of 5 mm was chosen to represent a positive TST result for all cases (1). Performance and data analysis of TS-TB and QFT-IT were realized according to the instructions of the manufacturers. Spot-forming cells were counted with an automated ELISPOT reader (AID Systems, Strassberg, Germany). IFN- γ concentrations (expressed in international units per milliliter) were measured with an automated ELISA reader. IGRAs were performed by highly specialized laboratory staff with more than 3 years of experience in the field. The local Ethics Committee approved the study, and the patients or their parents provided oral consent.

Test performance comparisons were realized with the Cochran test. The kappa (κ ; Cohen test) measurement method was used to determine test agreement. Determinations of correlations between tests and clinical parameters were performed using a chi-square test or Fisher's exact test. A P of <0.05 was considered significant.

TST and QFT-IT gave a positive result for one (1.2%) pa-

tient, while a significantly higher (9.4%) proportion of cases were positive by TS-TB ($P = 0.02$). Mean analytical TS-TB and QFT-IT results are shown in Table 2 and Table 3. The rate of TS-TB-positive results was higher for patients affected by rheumatic diseases compared with patients who had undergone a liver transplant (5/19 [26.3%] versus 2/54 [4%]; $P = 0.01$) and for patients treated with TNF- α blockers compared with those receiving other medications (4/15 [26.6%] versus 3/59 [5.1%]; $P = 0.026$). TS-TB and QFT-IT yielded a high number of indeterminate results (13.5% and 20%, respectively; $P = 0.3$). IGRA result agreement was found in 62.1% of cases ($\kappa = 0$; $P = 0.6$). Excluding indeterminate results, IGRA agreement with TST was poor ($\kappa = -0.028$ [$P = 0.89$] for TS-TB and $\kappa = -0.016$ [$P = 0.89$] for QFT-IT). IGRA performance was not associated with age, gender, blood leukocyte count, or treatment duration. No active TB cases were detected during the whole study period (median follow-up, 12 months).

The increasing number of iatrogenic immune-compromised children emphasizes the need of early TB screening, even in countries in which the prevalence of the disease is low (7, 10). Due to poor TST reliability, the performance of two commercial IGRAs was evaluated for TB infection-screening purposes for children receiving immune-suppressive therapies, including TNF- α blockers.

Unlike TST and QFT-IT, TS-TB yielded a surprisingly high proportion of positive results, approaching a worrying 10% of patients. A recent meta-analysis by Pai et al. has shown that IGRA specificity does not differ significantly from TST specificity for immune-competent patients with no confounding factors, such as BCG vaccination. Pooled IGRA sensitivity is also equivalent to TST sensitivity but, in certain studies, was higher TS-TB sensitivity (15). Although in our case series such a finding may represent the expression of higher TS-TB sensitivity, the possibility of lower specificity due to the lack of a gold standard for latent TB infection diagnosis cannot be excluded. To this end and to ensure that the IGRAs were properly performed, we tested 18 children with active pulmonary TB (3). Both commercial IGRAs scored a positive result in 77% (14/18) of the cases, whereas TST gave positive results in 72% of the cases. Only one indeterminate result was observed with QFT-IT. Discrepancies between commercial IGRA results are a recent issue of concern (9). Poor agreement between TS-TB and QFT-IT may stem from technical differences in test formats, with the ELISPOT-based assay accounting for an increased intrinsic sensitivity (11). Conversely, ELISA-based assays (mainly QuantiFERON TB Gold) more frequently score indeterminate results for immune-compromised patients, including children (11, 15). This, however, was not the case in our setting, as high rates of indeterminate results were scored by both IGRAs (P , not significant). Cross-reactivity with other mycobacteria may also explain TS-TB-positive results. Indeed, *M. kansasii* and *M. bovis*, the latter of which exhibits quite diffuse circulation in our geographic area, also express ESAT-6 and CFP-10 (11). In addition, although no TB risk factors were recorded for any patient, repeated hospitalization may have accounted for unrecognized TB exposure in some

TABLE 1. Demographics and clinical characteristics of the study population

Patient characteristic	Value
No. of patients	80
Gender (male/female)	36/44
Median age (range) in yrs	12.5 (2–24)
No. of patients with TB risk factors ^a	0
No. of cases of BCG vaccination	0
No. of patients with an indicator for immune-suppressive therapy such as:	
Juvenile rheumatoid arthritis	19
Nodose panarthritis	2
Liver transplantation	59
No. of cases currently receiving immune-suppressive treatment ^b	80
Median duration of therapy (range) in mos	80 (2–225)
Blood leukocyte count/mm ³ (mean ± SD)	2,130 ± 980

^a TB risk factors included recent exposure to active TB cases, a recent stay in a country with a high prevalence of TB, and previous active TB or X-ray sequelae.

^b Immune-suppressive treatment included the use of etanercept ($n = 17$), tacrolimus ($n = 42$), cyclosporine ($n = 22$), prednisone ($n = 13$), mycophenolate ($n = 9$), methotrexate ($n = 8$), thalidomide ($n = 1$), and azathioprine ($n = 1$). A total of 55 (69%) children were on single-drug regimens, 18 (22%) on two-drug regimens, and 7 (9%) on three-drug regimens.

instances. Finally, the finding that most of the TS-TB-positive results were found among patients on TNF- α blockers was surprising, since these drugs suppress T-cell responses (16), and unspecific immune reactivity of underlying rheumatic diseases may offer only a partial explanation.

From a clinical point of view, the main issue that arises from our study concerns evaluation of “isolated” IGRA (TS-TB)-positive results in accordance with the patient characteristics and the epidemiological setting. Are these true-positive or false-positive results? This is a key question, as it implies critical decisions in patient management. In the case of suspected TB infection, likely strategies may be (i) to stop immune-suppressive treatment for patients affected by severe immune disorders for whom few alternatives are available, or (ii) to

TABLE 2. Mean analytical data showing TS-TB performance^a

Assay	No. of SFC ^c representing the indicated IFN- γ test result		
	Positive ^b ($n = 7$)	Negative ^d ($n = 57$)	Indeterminate ^e ($n = 10$)
Control	7 ± 8 [4]	4 ± 4 [2]	17 ± 19 [8]
ESAT-6	34 ± 57 [7]	4 ± 5 [3]	16 ± 23 [8]
CFP-10	31 ± 50 [11]	3 ± 4 [1]	18 ± 22 [11]
PHA ^f	145 ± 100 [158]	92 ± 84 [52]	36 ± 39 [18]

^a Data are expressed as means ± standard deviations [median]. n , number of patients.

^b A result showing a total of 0 to 5 spots was designated a positive result for the control; a result showing ≥6 spots was designated a positive result for TB antigen-stimulated samples. A result showing a total of 6 to 10 spots was designated a positive result for the control; a result showing at least twice the number of spots seen with the control was designated a positive result for TB antigen-stimulated samples. A result showing a total of 11 to 20 spots was designated a positive result for the control; a result showing at least triple the number of spots seen with the control was designated a positive result for TB antigen-stimulated samples.

^c SFCs, spot-forming cells.

^d Results for which the positive criteria not met were designated negative results.

^e Results showing that the TB antigen-stimulated samples and positive control were not responsive were designated indeterminate results.

^f PHA, phytohemagglutinin.

TABLE 3. Mean analytical data showing QFT-IT performance

Assay antigen	IFN- γ test result ^a (IU/ml)		
	Positive ^b ($n = 1$)	Negative ^c ($n = 63$)	Indeterminate ^d ($n = 16$)
Control	1.3	0.5 ± 2.0 [0.1]	0.1 ± 0.05 [0.1]
ESAT-6/CFP-10/TB7.7	2.7	0.4 ± 1.4 [0.1]	0.1 ± 0.07 [0.1]
PHA ^e	57.6	36.4 ± 32.4 [25.5]	0.2 ± 0.2 [0.1]

^a Data are expressed as means ± standard deviations [median]. n , number of patients.

^b IFN- γ in TB antigen-stimulated sample ≥ 0.35 IU/ml with valid positive control represented a positive result.

^c IFN- γ in TB antigen-stimulated sample < 0.35 IU/ml with positive control ≥ 0.5 IU/ml represented a negative result.

^d IFN- γ in TB antigen-stimulated sample < 0.35 IU/ml with positive control < 0.5 IU/ml represented an indeterminate result.

^e PHA, phytohemagglutinin.

start patients on anti-TB therapy, which is, in turn, associated with important side effects. Our position has been to take a “wait and see” approach until further evidence of TB infection becomes available. As we have not yet detected any cases of active TB, it could be speculated that TS-TB yielded false-positive results.

In conclusion, due to high rates of discordant and indeterminate results, IGRAs are of little help for TB infection management for immune-compromised children in a country in which the prevalence of the disease is low.

REFERENCES

- American Academy of Pediatrics. 2006. Tuberculosis, p. 593–613. Red book: report of the Committee on Infectious Diseases, 27th ed. American Academy of Pediatrics, Elk Grove Village, IL.
- Barnes, P. F. 2001. Diagnosing latent tuberculosis infection: the 100-year upgrade. *Am. J. Respir. Crit. Care Med.* **163**:807–808.
- Centers for Disease Control and Prevention. 1990. Case definitions for public health surveillance. *MMWR Morb. Mortal. Wkly. Rep.* **39**:39–40.
- Connell, T. G., N. Curtis, S. C. Ranganathan, and J. P. Buttery. 2006. Performance of a whole blood interferon gamma assay for detecting latent infection with *Mycobacterium tuberculosis* in children. *Thorax* **61**:616–620.
- Detjen, A. K., T. Keil, S. Roll, B. Hauer, H. Mauch, U. Wahn, and K. Magdorf. 2007. Interferon-gamma release assays improve the diagnosis of tuberculosis and nontuberculous mycobacterial disease in children in a country with a low incidence of tuberculosis. *Clin. Infect. Dis.* **45**:322–328.
- Dogra, S., P. Narang, D. K. Mendiratta, P. Chaturvedi, A. L. Reingold, J. M. Colford, L. W. Riley, and M. Pai. 2007. Comparison of a whole blood interferon-gamma assay with tuberculin skin testing for the detection of tuberculosis infection in hospitalized children in rural India. *J. Infect.* **54**:267–276.
- Joint Tuberculosis Committee of the British Thoracic Society. 2005. BTS recommendations for assessing risk and for managing *Mycobacterium tuberculosis* infection and disease in patients due to start anti-TNF-alpha treatment. *Thorax* **60**:800–805.
- Lalvani, A., A. A. Pathan, H. McShane, R. J. Wilkinson, M. Latif, C. P. Conlon, G. Pasvol, and A. V. Hill. 2001. Rapid detection of *Mycobacterium tuberculosis* infection by enumeration of antigen specific T cells. *Am. J. Respir. Crit. Care Med.* **163**:824–828.
- Mandalakas, A. M., A. C. Hesseling, N. N. Chegou, H. L. Kirchner, X. Zhu, B. J. Marais, G. F. Black, N. Beyers, and G. Walzl. 2008. High level of discordant IGRA results in HIV-infected adults and children. *Int. J. Tuberc. Lung Dis.* **12**:417–423.
- Marais, B. J., and M. Pai. 2007. New approaches and emerging technologies in the diagnosis of childhood tuberculosis. *Pediatr. Respir. Rev.* **8**:124–133.
- Menzies, D., M. Pai, and G. Comstock. 2007. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. *Ann. Intern. Med.* **146**:340–354.
- Moiton, M. P., C. Richez, C. Dumoulin, N. Mehzen, J. Dehais, and T. Schaefferbeke. 2006. Role of anti-tumour necrosis factor-alpha therapeutic agents in the emergence of infections. *Clin. Microbiol. Infect.* **12**:1151–1153.
- Mori, T., M. Sakatani, F. Yamagishi, T. Takashima, Y. Kawabe, K. Nagao, E. Shigetou, N. Harada, S. Mitarai, M. Okada, K. Suzuki, Y. Inoue, K. Tsuyuguchi, Y. Sasaki, G. H. Mazurek, and I. Tsuyuguchi. 2004. Specific detection of tuberculosis infection: an interferon-gamma-based assay using new antigens. *Am. J. Respir. Crit. Care Med.* **170**:59–64.

14. **Okada, K., T. E. Mao, T. Mori, T. Miura, T. Sugiyama, T. Yoshiyama, S. Mitarai, I. Onozaki, N. Harada, S. Saint, K. S. Kong, and Y. M. Chhour.** 2007. Performance of an interferon-gamma release assay for diagnosing latent tuberculosis infection in children. *Epidemiol. Infect.* **8**:1–9.
15. **Pai, M., A. Zwerling, and D. Menzies.** 2008. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann. Intern. Med.* **149**:177–184.
16. **Saliu, O. Y., C. Sofer, D. S. Stein, S. K. Schwander, and R. S. Wallis.** 2006. Tumor-necrosis-factor blockers: differential effects on mycobacterial immunity. *J. Infect. Dis.* **194**:486–492.
17. **Tsiouris, S. J., J. Austin, P. Toro, D. Coetzee, K. Weyer, Z. Stein, and W. M. El-Sadr.** 2006. Results of tuberculosis-specific IFN- γ assay in children at high risk of tuberculosis infection. *Int. J. Tuberc. Lung Dis.* **10**:939–941.

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^v Published ahead of print on 6 May 2009.