

Association of age with the level of response in the QuantiFERON-TB Gold In-Tube assay for children with active tuberculosis

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SUMMARY

QuantiFERON-TB data from 50 children with tuberculosis were analysed to evaluate age related effects. Significantly higher IFN- γ responses to TB-specific antigens were associated with younger age, but no difference was found with Mitogen responses. Extrapolating IGRA responses to a Mitogen does not reflect those induced by an antigen-specific stimulus. QFT-IT responses to TB-specific antigens are not compromised with young age.

KEY WORDS: Children, Tuberculosis, QuantiFERON, IGRA

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INTRODUCTION

Interferon-gamma release assays (IGRAs) for the diagnosis of *Mycobacterium tuberculosis* infection are becoming commonly used worldwide. While there is a large body of evidence on their performance in many situations, data on their performance in young children are limited (Bocchino *et al.*, 2009).

This has led to a number of national guidelines on the use of IGRAs suggesting that the tests should not be used in place of the TST in children under 5 years of age (Anonymous [Guideline for the use of Quantiferon TB-2G] *Kekkaku* 2006, Mazurek *et al.*, 2005, NICE Guidelines, 2006). Moreover, a re-

cent publication by Hausteine and colleagues reported that indeterminate responses in the QuantiFERON[®]-TB Gold In-Tube test (QFT-3G) were more common in younger children, although in the same study, and despite the indeterminate responses, QFT was more sensitive than the TST in detecting children with active TB (Hausteine *et al.*, 2009).

We have routinely been using QFT-IT to aid the diagnosis of active tuberculosis in Bulgarian children for 4 years which has allowed us to examine the association between age and the magnitude of QFT-IT responses. We have analysed not only responses to the Mitogen control, but more importantly, to the TB-specific antigens, to establish the magnitude of age related effects.

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METHODS

Study population

Children hospitalised at the University Clinic for Lung Diseases in Children, Hospital "St. Sofia", Sofia, Bulgaria, were enrolled in the study dur-

ing the period December 2007 to May 2009. Clinical characteristics are presented in Table 1. The inclusion criteria were clinical and/or radiological features compatible with active tuberculosis infection and age 16 years or less. There were no exclusion criteria. QFT-IT (and TST for most) was performed for all children, prior to initializing anti-tuberculosis therapy and after obtaining informed consent from their parents. In Bulgaria children are immunized at birth with BCG produced in Bulgaria (BulBio-NCIPD Ltd). All children in Bulgaria are tested by TST (Mantoux) at the age of 7 months, 7, 11 and 16 years, and in cases where the TST is <5 mm, BCG re-vaccination is performed.

TABLE 1 - Clinical characteristics of children enrolled in the study (n=68) all BCG vaccinated at childbirth.

	N.	MTB culture-positive	MTB culture-negative
	68	17	51
Age (years):			
0 - 3	15	3	12
3 - 7	13	3	10
8 - 16	40	11	29
Sex:			
Male	29	9	20
Female	39	8	31
TST			
Yes	64	17	47
No	4	0	4
Clinical presentation (n=68)			
- Lymphadenopathia	24	4	20
- Unclear subfebrile temperature	9	0	9
- Bronchitis	3	0	3
- Pleuritis	3	0	3
- Pneumonia	24	11	13
- Meningitis	2	2	0
- Uveitis	3	0	3
BCG			
- Scar present	41	10	31
- History but no scar	27	7	20

Tuberculin skin test

The TST was performed using 5 TU of PPD Tuberculin (BulBio-NCIPD Ltd.) injected into the volar aspect of the forearm and transverse induration measured 48 to 72 hours later. Following Bulgarian guidelines for use of the test in children, the TST was interpreted as negative if induration was <5 mm and positive if ≥ 15 mm. Induration diameters between 5 and 14 mm are considered normal in previously BCG vaccinated individuals and interpreted as negative. Results are presented using this interpretation, but data are also presented to allow the reader to interpret the results if different TST cut-offs were applied.

QuantiFERON®-TB gold In-Tube

The QuantiFERON®-TB Gold In-Tube assay (QFT-IT) was performed as per the manufacturer's instructions (Cellestis Limited, Carnegie, Australia). Samples with IFN- γ ≥ 0.35 IU/mL following stimulation with tuberculosis-specific antigens (corrected for the Nil control) were considered positive. The QFT-IT test result was considered indeterminate if negative to the TB-antigen and production of IFN- γ after stimulation with Mitogen - Phytohemagglutinin (PHA) (minus the Nil control) was <0.5 IU/mL. The maximum IFN- γ response recorded for any sample was 10U/mL, due to that being the limit of accuracy of the QFT-IT ELISA (QFT Package Insert: www.cellestis.com).

Statistical analysis

Data were analysed using Spearman rank order correlation test (GraphPad Prism software, Version 4.0; San Diego, CA, USA).

RESULTS

A total of 68 therapy-free children were enrolled in the study. Active tuberculosis was confirmed by the isolation of *M. tuberculosis* from sputum or other bodily samples for 17 (25%) children, with QFT-IT being positive for 13 (76.5%) and TST for 10 (58.8%). Using a 5 mm or 10 mm TST cut-off, 15/17 and 14/17 were positive, respectively. Of the remaining 51 children, a diagnosis of *M. tuberculosis* infection, based on clinical grounds, was made for a further 33 (65%) - 20 of

these were QFT-IT positive (14 also TST positive and for 4 TST was not performed) and 13 were TST positive only. Clinical diagnosis was made on the basis of a positive QFT-IT and/or TST result along with clinical and radiological features. All 50 children with confirmed or clinically diagnosed active tuberculosis were treated with standard drug regimens and all improved. Clinicians excluded *M. tuberculosis* infection in the 18 children who were negative both by TST and QFT-IT and all were diagnosed with other infections/conditions. These children were treated with medications appropriate to their diagnosis and all 18 improved clinically. Results from these children were excluded from analysis of associations with age and QFT-IT responses.

For the 50 children with a final diagnosis of active TB, their mean age was 9.7 years (median 9 years, range 7 months to 16 years), 13 (26%) were aged less than 5 years, and 8 (16%) aged less than 2 years. There was no evidence of gender bias (27 were female) and all children had a history of

BCG vaccination and/or re-vaccination. No indeterminate results were found with the QFT-IT assay. TST results were not available for 4 of the children diagnosed with tuberculosis.

Figure 1 shows the results for the nil corrected tuberculosis antigen and mitogen responses as a function of age for the 50 children diagnosed with active tuberculosis. Linear regression identified that for the subset of 17 children with culture-confirmed tuberculosis there was a significantly lower IFN- γ response to the tuberculosis antigens with increasing age ($p=0.0019$). Similarly, there was evidence of lower IFN- γ responses to the tuberculosis antigens with increasing age when analysing QFT-IT data from all 50 children with a diagnosis of active tuberculosis ($p=0.0014$).

In contrast, there was no significant association with age and the level of response to mitogen for those with confirmed tuberculosis ($p=0.505$) or for all 50 with a diagnosis of active tuberculosis ($p=0.745$). However, any effect of age could have been masked by the fact that 34 of the 50 re-

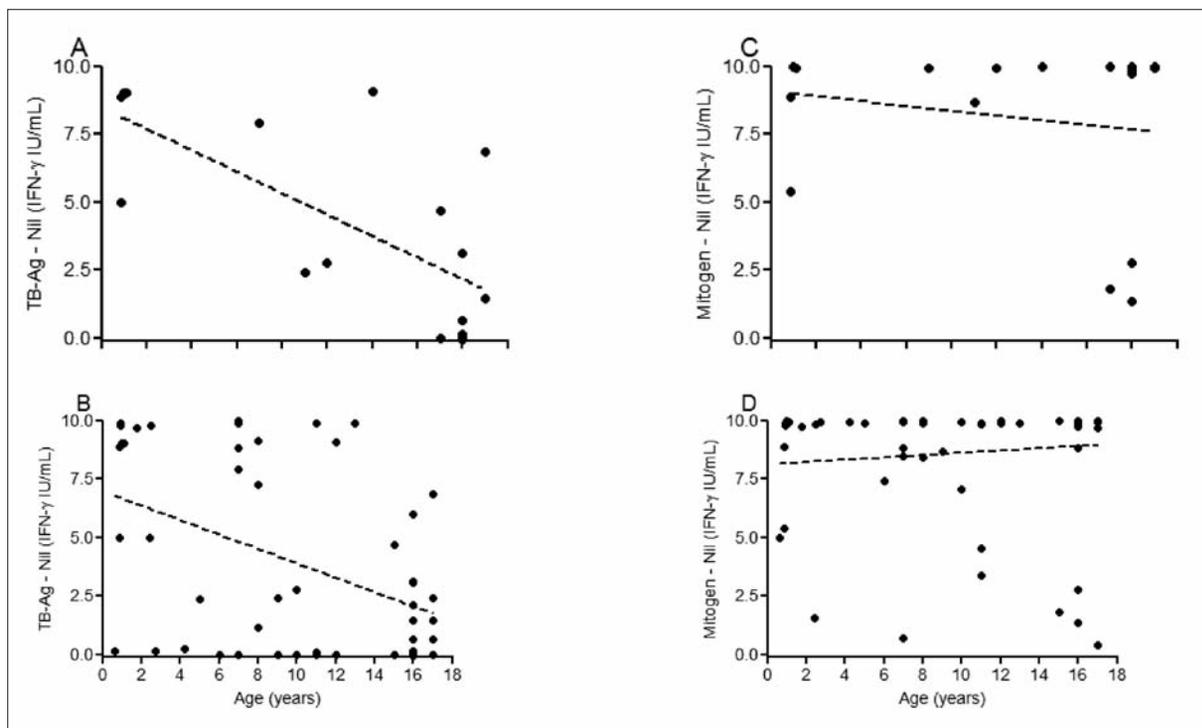


FIGURE 1 - Interferon- γ responses in the QFT-IT assay to the tuberculosis-specific antigens and Mitogen as a function of age. TB-antigen values for 17 children with culture-confirmed tuberculosis are shown in panel A, and their Mitogen responses in panel C. Data from all 50 children with a diagnosis of active tuberculosis are shown in panel B for their TB-antigen responses and panel D for Mitogen responses. The dotted lines are the linear regression for each data set.

sponses to mitogen were analysed as 10 IU/mL, but could have been higher.

DISCUSSION

There is some controversy in the literature about the use of IGRAs for detecting *M. tuberculosis* infection in young children. This is largely due to relatively limited published data available from those less than 5 years of age and understandable conservatism of doctors when dealing with young children who may develop severe forms of tuberculosis if incorrectly diagnosed. While some studies suggest commensurate high performance of QFT-IT in children and adults (Bianchi *et al.*, 2009, Detjen *et al.*, 2007, Kampmann 2009, Lighter *et al.*, 2009), others suggest the rate of indeterminate results increases with younger age (Haustein *et al.*, 2009, Bergamini *et al.*, 2009, Ferrara *et al.*, 2006), thereby making the test less useful in this age group.

Indeterminate QFT results generally arise from insufficient response to the mitogen positive control, which, apart from being a control for correct test performance, is a surrogate marker for the immune status of the individual. Thus, the mitogen control may provide a benefit for QFT-IT over the TST as it potentially reduces the possibility of false-negative results in children with under-developed immune responses. However, a number of publications suggest indeterminate QFT responses limit the test's utility in young children (Haustein *et al.*, 2009, Bergamini *et al.*, 2009, Ferrara *et al.*, 2006) which could result in reduced sensitivity of the test in young children. This suggestion may have merit if low response to mitogen is representative of low response to the TB-Antigens, but this has not been demonstrated to our knowledge.

Additionally, the mechanism of T-cell activation is entirely different for an antigen-stimulated response from that caused by direct binding of the phytohaemagglutinin mitogen to T-cell receptors, so assuming the two responses are aligned may be incorrect.

We chose to investigate the association between TB-specific antigen and mitogen responses in children with confirmed or clinically diagnosed tuberculosis disease. In contrast to prior studies, we found no evidence for QFT mitogen respons-

es varying with age of children, and none of the 68 children had an indeterminate result by QFT-IT. However, the response to the TB-specific antigens was a different profile to that to the mitogen. There was a significant increase in IFN- γ responses to the TB-specific antigens with decreasing age - the opposite of what has been postulated. This was apparent for children with confirmed tuberculosis and also for those with a clinical diagnosis (Figure 1).

Our findings question the validity of extrapolating responses to mitogen, as a factor of age, with antigen-specific responses in the QFT-IT test. We found evidence of increasing sensitivity of the QFT test with decreasing age, a significantly different response profile to that seen for mitogen. Our results are, in part, supported by those of Bianchi and colleagues who also found no evidence for lower QFT responses in younger children (Bianchi *et al.*, 2009).

Our study had limitations, including the fact that many of the children had a clinical diagnosis of active tuberculosis that could not be confirmed by isolation of *M. tuberculosis*, although this would be unlikely to have an effect on our analysis of effect of age. Although the regression analysis showed statistically significant associations with age, only 13 (26%) of the children studied were less than 5 years old. Our findings should be confirmed in larger studies of young children with confirmed tuberculosis.

In conclusion, care should be taken when extrapolating variations in IGRA responses to a mitogen, to those induced by an antigen-specific stimulus. Our data suggest that QFT-IT responses to the test's TB-specific antigens are not compromised in children less than 5 years of age and that QFT may have enhanced sensitivity in young children who, as a result of their young age, have only recently acquired their infection. QFT-IT was more sensitive than the TST in our BCG-vaccinated cohort of children, but both tests detected infected children that were missed by the other test. Given this, and the serious nature of active tuberculosis in children under 5 years of age, we would suggest that the optimal approach in this age group would be to use both TST and QFT-IT. With our current level of knowledge, the use of either TST or IGRA alone appears suboptimal and thus improper as a diagnostic aid for active tuberculosis in young children.

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