EFFECT OF BCG VACCINATION AND NON-TUBERCULOUS MYCOBACTERIUM INFECTION ON INTERFERON GAMMA SPECIFIC ASSAY AND A TUBERCULIN SKIN TEST AMONG CHILDREN WITH A TUBERCULOSIS CONTACT IN SURABAYA, INDONESIA

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Abstract. The tuberculin skin test (TST) as a diagnostic tool for tuberculosis (TB) infection is used in many countries, including Indonesia, but lacks specificity. Interferon-γ is a highly specific assay because it is not influenced by previous BCG vaccination or non-tuberculous mycobacteria (NTM) infections. We aimed to study the effect of BCG vaccination and NTM infection on the results of the interferon-γ specific assay and TST among children with a TB contact. We carried out a cross-sectional study of children at an outpatient clinic in Surabaya, Indonesia. We studied 37 children aged 1-15 years having a household contact with an acid-fast bacilli positive adult index case. BCG vaccination was determined by the presence of a BCG scar. A PPD RT23 2 tuberculin test was used for the TST. ESAT-6, CFP-10, and TB 7.7(p4) antigens were used for the interferon-γ assay by ELISA. Gastric aspirates were cultured in Lowenstein-Jensen media. A comparison of the two diagnostic tools among children aged 1-5 years without a BCG scar, revealed high agreement, while children with a BCG scar it revealed disagreement. Among children aged >5 years with or without a BCG scar the comparisons revealed disagreement. Among children aged >5-10 years, a comparison of the two diagnostic tools among NTM positive and negative children, there was a disagreement in results. Among children aged 1-5 years, the TST was influenced by a BCG scar. Infection with NTM had no influence on the results of the TST among children aged >5-10 years, while in children aged 1-5 years and >10 years the results could not be determined in this study.

Keywords: tuberculin skin test, interferon-γ specific assay, BCG scar, non-tuberculous mycobacteria

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INTRODUCTION

The tuberculin skin test is a major tool to diagnose tuberculosis (TB) although it has drawbacks (Garcia-Sancho *et al*, 2006; Nahid *et al*, 2006). Purified protein deriva-

tive (PPD) is used for the TST but it shares antigens with BCG vaccine strains and some non-mycobacterial strains (NTM). A positive TST could be due to true infection with *Mycobacterium tuberculosis* (MTB), prior BCG vaccination, or NTM infection (Lee and Holzman, 2002; Shigadia and Novelli, 2003; Nahid *et al*, 2006). As a consequence, a lower specificity of the TST is found in populations with high BCG immunization coverage and NTM exposure (Pai *et al*, 2004; Brodie and Schluger, 2005).

As an alternative tool for detecting TB the interferon-γ assay using a specific antigen has an advantage: it is not influenced by BCG vaccination or NTM infection. This advantage has been reported previously (Pai *et al*, 2004). Every region has factors that influence the effect of the BCG vaccine and NTM infection on the TST (Menzies, 2000; Floyd *et al*, 2002; Wang *et al*, 2002). The effect of BCG vaccination and NTM infection on the TST in Indonesia is not known. The interferon-γ specific assay requires a more advanced laboratory infrastructure and a higher cost (Pai and Menzies, 2007). We studied the effect of BCG vaccination and NTM infection on the results of the TST.

MATERIALS AND METHODS

We conducted a cross-sectional study of children at the outpatient clinics of Dr Soetomo Hospital and Karang Tembok Hospital, Surabaya, Indonesia, between April and June 2009. Written informed consent was obtained from the parents of the study subjects after the study was explained to them. The study was approved by the local ethics committee at the hospital. The subjects were 37 children aged 1-15 years, living in the same house for at least 8 weeks with an adult index case of TB seen at the Pulmonology Outpatient Clinics of Dr Soetomo Hospital and Karang Tembok Hospital who were positive for acid-fast bacilli (AFB) sputum examinations. The sample size was calculated based on the number of adult TB patients during 2007 at Soetomo Hospital (210 patients); each patient came in contact with 4.01 people, 47.5% were aged 0-14 years with a proportion of TB infection among the children of 0.72 (Dhingra, 2004), and $\alpha = 0.05$. Data regarding the acid-fast bacilli (AFB) sputum results among index cases was obtained from their medical records and graded according to the grading system of the International Union Against Tuberculosis and Lung Diseases, as +, ++, or +++. Subjects were excluded from the study if they had a history of TB and had received antituberculosis drugs, had performed TST in the previous 2 weeks, had received corticosteroid therapy for ≥2 weeks during the previous 2 weeks, or had an incomplete examination.

Clinical symptoms were obtained by interview. A physical examination was carried out to include evidence of malnutrition, lymph node enlargement or bone or joint lesions. Evidence of BCG vaccination was determined by the presence of a BCG scar. Nutritional status was determined using a National Center for Health Statistics (NCHS) growth chart. The subject was categorized as well-nourish if the subjects weight was >90% of the ideal body weight, moderately malnourished if the subjects weight was 90-70% of the IBW and severely malnourished if the subjects weight was <70% of the IBW. Each subject in this study also had a complete blood count, interferon-γ assay, TST and a chest X-ray performed.

The interferon-γ assay was performed using ESAT-6, CFP-10, and TB 7.7(p4) antigens by an ELISA (Quantiferon Gold-In tube™, Cellestis Chadstone, Victoria,

Australia) prior to the TST. Venous blood was collected in 3 tubes (1 ml each): a TB antigen tube, a mitogen positive control tube and a negative control tube. They were then incubated at 37ºC for 24 hours. After incubation, the tubes were centrifuged, plasma was harvested and stored at <-70ºC until the ELISA was performed. A result was considered positive if the interferon-γ level with the TB antigen minus the nil value was ≥0.35 IU/ml and >25% of the nil value and negative if the TB antigen minus the nil value was <0.35 IU/ml.

The tuberculin skin test was performed using the PPD RT23 2 tuberculin unit (Biofarma, Neuilly-sur-Seine, Franch), using the Mantoux technique. Induration was read at 48-72 hours, and a positive test was a diameter ≥10 mm.

Gastric aspirate (5-10 ml) was decontaminated using N-acetyl-L-cysteine sodium hydroxide (NALC-NaOH) solution (BBL™ MycoPerp™, Franklin Lakes, NJ) and cultured in Lowenstein-Jensen media.

Tuberculosis was categorized according to Indonesian Respirology Working Group criteria: TB class 1 was determined if there was an exposure to an index case and the TST was negative. TB class 2 was determined if there was exposure to an index case and the TST was positive. TB class 3 was determined if there was exposure to an index case, the TST was either positive or negative and clinical manifestations or radiology abnormalities were present.

Agreement between the interferon-γ specific assay and the TST results was analyzed using the proportion of agreement, the McNemar, and Kappa statistic, $\alpha = 0.05$. Agreement was obtained if the McNemar was not significant and the Kappa statistic was significant. The degree of agreement was high if the kappa value was > 0.75, moderate if it was 0.4-0.75, low if it was <0.4.

RESULTS

Forty-two subjects were enrolled in this study, 5 were excluded due to incomplete examinations. Of the 37 remaining children, 43.2, 43.2, and 13.5% were aged 1-5 years, >5-10 years, and >10 years, respectively. A BCG scar was present in 37.8% of children. The bacillary loads of the index cases were +, ++, and +++ in 27.0, 56.8, and 16.2% of subjects, respectively. The symptoms of the patients included chronic cough (32.4%) and fever (24.3%). The signs on examination included moderate malnutrition (45.9%), severe malnutrition (2.7%) and lymph node enlargement (24.3%). Chest X-ray abnormalities were seen in 18.9% of subjects. Thirteen subjects (35.1%) were TST positive, and an equal number of interferon-γ assay results were also positive. Following the Indonesian respirology working group criteria, 51% of subjects had TB class 1, 35% had TB class 3 and 14% had TB class 2.

A comparison of the TST and interferon-γ assay results revealed a discordance in 8 samples, consisting of 4 samples in each discordant group. The overall level of agreement between the two tests was moderate (McNemar p=1.000; Kappa 0.526, p=0.001). In the group without BCG scars, there was a high level of agreement between the TST and interferon-γ assay results. In the group with BCG scars, there was no significant agreement between the TST and interferon-γ assay results (Table 1).

In children aged 1-5 years without a BCG scar, there was a high level of agreement between the TST and interferon-γ

Table 1
Agreement between TST and interferon-γ specific assay results according to BCG scar status.

TST	Interferon-γ assay			McNemar	Kappa
	Negative	Positive	Total		
BCG scar negative					
TST negative	7	0	7		
TST positive	1	6	7		
Total	8	6	14	p=1.000	0.857 (p=0.001)
BCG scar positive					
TST negative	13	4	17		
TST positive	3	3	6		
Total	16	7	23	p=1.000	0.251 (p=0.226)

assay results. In children aged 1-5 years with a BCG scar, there was no agreement between the TST and interferon-γ assay results (Table 2). In children aged >5-10 years with a BCG scar, there was no agreement between the TST and interferon-γ assay results. In children aged >5-10 years without a BCG scar there was no agreement between TST and interferon-γ assay results (Table 2). In children aged >10 years with a BCG scar, there was no agreement between the TST and interferon-γ assay results. In children aged >10 years without a BCG scar, there was no agreement between TST and interferon-γ assay test results (Table 2).

Gastric aspirate culture results were positive in 6 samples (16.2%), all were NTM: 3 samples were from children aged 1-5 years and 3 samples were from children aged >5-10 years. No *Mycobacterium tuberculosis* was found (Table 3). The agreement of the two test results in children aged 1-5 years with a negative culture was 92.3%, the McNemar test was not significant (p=1.000) but the Kappa test was significant (0.847; p=0.002), which indicates a high levels of agreement between the interferon-γ test and the TST in this group (Table 3). Agreement between the two methods in the subjects aged 1-5 years with a positive culture could not be analyzed with statistics, because the TST results obtained were constant (Table 3). Agreement between the two methods among subjects aged >5-10 years with a negative culture was 69.2%; the McNemar was not significant (p=0.625) and the Kappa was not significant (0.161; p=0.522), which indicates no agreement between the two test methods in this group (Table 3). Agreement between the two test methods in subjects aged > 5-10 years with a positive culture was 100%; the McNemar was not significant (p=1.000) and the Kappa was not significant (1.000; p=0.083), which indicates no agreement between the two test methods in this group (Table 3). The sensitivity for detecting NTM could not be determined, because no gold standard for detecting NTM was performed in this study.

DISCUSSION

To study the effect of BCG vaccina-

Table 2
Agreement between TST and interferon-γ specific assay results according to BCG scar status by age group.

TST	Interferon-γ assay			McNemar	Kappa
	Negative	Positive	Total		
Age 1-5 years					
BCG scar negative					
TST negative	3	0	3		
TST positive	0	4	4		
Total	3	4	7	p=1.000	1.000 (p=0.008)
BCG scar positive					
TST negative	5	2	7		
TST positive	0	2	2		
Total	5	4	9	p=0.500	0.526 (p=0.073)
Age > 5-10 years					
BCG scar negative					
TST negative	3	0	3		
TST positive	1	1	2		
Total	4	1	5	p=1.000	0.545 (p=0.171)
BCG scar positive					
TST negative	7	1	8		
TST positive	2	1	3		
Total	9	2	11	p=1.000	0.233 (p=0.425)
Age >10 years					
BCG scar negative					
TST negative	1	0	1		
TST positive	0	1	1		
Total	1	1	2	p=1.000	1.000 (p=0.386)
BCG scar positive					
TST negative	1	1	2		
TST positive	1	0	1		
Total	2	1	3	p=1.000	-0.500 (p=0.157)

tion and NTM infection on the results of a TST, we compared the TST results with those of an interferon-γ specific assay. In this study determination of previous BCG immunization was based on the presence of a BCG scar, because of the difficulty of obtaining accurate records of immunization (Hill *et al*, 2006), although a BCG scar is not found in 6-17% of children who have received BCG vaccination (Guwatudde *et al*, 2003).

In this study the interferon-γ assay and TST results had moderate agreement. Four samples were negative with the interferon-γ assay and positive on the TST. This discrepancy is due to shared antigen among the PPD test MTB, the BCG vaccine and NTM (Brodie and Schluger, 2005; Pai and Menzies, 2007). On the TST, an induration of 10-15 mm was probably caused by the effect of the BCG vaccine. An induration ≥ 15 mm is suggestive of

Table 3
Agreement between TST and interferon-γ specific assay results according to NTM infection status by age group.

TST	Interferon-γ assay			McNemar	Kappa
	Negative	Positive	Total		
Age 1-5 years					
Culture negative:					
TST negative	6	1	7		
TST positive	0	6	6		
Total	6	7	13	p=1.000	0.847 (p=0.002)
Culture positive:[a]					
TST negative	2	1	3		
TST positive	0	0	0		
Total	2	1	3		
Age >5-10 years					
Culture negative:					
TST negative	8	1	9		
TST positive	3	1	4		
Total	11	2	13	p=0.625	0.161 (p=0.522)
Culture positive:					
TST negative	2	0	2		
TST positive	0	1	1		
Total	2	1	3	p=1.000	1.000 (p=0.083)
Age >10 years					
Culture negative:					
TST negative	0	0	0		
TST positive	0	0	0		
Total	0	0	0		
Culture positive:					
TST negative	0	0	0		
TST positive	0	0	0		
Total	0	0	0		

[a]Statisic could not be calculated (both assays were constant).

actual infection (American Academy of Pediatrics, 1974; Wang *et al*, 2002; Rahajoe *et al*, 2007), including NTM infection, although most NTM do not cause disease (Arend *et al*, 2002). A positive interferon-γ assay and negative TST result was obtained in 4 samples. In previous studies, this discrepancy was due to an interferon-γ assay response earlier than a TST response (Whalen *et al*, 2006) and reached a peak at 6 months after exposure (Hussain *et al*, 2007). Another study revealed some MTB strains have different abilities to induce cytokines involved in delayed type hypersensitivity (Anderson *et al*, 2006).

Comparing interferon-γ assay and TST results in all age groups without a

BCG scar revealed a high levels of agreement, but in the groups with a BCG scar there was no agreement (Table 1). Several studies have reported low agreement between the interferon-γ assay and the TST in children with BCG scars. Nakaoka *et al* (2006) compared the interferon-γ assay with the TST in Nigeria among people with a BCG scar; there was 90% agreement (Kappa 0.498; $p < 0.05$). Dogra *et al* (2007) compared the interferon-γ assay with the TST in India among people with a BCG scar and found 80% agreement. In the group without a BCG scar there was higher agreement (Kappa 1) than the BCG scar group (Kappa 0.63). Lighter *et al* (2009) compared the interferon-γ assay with the TST in people without a BCG scar (Kappa 0.31) and found higher agreement than in people with a BCG scar (Kappa 0.17).

In our study, comparing the interferon-γ assay with the TST among subjects aged 1-5 years with a BCG scar there was no agreement, whereas in the group without a BCG scar there was high agreement. In this study BCG vaccination influenced the results comparing the interferon-γ assay with the TST in children aged 1-5 years (Table 2). In children aged >5-10 years, comparing the interferon-γ assay with the TST groups both with and without BCG scars had no agreement. Thus BCG vaccination does not affect test results in children aged >5-10 years (Table 2). Similarly, in children aged >10 years, the BCG vaccination did not affect test results (Table 2).

The influence of BCG vaccination on the TST is a complex phenomenon. It is not only due to the antigen factor, but the age of vaccination and the interval between BCG immunization and the TST (Wang *et al*, 2002; Pai *et al*, 2004; Menzies *et al*, 2007). Wang *et al* (2002) in a meta-analysis found BCG vaccination affected the results of TST until 15 years after vaccination. Farhat *et al* (2006) in a meta-analysis found BCG vaccination in infants lead to a 6.3% false positive TST rate (induration ≥ 10 mm). If the TST was performed among subjects ≥ 10 years the false positive rate is only 1%. In contrast, if BCG vaccination is given to children aged ≥ 2 years, the false positive rate is 40%, and if the TST performed in children aged ≥ 10 years the false positive rate is 10% (Farhat *et al*, 2006). In Indonesia, the BCG vaccine is recommended for children < 2 months old (Rahajoe *et al*, 2007).

Other factors may influence the effect of the BCG vaccine on TST results. In some countries, the effect of the BCG vaccine on TST results decreases earlier, presumably because the high exposure to other infections leads to bias in the TH1 immune response (Floyd *et al*, 2002). In regions with high TB exposure, the influence of BCG vaccination in infants on the TST is temporary. This influence decreases with age, starting at about $2^1/_2$ months (Menzies *et al*, 2000; Floyd *et al*, 2002; Wang *et al*, 2002). Hill *et al* (2006) studied children aged 6 months to 14 years in Gambia and found no significant differences in TST results among individuals with or without a BCG scar. This is similar to a study in Uganda which revealed no influence of BCG immunization on the TST (Mudido *et al*, 1999). Hasanabadi *et al* (1998), in Iran, found TST results significantly greater in children with a BCG scar.

The main difference between the interferon-γ assay and the TST is the antigens used for the tests. Interferon-γ uses a specific antigen which is not shared with PPD or NTM. In this study the influence of NTM infection on the interferon-γ assay and TST results among children 1-5 years

old could not be determined, because the test results for the interferon-γ assay and the TST among all samples with positive cultures for NTM were the same. In the >5-10 year old age group no influence of NTM was found when comparing the two test results. Among children >10 years old no culture positive NTM was found.

NTM are commonly found in the environment. Humans are commonly exposed to 50-500 NTM bacilli per day (Primm *et al*, 2004). Because the magnitude of NTM exposure varies by region, the influence of NTM on the results of the TST also varies (Wang *et al*, 2002; Pai *et al*, 2004). In Montreal and France, NTM causes a 0.1% false positive rate on the TST, while in India false positive rate is 2.3% (Farhat *et al*, 2006).

This study was limited since the setting was an outpatient clinic, gastric aspiration was not performed since it requires subjects to be in a lying position overnight. The influence of NTM infection could not be determined in any age group.

In summary, among children aged 1-5 years, TST results were influenced by BCG scar. Presence of NTM infection had no influence on TST results among children aged >5-10 years, but in children aged 1-5 years and >10 years the effect could not be determined.

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