SHORT COMMUNICATION

T-Cell Assay as a Diagnostic Tool for Tuberculosis

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This study aimed to estimate the specificity and sensitivity of a whole blood IFN-g assay (ELISPOT) test for diagnosis in childhood tuberculosis. 96 patients, less than 18 years of age, diagnosed and commenced on anti-tubercular therapy were enrolled and tested. 47 age and sex matched controls were also tested. 23 tests were deemed invalid and analysis done on the remainder. The sensitivity was 53.3% in confirmed cases and less in other groups. The specificity was high at 97.9%. This test can be an useful aid in the diagnosis of tuberculosis.

Key words: *Children, Diagnosis, Interferon assay, Tuberculosis.*

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Tuberculosis in children is paucibacillary in nature and bacteriological confirmation by culture of *Mycobacterium tuberculosis* rarely exceeds 30-40%(1). There is a need for an effective diagnostic test for childhood tuberculosis. An *in vitro* whole blood test that detects *M. tuberculosis* infection by measuring IFN-g responses to a number of specific proteins including culture filtrate protein (CFP-10) and early secreted protein 6 (ESAT-6) is now available. The two antigens mentioned are part of the *M. tuberculosis* genome, absent from genomes of all BCG sub strains and most non-tuberculous mycobacteria (NTM)(2).

We used one of the available assays, the enzyme linked immunospot assay (ELISPOT test) (Oxford Immunotec, Oxford, UK). The ELISPOT has been widely used for screening for latent infection(3-5) and has been compared with the tuberculin skin tests (TST)(6,7). In this study, we evaluated the sensitivity and specificity of ELISPOT in the diagnosis of childhood TB. Specificity was estimated in a group of BCG-vaccinated children with no known exposure to *M. tuberculosis* infection. Sensitivity was determined in three defined groups, confirmed, probable and possible tuberculosis.

METHODS

The study was conducted, after obtaining ethical approval, and with informed consent from the parent or patient between March 2005 and November 2006 at Christian Medical College, Vellore. Children and young people below the age of 18 years who were diagnosed and started on anti-tubercular treatment less than 2 weeks previously, were prospectively enrolled in the study.

The clinical diagnosis of tuberculosis was made when at least 2 of the following criteria were positive: positive tuberculin skin test (TST), defined as induration >10 mm in the horizontal plane; clinical findings compatible with diagnosis; positive history of contact; culture positivity; biopsy positivity of skin, lymph node or pleura; and, positive imaging studies. The cases were further categorized as (*i*) Confirmed tuberculosis: Culture or smear positive for *M.tuberculosis*; and/or biopsy-pathological features suggestive of tuberculosis; and

(*ii*) Probable tuberculosis if the chest *X*-ray was suggestive of active tuberculosis; presence of cervical lymphadenopathy of more than 2 cm, with a positive TST; abdominal mass, ascites, or lymphadenopathy on ultrasound; CSF changes consistent with TB meningitis, or presence of tuberculomas on neuroimaging, (*iii*) Possible tuberculosis: was considered with a history of contact, with either a positive TST or with failure of nutritional rehabilitation. Controls were matched for age and sex, had a diagnosis other than tuberculosis, had no history of contact with TB, and were well apart from the acute clinical condition for which health care was being accessed. TST (10 units per mL) was only done on controls if clinically indicated. They were reviewed in six months, to ensure that they had remained well, and were unlikely to have been harbouring early infection with tuberculosis.

All assays were performed by one of the two investigators. ELISPOT assays were done, following protocol, with kits provided by Oxford Immunotech. Quality control of the Vellore laboratory was done at the start of the study, and results validated. ELISPOT plates were read manually by an independent blinded observer and subsequently scored in Oxford with an automated ELISPOT counter. Settings for intensity and spot size were predefined and fixed. Statistical analysis was done using Epi-Info.

RESULTS

143 tests were done, 96 cases and 47 controls, 32 results were initially declared invalid and 9 subsequently manually verified and validated. Of the failed samples, 2 were from confirmed, 7 'probable' and 14 'possible' cases. All failures were due to 'positive control' failures, ie, less than 20 spots and less than 75% saturated.

There were 41 girls and 32 boys amongst valid cases, and 16 girls amongst controls. The cases were diverse: bone and joint involvement (*n*=6), cough and fever (*n*=13 and 27), hemoptysis (*n*=2), meningitis (*n*=15), lymph node enlargement (*n*=17), and weight loss (*n*=6). ***Table* I** shows the number of positive results in the different groups of patients. Sensitivity in cases were 53.3%, 36.8%, and 30% in the confirmed, 'probable' and 'possible' groups, respectively. The specificity was 97.9%.

TABLE I CHILDREN IN EACH GROUP AND POSITIVE RESULTS

	Definitive group	Probable group	Possible group	Controls group
Total cases	15	38	20	47
Positive results	8	14	6	1
Percentage positive	53.3%	36.8%	30%	2.1%

DISCUSSION

Validation of a diagnostic tool in tuberculosis is a challenge. The gold standard of a positive Mycobacterial culture rarely exceeds 30-40%. Factors such as tests not being done in appropriate populations, bias in patient selection, bias due to lack of blinding have been addressed in the selection of patients and controls.

A study from South Africa has shown higher sensitivity, i.e., two thirds in children with a clinical diagnosis of tuberculosis and in 83% with culture-confirmed disease(8). Our results showed a lower sensitivity; 53% in confirmed cases and lower in the other categories, perhaps because of poor specificity of the diagnostic criteria in the other groups. The specificity of the test was very high, 97.8% (46/47). All but 11 of the 143 children had a BCG scar, indicating that the BCG does not have an effect on the ELISPOT result. The presence in the control patients of a variety of infectious, inflammatory, and granulomatous diseases indicates that the assay is not confounded by nonspecific activation of the cellular immune system.

The ELISPOT assay has some limitations. It is expensive, requires trained laboratory personnel and sophisticated laboratory equipment. The blood needs processing within 4 hours of collection. In our series, there was some failure of the positive control wells.

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WHAT THIS STUDY ADDS?

- Enzyme linked immunospot assay (ELISPOT) has low sensitivity but high specificity for the diagnosis of pediatric tuberculosis.

project: Dr Leni Mathew, Dr Indira Agarwal, Dr Mona Baskar, Dr Joy Mammen, Dr Maya Thomas.

Contributors: AB designed the study, in consultation with KRJ. AW and SG were the Research Officers on the project, identified the patients, assisted in sample collection. All authors provided critical input into the preparation of the manuscript.

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