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Prognostic Value of a T-Cell-Based Interferon-Gamma Biomarker in Child Tuberculosis Contacts

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Abstract

Background—Enzyme-linked-immunospot (ELISpot) is an increasingly widely-used interferon-gamma release assay (IGRA) for diagnosing tuberculosis infection but it is unknown whether positive results are prognostic of active tuberculosis.

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Author Contributions

The first two authors contributed equally to this work. A Lalvani and M Bakir designed the study and coordinated it together with A Soysal. M Bakir, A Soysal and Y Aslan enrolled and clinically assessed the children and created the clinical and demographic database. S Efe did the ELISpot assays. K Millington and D Dosanjh scored and analysed ELISpot assay results, and K Millington and D Dosanjh unblinded and combined the clinical and ELISpot datasets. The analysis was designed by J Deeks, K Millington, A Lalvani and M Bakir and the statistical analysis done by J Deeks and K Millington. K Millington and A Lalvani wrote the paper, with critical appraisal from M Bakir, J Deeks and A Soysal. All researchers reviewed the final report.

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Data: Can be made available to academic investigators on written request by agreement with Prof A. Lalvani at a.lalvani@imperial.ac.uk

Competing Interests

The Lalvani ELISpot was commercialised by an Oxford University spin-out company (T-SPOT.*TB*®, Oxford Immunotec Ltd, Abingdon, United Kingdom) in which Oxford University and Professor Lalvani have minority shareholdings and to which Professor Lalvani previously acted as non-executive director (2003-07). Professor Lalvani and Dr Millington have potential financial conflicts of interest arising from patents relating to T cell-based diagnosis.

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Objective—To determine the prognostic value of this T-cell-based interferon-gamma biomarker.

Design—Longitudinal cohort study of child tuberculosis contacts recruited from October 2002 to April 2004.

Setting—Community-based contact investigations in Turkey.

Patients—908 children and adolescents with recent household tuberculosis exposure.

Intervention—ELISpot, incorporating Early Secretory Antigenic Target-6 and Culture Filtrate Protein-10, and tuberculin skin test (TST) were performed at baseline.

Measurements—Incidence rates of progression to active tuberculosis for contacts with positive TST and ELISpot results and relative incidence rates comparing test-positive and test-negative contacts.

Results—688 (76%) contacts received isoniazid preventive therapy in accordance with local guidelines. Fifteen contacts developed active tuberculosis over 1201 person-years follow-up. Of 381 ELISpot-positive contacts, 11 developed active tuberculosis over 536 person-years follow-up (incidence rate 21 per 1000 person-years [95% CI 10.2, 36.7]) and of 550 TST-positive contacts, 12 developed active tuberculosis over 722 person-years of follow-up (17 per 1000 person-years [95% CI 8.6, 29.0]).

Limitations—Only 3 of the 15 incident cases were culture-confirmed.

Conclusion—Although tuberculosis contacts with positive ELISpot results have a similar incidence rate of tuberculosis compared to contacts with positive TST results, ELISpot testing could allow more focussed targeting of preventive therapy to fewer contacts.

Introduction

The use of the tuberculin skin test (TST) to diagnose latent tuberculosis infection was established and validated in large studies which observed that persons with a positive TST had a higher risk of developing active tuberculosis compared with TST-negative persons (1-7). However, diagnostic specificity of the TST is confounded in Bacille Calmette-Guérin (BCG)-vaccinated persons and sensitivity is reduced in vulnerable populations including immunocompromised persons and children (8).

The advent of T-cell interferon-gamma release assays (IGRAs) may offer a realistic alternative to the TST (9-15). Currently, there are two forms of IGRAs using *M. tuberculosis*-specific antigens: interferon-gamma secretion measured in whole-blood by the enzyme-linked immunosorbant (ELISA) assay and the enumeration of T-cells secreting interferon-gamma measured by the enzyme-linked immunospot (ELISpot) assay. Much published data indicates that IGRAs are more specific than the TST (12, 15, 16) and cross-sectional studies have shown good correlation of IGRA results with tuberculosis exposure in contacts (17-22).

Treatment of recent tuberculosis contacts with positive IGRA results, as recommended by some national guidelines, (23, 24) will only provide clinical benefit if these individuals are at an increased risk of progression to tuberculosis compared to those with negative results. Thus, there is a widely-recognised urgent need for large longitudinal studies with clinical outcomes to determine the prognostic value of positive IGRA results for development of tuberculosis (12, 15, 23-28). A recent report of predominantly immunocompetent adult tuberculosis contacts who refused preventive therapy showed that a higher proportion of those who were initially positive by whole-blood ELISA (Quantiferon-Gold in-tube, Cellestis Inc, Carnegie, Victoria, Australia) developed active tuberculosis over two years follow-up than TST-positive contacts using a 5 mm cut-off point (29). However, this

important observation, which was based on 6 incident tuberculosis cases, cannot be extrapolated to ELISpot because of substantial discordance between the two assays (12), nor to contact investigations involving high-risk populations (30).

We conducted a longitudinal cohort study to determine the prognostic value of positive interferon-gamma ELISpot results for development of active tuberculosis in a key high-risk population: children and adolescents with recent household exposure to tuberculosis. Since tuberculosis infection in children is usually recently acquired, it serves as a key marker for *M. tuberculosis* transmission in the general population and triggers extensive contact investigations to identify infectious adult source cases. Moreover, prompt isoniazid preventive therapy in infected children reduces the risk of development of active tuberculosis, which is associated with high morbidity and mortality (31, 32). Validation of IGRA for diagnosis of asymptomatic tuberculosis infection by determining the predictive value of positive test results in this population is therefore a global public health priority.

Methods

Study participants

All adults newly diagnosed with sputum smear-positive pulmonary tuberculosis at the 7 government-funded tuberculosis clinics on the Anatolian side of Istanbul over 18 months from October 2002 were asked if they had children living in the household and were invited to participate in the study. 443 patients had one or more child household contacts and all agreed to participate and gave written informed consent on behalf of their children (17). Where the index case was not the parent, consent was given by the child's grandparents or legal guardian. Contacts were included if they were 16 years or younger, and there were no exclusion criteria for enrolment. Follow-up comprised 6-monthly symptom review and continued until incident tuberculosis was diagnosed or planned 2 years follow-up completed. Parents or guardians not attending clinical follow-up appointments were contacted by telephone and the health status of the child assessed using a standardised questionnaire. In addition, all children had a repeat ELISpot assay at 6 months follow-up. Ethical approval for the study was granted by the Institutional Review Board of Marmara University School of Medicine, Istanbul, The Turkish Ministry of Health, Ankara and the WHO Steering Committee on Research Involving Human Subjects, Geneva.

Children were vaccinated intradermally with BCG Pasteur 1173-P2 between 2 and 3 months of age and a booster vaccination administered in the first year of primary school, and at 6 to 7 years of age as recommended by the Turkish Ministry of Health guidelines. BCG vaccination was documented by number of BCG scars. Prevalent cases of active tuberculosis were diagnosed by history, physical examination, chest radiography and microbiological investigations at enrolment and were excluded from our cohort.

Three groups of contacts were given a 6 month course of isoniazid preventive therapy on the basis of age and TST results interpreted in accordance with Turkish Ministry of Health guidelines (33): i) all children younger than 6 years old regardless of baseline or subsequent TST results, ii) children 6 years or older positive to the first TST (i.e. ≥10 mm in unvaccinated children and ≥15 mm in vaccinated children) and iii) children 6 years or older negative to the first TST but who converted their TST result (i.e. induration increased by ≥6 mm from the first TST result and the second TST induration was ≥10mm in unvaccinated children and ≥15mm in vaccinated children). Isoniazid was administered to children by their parents or guardians who were provided with tablets at 2 monthly intervals. All parents or guardians who returned for follow-up were questioned about the compliance of their children with preventive treatment and all reported full compliance.

Ex-vivo interferon-gamma ELISpot assays

ELISpot assays using peptides spanning early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) (10 μg/mL final concentration of each peptide, Core Labs LSUHSC, New Orleans, LA, USA) and recombinant ESAT-6 (rESAT-6 10 μg/mL, Veterinary Laboratories Agency, Weybridge, U.K.) and recombinant CFP-10 (rCFP-10, 10 μg/mL, Lionex Diagnostics and Therapeutics, Braunschweig, Germany) were performed as previously described (17, 21). The peptide assay was subsequently commercialised into the regulatory-approved T-SPOT®.*TB* assay (Oxford Immunotec, Abingdon, United Kingdom). Because the diagnostic potential of ESAT-6 and CFP-10-derived peptides and recombinant antigens has been assessed in published work, we determined the prognostic value of both forms of these *M. tuberculosis*-specific antigens.

ELISpot plates were counted and scored using an automated ELISpot counter (AID-GmbH, Strassberg, Germany) using pre-defined size and intensity spot settings. Responses to peptides were scored positive if duplicate test wells contained a mean of ≥5 spot-forming cells (SFCs) more than the mean of the negative control wells, and, in addition, this number was at least twice the mean of the negative control wells. This predefined threshold was used in all our previous studies (17, 20-22, 34-38). Responses to antigen were scored positive if duplicate test wells contained a mean of ≥10 SFCs more than the mean of the negative control wells, and, in addition, this number was at least twice the mean of the negative control wells. For simplicity, cells stimulated with ESAT-6/CFP-10 peptides will be referred to as “ELISpot”.

Tuberculin skin testing

TST was administered by the Mantoux method using 0.1 mL (2 tuberculin units) of purified protein derivative (PPD) RT23 (Statens Serum Institut, Copenhagen, Denmark). The test was performed and read by the study pediatrician who was blinded to ELISpot results. The cutaneous appearance of peau d’orange was noted in all participants, confirming intradermal inoculation of PPD. Induration was measured after 72 to 96 hours with a ruler. For our analysis a TST response was scored positive if the induration diameter was ≥5 mm induration as recommended by American Thoracic Society and Center for Disease Control and Prevention guidelines (39).

Assessment of outcome

Each child contact was to be clinically followed-up every 6 months for 2 years but parents were asked to return with the child immediately for further clinical assessment if they developed intercurrent symptoms. Diagnosis of incident tuberculosis was made by the study pediatrician, blinded to ELISpot results, based on clinical, radiological and microbiological criteria. The clinical and radiological evidence for culture-negative incident cases was assessed separately by 2 independent clinicians and in each case the diagnosis was further confirmed by a documented successful clinical and radiological response to anti-tuberculosis treatment. Children diagnosed with tuberculosis were treated with 6 months standard chemotherapy and the continuation phase of isoniazid and rifampicin was extended to 10 months for children with miliary tuberculosis. Children diagnosed with multi-drug resistant tuberculosis were treated with second line agents, based on antibiotic susceptibility results.

Statistical analysis

Each child’s entry into the study was defined as the date first examined and tested with the ELISpot and TST (baseline). The endpoint of the study for each child contact was when the child developed active tuberculosis or the last occasion on which they were assessed at follow-up (whether by telephone or a clinic visit). Differences in baseline characteristics of

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participants who developed active tuberculosis and those that did not were compared using the chi-squared test for binary variables and the Mann-Whitney test for continuous variables. Poisson regression was used to estimate incidence rates of progression to active tuberculosis per 1000 person years of follow-up, together with 95% confidence intervals (CI). Incidence rate ratios were estimated to compare the prognostic value of test positive to test negative results, both unadjusted and adjusted for preventive therapy. Differences in SFCs at baseline were compared using the Mann-Whitney test. All analyses were undertaken in Stata version 9.1 (Stata Corporation, College Station, Texas, USA).

Role of the Funding Source

UNICEF/UNDP/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases (TDR) and the Wellcome Trust had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Participant characteristics and follow-up

The final cohort comprised 908 children who were followed-up for 1201 person years; thus, the mean duration of follow-up was 1.3 years (Figure 1). 456 (50%) child contacts were male and the mean age of the cohort was 7.5 years (range 1 month to 16 years). 728 (80%) children were BCG-vaccinated.

All 908 children in the analytic dataset had complete baseline ELISpot and TST results available. 381 (42%) children were positive by ELISpot and 550 (61%) children were positive by TST. The prevalence of positive TST results was statistically significantly higher among BCG-vaccinated contacts than among unvaccinated contacts (462/728 versus 88/180; P<0.0001).

63 children were lost from the cohort during follow-up after clinical assessment at 6 months and drop-out rates did not differ according to ELISpot status (6% of ELISpot-positive contacts versus 7% of ELISpot-negative contacts). 270 children completed 24 months follow-up with 6 monthly clinical review and 560 children who did not attend clinical follow-up beyond 12 months were followed-up 6 monthly with a standardized telephone questionnaire administered by the study nurse.

Fifteen contacts developed active tuberculosis during follow-up. Development of active tuberculosis was not related to gender (incidence in females 9/452 (2.0%) versus males 6/456 (1.3%); P=0.43) although incident cases tended to be younger (median (inter-quartile range): incident cases 4 (1 to 8) versus 8 (4 to 11) years; P=0.07).

Incident tuberculosis cases

Demographic characteristics and test results of the 15 incident cases are listed in Table 1. At enrolment all incident cases were asymptomatic with a normal chest radiograph and had no past history of tuberculosis; clinical manifestations at diagnosis are shown in Table 2. Eight cases were detected at scheduled follow-up visits and the remaining 7 presented to clinic outside of scheduled follow-up appointments. The proportion of ELISpot-positive incident cases diagnosed at scheduled clinical follow-up visits (5 out of 11 cases) was not higher than the corresponding proportion of ELISpot-negative incident cases diagnosed at scheduled clinical follow-up (3 out of 4 cases). All 15 cases had clinical and radiological findings strongly suggestive of tuberculosis, 3 of whom also had positive cultures for *M. tuberculosis* from clinical specimens (Table 2). All cases responded fully to anti-tuberculosis treatment,

including those treated with second line drugs for multi-drug resistant tuberculosis, with complete clinical and radiological resolution.

11 incident cases were ELISpot-positive and 12 were TST-positive at recruitment. 10 incident cases were positive to both ELISpot and TST, 1 incident case was ELISpot-positive TST-negative, 2 were TST-positive ELISpot-negative and 2 were negative to both ELISpot and TST (Table 1). 2 of the 4 cases that were initially ELISpot-negative turned ELISpot-positive when re-tested 6 months later. Although the quantitative definition of ELISpot conversion is an evolving area because of the natural biological variability around the threshold for a positive result, both converted results were strongly positive (376 and 140 SFC/10^6 peripheral blood mononuclear cells respectively). Of the 3 cases that were TST-negative all were retested 2 to 4 months later and 1 converted their result to 15 mm induration (Table 1).

Incidence of tuberculosis

Of the 381 contacts that were ELISpot-positive, 11 progressed to active tuberculosis within 536 person-years of follow-up (incidence rate 20.5 per 1000 person-years [95% CI 10.2, 36.7]). Of the 550 TST-positive contacts, 12 progressed to tuberculosis within 722 person-years (incidence rate 16.6 per 1000 person years [95% CI 8.6, 29.0]) (Table 3). Thus, ELISpot detected a similar number of incident cases from a smaller group of test positive results. Moreover, a statistically significantly higher proportion of contacts were TST-positive than ELISpot-positive (550/908 versus 381/908 respectively; P<0.0001), consistent with the higher specificity of ELISpot.

Children who were ELISpot-positive were 3.4 times more likely to develop active disease than those who were ELISpot-negative (P=0.04) (Table 3). TST-positive children were 2.7 times more likely to develop disease than TST-negative children, the difference not being statistically significant (P=0.13) (Table 3).

688 children were prescribed isoniazid preventive therapy. Because isoniazid was partly administered on the basis of TST results interpreted in accordance with the Turkish Ministry of Health guidelines, and because of the high agreement between TST and ELISpot results (k=0.65), a higher proportion of ELISpot-positive contacts received isoniazid than ELISpot-negative contacts (353/381 (93%) versus 335/527 (64%) P<0.0001). Thus administration of isoniazid preventive therapy is partially related to the test result and could confound the incidence rate. Adjusting for treatment mildly increased the relative incidence rates between test-positive and test-negative children (Table 3).

Because a high proportion (71%) of contacts exposed to index cases infected with *M. tuberculosis* susceptible to isoniazid received preventive therapy, including 10 of the 15 incident cases, we had limited data on contacts who had not received preventive therapy from which to estimate the incidence rate. In these untreated contacts, 54 were ELISpot-positive, of whom 4 progressed to active tuberculosis within 93 person-years of follow-up (incidence rate 43.0 per 1000 person-years [95% CI 11.7, 110.2]). ELISpot-positive contacts were statistically significantly more likely to develop active tuberculosis than ELISpot-negative contacts (incidence rate ratio 10.6 [95% CI 1.19, 95.2, P=0.03]). 83 contacts were TST-positive, of whom 3 progressed to tuberculosis within 114 person-years of follow-up (incidence rate 26.2 per 1000 person years [95% CI 5.4, 76.6]).

333 contacts were ELISpot-positive to recombinant ESAT-6/CFP-10 antigen, of whom 11 progressed to active tuberculosis within 462 person-years of follow-up (incidence rate 23.8 per 1000 person-years [95% CI 11.9, 42.6]).The incidence rate in contacts positive to PPD

was similar to that in contacts positive to the non-tuberculosis control antigen SKSD (Table 3).

Of the 337 contacts that were positive to both tests, 10 progressed to tuberculosis within 451 person-years of follow-up (incidence rate 22.2 per 1000 person years [95% CI 10.6, 40.8]) (Figure). The low number of cases with discordant test results (ELISpot-positive TST-negative: 1 case from 44 contacts; ELISpot-negative TST-positive: 2 cases from 213 contacts) resulted in poor estimates of incidence rates with very wide confidence intervals for these test result combinations.

Magnitude of baseline ELISpot results and incidence of tuberculosis

Within ELISpot-positive contacts, the size of the baseline interferon-gamma response to peptides was not statistically significantly different between contacts who developed tuberculosis and those who remained well (SFC/10^6 peripheral blood mononuclear cells median [interquartile range]: 176 [88, 1332] versus 182 [70, 534] P=0.27), and likewise for responses to recombinant antigen (SFC/10^6 peripheral blood mononuclear cells median [interquartile range]: 246 [200, 918) versus 264 [124, 526] P=0.18).

Discussion

We found that child tuberculosis contacts with positive ELISpot results had a statistically significant 3 to 4-fold increased risk of progression to active tuberculosis compared to ELISpot-negative contacts. The incidence rate of tuberculosis in ELISpot-positive contacts was similar to the incidence rate in TST-positive contacts, but ELISpot detected a similar number of incident cases from a smaller number of contacts with positive test results.

ELISpot is a quantitative assay, thus interpretation of ELISpot results is not restricted to a binary read-out. There was no statistically significant difference in the size of the baseline interferon-gamma response between incident cases and contacts that did not develop tuberculosis suggesting that whilst a positive ELISpot result is prognostic of progression to tuberculosis, the magnitude of this response does not further refine the risk of progression. This is in contrast to the TST, where the size of the tuberculin reaction correlates with the risk of subsequent progression to tuberculosis (1-7).

None of the incident cases had a history of tuberculosis and tuberculosis was excluded at recruitment by the absence of symptoms and normal radiological and clinical examinations. Thus, a positive ELISpot result at recruitment reflected asymptomatic *M. tuberculosis* infection and not active tuberculosis or a relapse of tuberculosis. All household members were evaluated clinically and with chest radiography and any secondary cases of tuberculosis were treated promptly at the time of recruitment of child contacts. Hence re-exposure of child contacts within the household would have been minimal, although re-infection arising from exposure in the community post-recruitment cannot be completely excluded.

Very few prospective studies have assessed the relationship between IGRA results and clinical outcome, and the present study is the first to focus on a key high-risk group. In a small study of 24 household contacts of sputum smear-positive pulmonary tuberculosis in Ethiopia, 6 of the 7 incident cases were positive to rESAT-6 in a 5 day interferon-gamma research ELISA compared to 3 of 17 contacts that did not develop active tuberculosis (40). In a more recent household contact study in Germany, a statistically significantly higher proportion of untreated household contacts with positive ELISA results progressed to active tuberculosis than contacts with positive TST results when using the 5 mm threshold (P<0.003) (29). However, this proportion was not statistically significantly higher when

compared to the proportion of contacts with positive TST results that progressed to tuberculosis when using a 10 mm threshold (P=0.1). In The Gambia, Hill et al identified 26 incident cases during two years follow-up of household contacts and, although contacts did not receive isoniazid preventive therapy, neither TST (using a 10 mm threshold) nor ELISpot (using a substantially higher threshold for positive test results than the threshold used in our current and previous studies and in the commercial kit), were prognostic of subsequent progression to tuberculosis. The reason for the lack of prognostic power of both TST and ELISpot in the African study is currently unclear but may relate to the highly endemic setting in which community transmission outside households may cause a substantial proportion of tuberculosis even in household contacts (41).

Our study has several limitations. Only three of the incident cases in this study were culture-positive; however, clinical specimens from children are difficult to obtain and are usually culture-negative (42, 43). For the culture-negative cases the clinical and radiological findings were strongly suggestive of active tuberculosis in each case and all responded well to anti-tuberculosis treatment. Although the number of incident cases in our study was small, and hence the confidence intervals wide, the risk of subsequent tuberculosis in the ELISpot-positive recent child contacts was statistically significant and was robust to adjustment for confounding factors including isoniazid preventive therapy.

Because a high proportion of contacts and incident cases received isoniazid preventive therapy we had very limited data on contacts who had not received preventive therapy. As a result, our estimates of incidence rates in untreated ELISpot-positive and TST-positive contacts were less robust but did suggest a stronger prognostic value of each test result in this group. Given the ethical imperative for prompt isoniazid preventive therapy in young tuberculosis-exposed children, it may not be possible to robustly assess whether IGRAs are more strongly prognostic of subsequent tuberculosis than TST in the absence of preventive treatment.

13 incident cases developed tuberculosis despite being prescribed isoniazid, 3 of whom were exposed to isoniazid-resistant *M. tuberculosis*. Reasons for progression to disease in the remaining 10 children include the fact that 6 months isoniazid preventive therapy is only 60% effective (31, 32) and the possibility that compliance may not have been complete in all children, even though parents/guardians reported full compliance of their children when they returned to collect more tablets every 2 months. Although there may theoretically have been a potential bias of greater failure to complete the isoniazid regimen among those with TST results deemed negative by Turkish national guideline criteria and among those who had received BCG, all parents were informed that negative TST results do not rule out infection and BCG vaccination does not adequately protect against disease. In any event, our finding that ELISpot is prognostic of active tuberculosis holds irrespective of the extent of compliance with isoniazid preventive therapy in the study population.

A positive interferon-gamma ELISpot result is a useful and valid marker of latent tuberculosis infection because it predicts the subsequent development of active tuberculosis which suggests that contacts diagnosed with latent tuberculosis infection on the basis of ELISpot results could benefit from preventive therapy. Whilst the risk of progression to active tuberculosis in contacts with a positive ELISpot result was similar to contacts with a positive TST result, if test-positive contacts were targeted with preventive treatment, a statistically significantly higher number of TST-positive contacts compared to ELISpot-positive contacts would need to be treated to prevent a similar number of incident cases; thus ELISpot testing could allow more focussed targeting of preventive therapy to fewer contacts. Moreover, contacts' awareness of the risk of progression may improve adherence to the preventive treatment regimen (44). Although our study is generalisable as it was

conducted in unselected child household tuberculosis contacts in community-based contact investigations, similar studies are now required to validate and quantify the prognostic power of ELISpot in other high risk groups including HIV co-infected persons (45).

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References

1. Ferebee SH, Mount FW. Tuberculosis morbidity in a controlled trial of the prophylactic use of isoniazid among household contacts. Am Rev Respir Dis. 1962; 85:490–510. [PubMed: 13892318]
2. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. Am J Epidemiol. 1974; 99(2):131–8. [PubMed: 4810628]
3. Fine PE, Sterne JA, Ponnighaus JM, Rees RJ. Delayed-type hypersensitivity, mycobacterial vaccines and protective immunity. Lancet. 1994; 344(8932):1245–9. [PubMed: 7967984]
4. Medical Research Council. BCG and vole bacillus vaccines in the prevention of tuberculosis in adolescence and early life. Bull World Health Organ. 1972; 46:371–85. [PubMed: 4537855]
5. Fine PE, Bruce J, Ponnighaus JM, Nkhosa P, Harawa A, Vynnycky E. Tuberculin sensitivity: conversions and reversions in a rural African population. Int J Tuberc Lung Dis. 1999; 3(11):962–75. [PubMed: 10587318]
6. Horsburgh CR Jr. Priorities for the treatment of latent tuberculosis infection in the United States. N Engl J Med. 2004; 350(20):2060–7. [PubMed: 15141044]
7. Menzies D. Interpretation of repeated tuberculin tests. Boosting, conversion, and reversion. Am J Respir Crit Care Med. 1999; 159(1):15–21. [PubMed: 9872812]
8. Huebner RE, Schein MF, Bass JB Jr. The tuberculin skin test. Clin Infect Dis. 1993; 17(6):968–75. [PubMed: 8110954]
9. Starke JR. Interferon-gamma release assays for diagnosis of tuberculosis infection in children. Pediatr Infect Dis J. 2006; 25(10):941–2. [PubMed: 17006293]
10. Richeldi L. An update on the diagnosis of tuberculosis infection. Am J Respir Crit Care Med. 2006; 174(7):736–42. [PubMed: 16799073]
11. Barnes PF. Diagnosing latent tuberculosis infection: turning glitter to gold. Am J Respir Crit Care Med. 2004; 170(1):5–6. [PubMed: 15220119]
12. Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. Ann Intern Med. 2007; 146(5): 340–54. [PubMed: 17339619]
13. Lalvani A, Millington KA. T cell-based diagnosis of childhood tuberculosis infection. Curr Opin Infect Dis. 2007; 20(3):264–71. [PubMed: 17471036]
14. Schluger NW. The diagnosis of tuberculosis: what's old, what's new. Semin Respir Infect. 2003; 18(4):241–8. [PubMed: 14679473]
15. Pai M, Zwerling A, Menzies D. Systematic Review: T-Cell-Based Assays for the Diagnosis of Latent Tuberculosis Infection: An Update. Ann Intern Med. 2008
16. Pai M, Riley LW, Colford JM Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. Lancet Infect Dis. 2004; 4(12):761–76. [PubMed: 15567126]

17. Soysal A, Millington KA, Bakir M, et al. Effect of BCG vaccination on risk of Mycobacterium tuberculosis infection in children with household tuberculosis contact: a prospective community-based study. Lancet. 2005; 366(9495):1443–51. [PubMed: 16243089]
18. Shams H, Weis SE, Klucar P, et al. Enzyme-linked immunospot and tuberculin skin testing to detect latent tuberculosis infection. Am J Respir Crit Care Med. 2005; 172(9):1161–8. [PubMed: 16081545]
19. Zellweger JP, Zellweger A, Ansermet S, de Senarclens B, Wrighton-Smith P. Contact tracing using a new T-cell-based test: better correlation with tuberculosis exposure than the tuberculin skin test. Int J Tuberc Lung Dis. 2005; 9(11):1242–7. [PubMed: 16333932]
20. Richeldi L, Ewer K, Losi M, et al. T cell-based tracking of multidrug resistant tuberculosis infection after brief exposure. Am J Respir Crit Care Med. 2004; 170(3):288–95. [PubMed: 15130907]
21. Ewer K, Deeks J, Alvarez L, et al. Comparison of T-cell-based assay with tuberculin skin test for diagnosis of Mycobacterium tuberculosis infection in a school tuberculosis outbreak. Lancet. 2003; 361(9364):1168–73. [PubMed: 12686038]
22. Lalvani A, Pathan AA, Durkan H, et al. Enhanced contact tracing and spatial tracking of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells. Lancet. 2001; 357(9273):2017–21. [PubMed: 11438135]
23. Mazurek GH, Jereb J, Lobue P, Iademarco MF, Metchock B, Vernon A. Guidelines for using the QuantiFERON-TB Gold test for detecting Mycobacterium tuberculosis infection, United States. MMWR Recomm Rep. 2005; 54(RR-15):49–55. [PubMed: 16357824]
24. National Collaborating Centre for Chronic Conditions. Tuberculosis: clinical diagnosis and management of tuberculosis, and measures for its prevention and control. Royal College of Physicians; London, UK: 2006.
25. World Health Organization. Global tuberculosis control: surveillance, planning, financing. Geneva, Switzerland: 2007. Publication WHO/HTM/TB/2007.376
26. Sterling TR, Haas DW. Transmission of Mycobacterium tuberculosis from health care workers. N Engl J Med. 2006; 355(2):118–21. [PubMed: 16837674]
27. Andersen P, Doherty TM, Pai M, Weldingh K. The prognosis of latent tuberculosis: can disease be predicted? Trends Mol Med. 2007; 13(5):175–82. [PubMed: 17418641]
28. Pai M, Dheda K, Cunningham J, Scano F, O'Brien R. T-cell assays for the diagnosis of latent tuberculosis infection: moving the research agenda forward. Lancet Infect Dis. 2007; 7(6):428–38. [PubMed: 17521596]
29. Diel R, Loddenkemper R, Meywald-Walter K, Niemann S, Nienhaus A. Predictive Value of a Whole-blood IFN-{gamma} Assay for the Development of Active TB Disease. Am J Respir Crit Care Med. 2008; 177:1164–1170. [PubMed: 18276940]
30. Stout J, Menzies D. Predicting Tuberculosis Does the IGRA Tell the Tale? Am J Respir Crit Care Med. 2008; 177:1055–1057. [PubMed: 18460459]
31. Marais BJ, Gie RP, Schaaf HS, Beyers N, Donald PR, Starke JR. Childhood pulmonary tuberculosis: old wisdom and new challenges. Am J Respir Crit Care Med. 2006; 173(10):1078–90. [PubMed: 16484674]
32. Hsu KH. Thirty years after isoniazid. Its impact on tuberculosis in children and adolescents. Jama. 1984; 251(10):1283–5. [PubMed: 6366257]
33. Rekmay Ltd.; Ankara, Turkey: 2003. Türkiye Cumhuriyeti Sağlik Bakanliği A. Türkiye'de tüberkülozun kontrolü için başvuru kitabi. http://www.istanbulsaglik.gov.tr/w/sb/bh/belge/tuberkitabi.pdf
34. Lalvani A, Pathan AA, McShane H, et al. Rapid detection of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells. Am J Respir Crit Care Med. 2001; 163(4):824–8. [PubMed: 11282752]
35. Liebeschuetz S, Bamber S, Ewer K, Deeks J, Pathan AA, Lalvani A. Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. Lancet. 2004; 364(9452):2196–203. [PubMed: 15610806]
36. Pathan AA, Wilkinson KA, Klenerman P, et al. Direct ex vivo analysis of antigen-specific IFN-gamma-secreting CD4 T cells in Mycobacterium tuberculosis-infected individuals: associations

with clinical disease state and effect of treatment. J Immunol. 2001; 167(9):5217–25. [PubMed: 11673535]

37. Chapman AL, Munkanta M, Wilkinson KA, et al. Rapid detection of active and latent tuberculosis infection in HIV-positive individuals by enumeration of Mycobacterium tuberculosis-specific T cells. Aids. 2002; 16(17):2285–93. [PubMed: 12441800]
38. Lalvani A, Nagvenkar P, Udwadia Z, et al. Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent Mycobacterium tuberculosis infection in healthy urban Indians. J Infect Dis. 2001; 183(3):469–77. [PubMed: 11133379]
39. American Thoracic Society. Targeted tuberculin testing and treatment of latent tuberculosis infection. Am J Respir Crit Care Med. 2000; 161(4 Pt 2):S221–S247. [PubMed: 10764341]
40. Doherty TM, Demissie A, Olobo J, et al. Immune responses to the Mycobacterium tuberculosis-specific antigen ESAT-6 signal subclinical infection among contacts of tuberculosis patients. J Clin Microbiol. 2002; 40(2):704–6. [PubMed: 11826002]
41. Verver S, Warren RM, Munch Z, et al. Proportion of tuberculosis transmission that takes place in households in a high-incidence area. Lancet. 2004; 363(9404):212–4. [PubMed: 14738796]
42. Schaaf HS, Beyers N, Gie RP, et al. Respiratory tuberculosis in childhood: the diagnostic value of clinical features and special investigations. Pediatr Infect Dis J. 1995; 14(3):189–94. [PubMed: 7761183]
43. Hesseling AC, Schaaf HS, Gie RP, Starke JR, Beyers N. A critical review of diagnostic approaches used in the diagnosis of childhood tuberculosis. Int J Tuberc Lung Dis. 2002; 6(12):1038–45. [PubMed: 12546110]
44. Shieh FK, Snyder G, Horsburgh CR, Bernardo J, Murphy C, Saukkonen JJ. Predicting non-completion of treatment for latent tuberculous infection: a prospective survey. Am J Respir Crit Care Med. 2006; 174(6):717–21. [PubMed: 16809632]
45. Altman DG, Royston P. What do we mean by validating a prognostic model? Stat Med. 2000; 19(4):453–73. [PubMed: 10694730]

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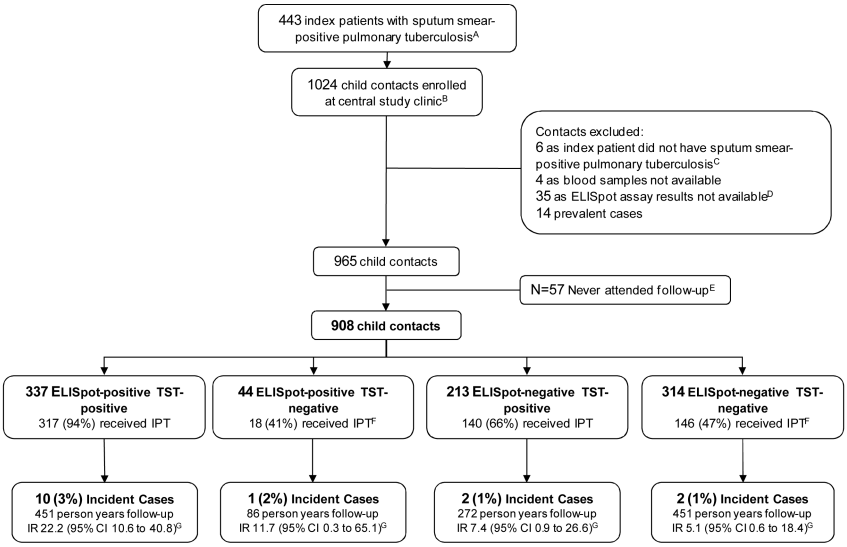

Figure 1.

Study flow chart

Study flow chart detailing the follow-up of 908 children with complete baseline results for ELISpot and TST.

ELISpot = enzyme linked immunospot, TST = tuberculin skin test

[A]Index patients and child contacts were recruited at the 7 government run tuberculosis clinics in the Anatolian side of Istanbul.

[B]Pediatric Infectious Disease Clinic at Marmara University Hospital, Istanbul.

[C]When sputum microscopy and culture reports for all 443 index cases were obtained and checked, it transpired that 4 contacts had index cases who were not sputum smear positive and 2 contacts had index cases whose sputum grew non-tuberculous atypical mycobacteria.

[D]2 contacts were removed due to the loss of ELISpot plates and 33 contacts were removed due to an episode of bacterial contamination of peptide pool reagents.

[E]20 (5%) contacts were ELISpot-positive and 37 (7%) contacts were ELISpot-negative at recruitment

[F]Isoniazid preventive therapy was administered on the basis of age and TST results interpreted in accordance with Turkish Ministry of Health guidelines (see Methods). A further 18 contacts ELISpot-positive TST-negative at recruitment and 49 contacts ELISpot-negative TST-negative at recruitment were given IPT because they converted their TST. In total, 688 contacts received IPT of whom 41 were exposed to index cases with multi-drug-resistant tuberculosis. 13 incident cases received IPT: 6 were ELISpot-positive TST-positive, 4 were ELISpot-positive TST-negative (2 of whom converted their TST) and 3 were ELISpot-negative TST-negative (2 of whom converted their TST). None of the incident cases were ELISpot-negative TST-positive.

[G]IR = incidence rate per 1000 person years of follow-up

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Table 1

Demographic characteristics and ELISpot and tuberculin skin test results of incident tuberculosis cases

	Demographics			Test results					Treatment
				TST		ELISpot			
Patient number	Age at recruitment (years)	Gender	#BCG vaccinations	Baseline induration, mm	Follow-up induration, mm	ELISpot magnitude SFC/10^6 PBMC[C]	Baseline result[D]	ELISpot 6 month result	Isoniazid preventive therapy[E]
66	4 months	F	0	0	15	0	Negative	Positive	Yes
134[A,B]	8	F	1	20	-	110	Positive	Positive	Yes
135[A,B]	7	F	0	19	-	788	Positive	Positive	Yes
138[A,B]	1	M	0	8	28	176	Positive	Positive	Yes
174	4	F	0	7	24	0	Negative	Negative	Yes
176	4 months	F	1	7	8	32	Positive	Positive	Yes
215[A]	7	F	1	7	17	166	Positive	-	Yes
218[A]	1	M	1	7	11	0	Negative	Positive	Yes
284	14	F	2	23	-	1906	Positive	-	Yes
290	3	M	0	6	10	1862	Positive	Positive	Yes
472	3	M	1	18	-	88	Positive	Positive	Yes
510	4	M	0	20	-	1332	Positive	Positive	Yes
573	3	F	1	16	-	766	Positive	Positive	Yes
608	8	F	2	0	4	0	Negative	Negative	No
874	15	M	1	0	4	52	Positive	Negative	No

TST= tuberculin skin test, ELISpot = enzyme linked immunospot, BCG= bacilli Calmette-Guerin, PBMC = peripheral blood mononuclear cells

[A] Patients 134, 135 and 138 and patients 215 and 218 were from the same household and exposed to the same index case.

[B] Patients 134, 135 and 138 were exposed to the same index case with TB resistant to isoniazid, rifampicin, ethambutol and streptomycin. Patient 134 was resistant to isoniazid and rifampicin but drug sensitive to ethambutol and streptomycin and patient 138 was resistant to isoniazid, rifampicin, ethambutol and streptomycin. Patient 135 was culture-negative.

[C] Summated SFCs from 6 pools of peptides derived from ESAT-6 and CFP-10

[D] ELISpot result is scored positive when test well ≥20 SFC/10^6 PBMC above negative control and is twice negative control. Patient 218 was ESAT-6 peptide-negative rESAT-6-positive. Patients 218 and 472 were CFP-10 peptide-negative rCFP-10-positive. Patients 215 and 573 were CFP-10 peptide-positive rCFP-10-negative. All other peptide/antigen results were concordant.

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[E] The following subgroups of contacts were given a 6 month course of isoniazid preventive therapy in accordance with Turkish Ministry of Health guidelines i) all children younger than 6 years old regardless of baseline or subsequent TST results, ii) children 6 years or older positive to the first TST (i.e. ≥10 mm in unvaccinated children and ≥15 mm in vaccinated children) and iii) children 6 years or older negative to the first TST but positive to the second TST (i.e. those that converted their TST result). Patients 134, 135, 284, 472, 510 and 573 had completed their regimen prior to developing active tuberculosis.

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Table 2

Clinical manifestations of all incident tuberculosis cases

Patient number	Culture sample	Culture result	Symptoms suggestive of TB[A]	Physical signs consistent with TB[B]	Chest radiography supportive of TB[C]	Chest CT supportive of TB[D]	Site of disease	# months between recruitment and diagnosis	Clinical & radiographic response to therapy
66	Gastric	Negative	Yes	No	Yes	Yes	Pulmonary	5	Yes
134[A,B]	Gastric	Positive	No	Yes	Yes	Yes	Pulmonary	28	Yes
135[A,B]	Gastric	Negative	Yes	Yes	Yes	Yes	Pulmonary	28	Yes
138[A,B]	Skin lesion	Positive	Yes	Yes	Yes	Yes	Miliary	18	Yes
174	Gastric	Negative	No	No	Yes	Yes	Pulmonary	6	Yes
176	Gastric	Negative	Yes	Yes	Yes	Yes	Miliary	3	Yes
215[A]	Gastric	Negative	Yes	No	Yes	Yes	Pulmonary	5	Yes
218[A]	Gastric	Negative	Yes	No	Yes	Yes	Pulmonary	17	Yes
284	Gastric	Negative	Yes	No	Yes	-	Pulmonary	7	Yes
290	Gastric	Negative	Yes	No	Yes	Yes	Pulmonary	7	Yes
472	BAL	Positive	Yes	No	Yes	Yes	Pulmonary	14	Yes
510	Gastric	Negative	Yes	Yes	No	Yes	Pulmonary	3	Yes
573	BAL	Negative	Yes	Yes	Yes	Yes	Pulmonary	12	Yes
608	Gastric	Negative	Yes	No	Yes	Yes	Pulmonary	24	Yes
874	Gastric	Negative	Yes	No	Yes	Yes	Pulmonary	18	Yes

TB = tuberculosis, CT = computed tomography, BAL = bronchoalveolar lavage

[A]Symptoms included cough for more than 7 days, chest pain, wheezing, pyrexia for more than 7 days, night sweats, malaise and weight loss

[B]Physical signs included chest crackles, decrease in chest sounds, ralles, erythematous nodular skin abscessess, prolonged chest sounds and wheezing

[C]Abnormal chest radiography findings consistent with a diagnosis of active tuberculosis included lung consolidation, cavitation, mediastinal enlargement, linear fibrotic opacities, para-cardiac infiltration, para-hilar infiltration, hilar enlargement, fibronodular infiltration, blunting of the phrenic sinus, and pleural effusion

[D]Abnormal chest CT scan findings consistent with a diagnosis of active tuberculosis included lung consolidation, mediastinal lymphadenopathy, cavitation, calcified nodules, miliary nodules, characteristic lung infiltration and pleural effusion. Patient 284 did not have a CT scan.

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Table 3

Incidence rates of tuberculosis and incidence rate ratios among child contacts, by ELISpot and TST result at recruitment

	ELISpot						TST	
Test	ESAT-6/CFP-10		PPD		SKSD[B]		5 mm threshold[C]	
Test result	positive	negative	positive	negative	positive	negative	positive	negative
N	381	527	658	250	588	319	550	358
TB incident cases, n	11	4	11	4	10	5	12	3
Person years at risk	536	664	875	326	759	441	722	478
Unadjusted analysis								
Incidence rate per 1000 person years (95% CI)	20.5 (10.2, 36.7)	6.0 (1.6, 15.4)	12.6 (6.3, 22.5)	12.3 (3.3, 31.4)	13.2 (6.3, 24.2)	11.3 (3.7, 26.5)	16.6 (8.6, 29.0)	6.3 (1.3, 18.3)
Incidence rate ratio (95% CI)	3.41 (1.08, 10.70)		1.03 (0.33, 3.22)		1.16 (0.40, 3.40)		2.65 (0.75, 9.39)	
P value	0.036		0.97		0.78		0.13	
Adjusted analysis[A]								
Incidence rate ratio (95% CI)	3.86 (1.19, 12.5)		1.09 (0.34, 3.56)		1.14 (0.39, 3.35)		3.28 (0.87, 12.4)	
P value	0.024		0.88		0.81		0.080	

ELISpot = enzyme linked immunospot, TST = tuberculin skin test, PPD = purified protein derivative, SKSD = streptokinase streptodomase, TB = tuberculosis, CI = confidence interval

[A] Adjusted for isoniazid preventive therapy given to contacts exposed to drug sensitive tuberculosis

[B] Streptokinase streptodornase (SKSD), a non-tuberculosis control antigen

[C] TST response was scored positive if the induration diameter was ≥5 mm in accordance with American Thoracic Society and Center for Disease Control and Prevention guidelines.