Tuberculin skin test and QuantiFERON in children

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SUMMARY

Until some time ago, the tuberculin skin test was the only available screening test for the diagnosis of tubercular infection. Now the new interferon- release assay QuantiFERON-TB Gold shows promise of greater accuracy in the detection of *Mycobacterium tuberculosis*-infected subjects. The aim of our study was to evaluate the use of QuantiFERON-TB Gold in children and to verify its agreement with the tuberculin skin test. A total of 27 children had a positive tuberculin skin test, 76 subjects were negative and the remaining 2 had a dubious Mantoux test. A positive QuantiFERON-TB Gold result was obtained in 21 children while in 84 it was negative. No statistically significant difference was detected between the two assays, which showed a concordance of 90.57%. Our results demonstrated a good concordance between the tuberculin skin test and the interferon- release assay, though the QuantiFERON-TB may have several advantages over the Mantoux test.

KEY WORDS: Tuberculosis, Quantiferon, Children, Mantoux.

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INTRODUCTION

Latent tubercular infection (LTBI) in children, as in adults, lacks a diagnostic gold standard. Until some time ago the only available test for the diagnosis of LTBI was the tuberculin skin test (TST) but it has drawbacks such as poor sensitivity and specificity (Pai *et al.,* 2008). QuantiFERON-TB Gold In-Tube (QFT-IT) has been approved for clinical use by the Food and Drug Administration and its major benefit is a high specificity even in BCG-vaccinated subjects (Pai *et al.,* 2008). The performance of QFT-IT in children has not been extensively explored but preliminary data suggest that it performs better than TST (Pai *et al.,* 2008). Few studies present paediatric data on an age-related performance assessment of QFT-IT, so reservations remain regarding its performance in very young children. This study evaluated the performance of QFT-IT for the diagnosis of tubercular infection in children of different age groups and we examined its agreement with TST assay.

MATERIALS AND METHODS

Between January 2009 and September 2011, 105 Italian children were investigated. The test was performed because a household or occasional contact with a patient with active pulmonary tuberculosis (TB) was reported. The parents of all the children provided their consent prior to screening procedures. Chest-x-rays and Mantoux test were carried out at the Sassari Paediatric Clinic of Infectious Diseases. All the children underwent a TST with 5 tuberculin units of purified protein derivative (Biocine test PPD, Chiron, Siena, Italy), according to the intradermal

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Mantoux method and a reaction with a diameter >5mm was considered positive. QFT-IT (Cellestis Limited, Carnegie, Victoria, Australia) and microbiologic examinations were carried out in the Mycobacteriology Laboratory, Department of Biomedical Science, University of Sassari.
QFT-IT test was executed as indicated by the manufacturer and an Interferon-gamma (IFN-γ) cut-off value for a positive test was ≥0.35 IU/ml. Gastric aspirates were collected from children who were chest-x-ray, TST and QFT-IT positive and microbiological tests were carried out according to standard protocols (Sechi *et al.,* 1998). Statistical analysis was performed with McNemar test and t-student test (Graph-pad Scientific calculator, $p<0.05$ was considered significant). Agreement between QFT-IT and TST was quantified by kappa (*k*) (*k* values >0.6 indicating a good concordance).

RESULTS

This study analyzed 105 Italian children: 63 females and 43 males with an age range from 3 months to 15 years; 33% (35/105) of the children had a close household contact with a TB patient and 67% (70/105) had an occasional TB contact. Two subjects (1.9%) were BCG-vaccinated, none of the children had an HIV infection or concomitant disease. 27 children (26%) had a positive TST, 76 subjects (72%) were negative (Table 1) and the remaining 2 (2%) had a dubious Mantoux test. 21 children (20%) had a positive QFT-IT and 84 (80%) were negative (Table 2). None of the children had an indeterminate QFT-IT. In children with positive QFT-IT, the concentration of IFN-γ ranged between 0.56-24 IU/ml. The child with an IFN-γ value of 0.56 IU/ml had a negative Mantoux test: QFT-IT was repeated after two months and its value was equal to 3.11 IU/ml, confirming the first positive result. The subject with the highest value of IFN-γ (24 IU/ml) had a dubious TST.
In 7 QFT-IT positive children younger than 5 years the mean IFN-γ concentration was 10.4 IU/ml, while in the remaining 14 (5 to 15 years of age) it was 10.55 IU/ml.
Chest-x-ray was performed only in 24% of the children (25/105) and it was positive in 36% (9/25) and negative in 64% (16/25) (Table 3). All the chil-

TABLE 1 - *Results of TST and QFT-IT.*

	TST Positive	*TST Negative*
QFT-IT Positive	19	2
QFT-IT Negative	8	75

One child with negative QFT-IT had dubious TST. No indeterminate QFT-IT results were observed.

TABLE 2 - Characteristic of the children assayed (N=105)

Characteristic	*Number and percentage*
Females	62
Males	43
Age (range)	3 months-15 years
Race	Italian
BCG status	2 (1.9%)
Household TB contact	35 (33%)
Occasional TB contact	70 (67%)
Active TB	3 (2.8%)
Therapy in LTBI	36 (34%)
HIV+	0
HIV-	105 (100%)
Concomitant pathology	None

TABLE 3 - *Results of TST, QFT-IT, Chest-x-ray.*

	TST	*QFT-IT*	*Chest-x-Ray*
Positive	27	21	9
Negative	76	84	16
Indeterminate	0	0	0
Dubious	2	0	0
No executed	0	0	80
Total	105	105	105

dren with positive chest-x-ray had positive TST and QFT-IT. Microbiological investigations were performed in all children positive at chest-x-rays, TST and QFT-IT. All direct microscopic tests were negative. Three children (2.8%) had a positive culture and *Mycobacterium tuberculosis* (*Mtb*) was identified by Nested-IS6110 PCR. Drug susceptibility assay was performed for 3 children with active TB, 2 of them received isoniazid, rifampicin, ethambutol and pyrazinamide for 12 months, while a *Mtb* strain was multi-drug-resistant (MDR). LTBI treatment was recommended in 34% of the children (36/105) (Table I). 28 received isoniazid for three months and 2 for nine; 3 received isoniazid, rifampicin, ethambutol and pyrazinamide for 12 months; 4 children were treated with rifampicin, ethambutol and pyrazinamide and 4 subjects received rifampicin (2 children for 3 months, 1 for 4 and 1 for 10 months). No statistically significant difference between the TST and QFT-IT was detected (two tailed P values=0.1138). No statistically significant difference in the mean positive IFN-γ concentration between children younger and older than 5 years was observed (p=0.96).

QFT-IT-positive/TST-positive concordance was found in 19/105 children (18%) (Table 1). QFT-IT-negative/TST-negative concordance was recovered in 75/105 subjects (72%) (Table 1). 11/105 children (10%) had discordant QFT-IT/TST results: 2 of them were only QFT-IT positive (1 had a dubious TST and 1 had a negative TST), 8 had only positive Mantoux test (2 BCG-vaccinated and 6 with weakly positive Mantoux) and one with negative QFT-IT had a dubious TST. These two assays showed an overall good concordance of 90.57% (k=0.753).

DISCUSSION

Agreement between TST and interferon-gamma-release-assays (IGRAs) in the diagnosis of LTBI in children appears to be between 55% and 95% (Lighter *et al.,* 2009). In our study a good concordance of 90.57% between QFT-IT and Mantoux test was observed, consequently both tests can be used to identify *Mtb*-infected children. The 2009 American Academy of Paediatrics Red Book recommendations on TB state that IGRAs cannot be recommended for routine use in children younger than 5 years or for immunocompromised children of any age, due to a lack of published data on their utility in these groups (American academy, 2009).

In our work we did not test immunocompromised children, but we analyzed 50 subjects younger than 5 years. We observed that in 48 of them (aged from 3 months to 4 years) both Mantoux test and QFT-IT assay gave the same results. In 2 cases we detected a discordant outcome: a TST-doubt/QFT-IT-negative (with negative chest-x-ray) and a TST-positive/QFT-IT-negative in a BCG-vaccinated child. Considering that no statistical difference was observed in the positive QFT-IT response among children younger and older than 5 years, and a good correlation between TST and QFT-IT also was demonstrated in other studies we think that IGRA assay can be used routinely in children when tuberculosis infection is suspected (Pavic *et al.,* 2011).

Few studies have explored the relationship between IFN-γ production and age. Some authors reported that indeterminate responses in the QFT-IT were more common in younger children (Austin *et al*., 2009; Connell *et al.,* 2006), others did not detect any indeterminate QFT-IT results (Mandalakas *et al*., 2008; Markova *et al.,* 2011). Moreover, Markova *et al.* found that there was a significant increase in IFN-γ responses to TB-specific antigens with decreasing age in children with active tuberculosis (Markova *et al.,* 2011). Our study found that none of the 105 children had an indeterminate QFT-IT result and that no statistically significant difference in the mean positive IFN-γ concentration between children younger and older 5 years was observed. Consequently our data suggest that IFN-γ production in QFT-IT assay is not compromised in very young children.

QFT-IT is useful in vaccinated individuals and thanks to the use of mitogen as a positive control the state of immunosuppression in young children can be detected avoiding false negative TST results.

Among discordant results, TST-positive and IGRA-negative combination prevailed (Menzies *et al.,* 2007). This is principally due to a previous exposure to non-tuberculous mycobacteria and a vaccination with BCG (Paim M. *et al*., 2005). Nevertheless the combination TST-positive/IGRA-negative might also indicate that the TST is more likely to detect resolved or old LTBI while the

IGRA mainly detects current or recent infections (De Vries *et al.,* 2006). In our study, 8 children only TST-positive were in two cases BCG-vaccinated and in 6 cases had a weakly positive Mantoux test. In the subjects where a chest-x-ray was executed it was negative and the repeat of QFT-IT in some children, 4 and 8 months after the first examination, confirmed the negative IGRA result. This outcome suggests that at least some of the TST results were false-positive, but it is also possible that some children had an old tubercular infection despite their young age or we might even suppose that the weakly positive Mantoux test was due to to the subjective reading of the TST. We observed that in a QFT-IT-positive/TST-negative child the repetition of IGRA 2 months later confirmed the initial QFT-IT result, with an IFN-γ concentration that increased from 0.56 to 3.11 IU/ml, indicating that QFT-IT was able to detect the tubercular infection as soon as possible. QFT-IT can be carried out several times without affecting the *Mtb* antigen specific host-immune response, so when a dubious Mantoux test is detected, the repetition of QFT-IT could be of great help in identifying LTBI. Since a good agreement between the two assays was found, both tests may be used in paediatrics, even together, especially to solve dubious TST results. Due to the boosting effect of TST any discrepancy between the two tests must be clarified by repeating only the QFT-IT, and IGRA must be the choice in BCG-vaccinated children.

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REFERENCES

American Academy of Pediatrics. Red Book: 2009 Report of the Committee on Infectious Diseases. <http://aapredbook.

Connell T.G., Curtis N., Ranganathan S.C., Buttery J.P. (2006). Performance of a whole blood interferon gamma assay for detecting latent infection with *Mycobacterium tuberculosis* in children. *Thorax.* **61**, 616-620.

De Vries G., Sebek M.M., Lambregts-van Weezenbeek C.S. (2006). Health-care workers with tuberculosis infected during work. *Eur. Respir. J.* **28**, 1216-1221.

Haustein T., Ridout D.A., Hartley J.C., et al. (2009). The likelihood of an indeterminate test result from a whole-blood interferon-gamma release assay for the diagnosis of Mycobacterium tuberculosis infection in children correlates with age and immune status. *Pediatr. Infect. Dis. J.* **28**, 669-673.

Lighter J., Rigaud M., Eduardo R., et al. (2009). Latent tuberculosis diagnosis in children by using the QuantiFERON-TB Gold In-Tube test. *Pediatrics.* **123**, 30-37.

Mandalakas A.M., Hesseling A.C., Chegou N.N., et al. (2008). High level of discordant IGRA results in HIV-infected adults and children. *Int. J. Tuberc. Lung. Dis.* **12**, 417-423.

Markova R., Drenska R., Minchev P., et al. (2001). Association of age with the level of response in the QuantiFERON TB Gold In-Tube assay for children with active tuberculosis. *New Microbiol.* **34**, 81-85.

Menzies D., Pai M., Comstock G. (2007). Meta-analysis: new tests for diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. *Ann. Intern. Med.* **146**, 340-352.

Pai M. (2005). Alternatives to the tuberculin skin test: interferon-gamma assays in the diagnosis of Mycobacterium tuberculosis infection. *Indian. J. Med. Microbiol.* **23**, 151-158.

Pai M., Zwerling A., Menzies D. (2008). Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann. Intern. Med.* **149**, 177-184.

Pavic I., Zrinski Topic R., et al. (2011). Interferon-gamma release assay for the diagnosis of latent tuberculosis in children younger than 5 years of age. *Pediatr. Infect. Dis. J.* **30**, 866-870.

Sechi L.A., Zanetti S., Dupre I., et al. (1998). Genotypic changes in DNA fingerprinting patterns of Mycobacterium tuberculosis strains from HIV-positive persons in Sardinia. *AIDS.* **12**, 2084-2086.