LTBI Diagnosis: Advances and Prospects

Madhukar Pai, MD, PhD
McGill University, Montreal
madhukar.pai@mcgill.ca
Why focus on LTBI diagnosis?

- Global Plan to Stop TB: by 2012, a test that will accurately identify people with LTBI and those at high risk of progression to active disease
- As active TB case rates decrease over time, LTBI Dx and Rx will become important to eliminate TB
- Even in resource-limited settings, high-risk populations may benefit from IPT (immunocompromised, children, and contacts)
- New LTBI tests are giving us a fresh perspective on LTBI, a poorly understood entity shrouded in fuzzy terminology!
How many ways to say TB infection???

- Latent infection
- Active infection
- Inactive infection
- Subclinical infection
- Acute infection
- Chronic infection
- Persistent infection
- Dormant infection
- Recent infection
- Remote infection
- Quiescent infection
- Incipient disease
Advances in Latent TB diagnosis

- Improving the interpretation of TST
- Improving the TST reagent
- Replacing the TST with in-vitro assays (IGRAs)
Tuberculin skin test (TST)

- **TST**
  - Measures cell-mediated immune response (CMI)
    - Uses PPD: a crude antigenic mixture

- **Limitations of TST:**
  - fairly high proportion of false positives and false negatives
  - technical problems in administration and interpretation
  - difficulty in separating true infection from the effects of BCG and non-tuberculous mycobacteria (NTM)
  - repeated TST boosts the immune response
  - requires a 3-dimensional interpretation
Effect of BCG on TST results

False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria?

M. Farhat,‡ C. Greenaway,‡ M. Pai,*§ D. Menzies*

* Respiratory Epidemiology and Clinical Research Unit, Montreal Chest Institute, McGill University, Montreal, Quebec, Canada; † Massachusetts General Hospital, Harvard University, Boston, Massachusetts, USA; ‡ Division of Infectious Disease and Microbiology, SMBD-Jewish General Hospital, McGill University, Montreal, § Joint Departments of Epidemiology & Biostatistics and Occupational Health, McGill University, Montreal, Quebec, Canada

- Analysis of 24 studies with N = 240,243 subjects
- When BCG is given in infancy, false-positive TST results due to BCG occur in 6% of vaccinated subjects
- When BCG is given after infancy, false-positive TST results due to BCG occur in 40% of vaccinated subjects

Currently the atlas includes information for over 140 countries from around the world. We have endeavoured to collect data on each country's current and past Bacille Calmette-Guerin (BCG) vaccination policies and practices.

As you know, variations in BCG vaccination practices impact the interpretation of TB diagnostics, such as the widely used Tuberculin Skin Test (TST). The World Atlas of BCG Policies and Practices will help clinicians in your country and around the world make better diagnostic decisions concerning TB infection. We have made the data available, for use as a searchable online tool for physicians and researchers alike.

If information for your country is missing, we encourage you to complete a very short questionnaire (should take only about 5 minutes to complete) concerning your country's BCG vaccination policy. The questionnaire is available on the website as a word form document. Please take the time to complete the questionnaire and contribute to the creation of a valuable resource for physicians and patients in your country.

Country: India
Codes: IND
Region: South Asia
Income group (World Bank): Low income
Category: A, B or C: A
First BCG who: birth
Second BCG who:
Third BCG who:
Fourth BCG who:
Current BCG vaccination?: Yes
Q2: A, B, C: A
Which year was vaccination introduced?: 1948
Year BCG stopped: N/A
Age of 1st BCG?: At birth
Multiple BCG?: No
Age BCG #2: N/A
Age BCG #3: N/A
Age of BCG #4: N/A
Multiple BCG in the past?: No
Age past BCG #2: N/A
Age past BCG #3: N/A
Year booster BCG stopped: N/A
BCG strain: BCGWL, Chennai strain, BCG Laboratory Guindy, Chennai, India
TST done post BCG?: No
BCG coverage year: 2006
BCG coverage: 99
Year of changes: 1948: BCG intro as pilot project, 1949: Immunization program in schools, 51-59 Mass immunization campaigns
Explain changes: 1978: extended program of immunization to be given at birth or within 1st mo, 1985: universal immunization program BCG vaccine policy continued as earlier
Special groups: No
Explain: N/A
Summary: A
Thinking in three dimensions: a web-based algorithm to aid the interpretation of tuberculin skin test results

D. Menzies,* G. Gardiner,** M. Farhat,** C. Greenaway,**‡ M. Pai‡

* Respiratory Epidemiology and Clinical Research Unit, Montreal Chest Institute, McGill University, Montreal,
** Massachusetts General Hospital, Harvard University, Boston, Massachusetts, USA; ‡ Division of Infectious Di
Microbiology, Sir Mortimer B Davis Jewish General Hospital, McGill University, Montreal, ‡ Department of Epic
and Biostatistics, McGill University, Montreal, Canada

Thinking in three dimensions:
An algorithm to aid interpretation of the tuberculin skin test
(Version 1.0 January 19, 2006)
Initial design: Maha Farhat, MD; Christina Greenaway, MD and Dick Menzies, MD;
Revisions and updates Dick Menzies, MD and Madhukar Pai MDPhD
Programming: Irena Sesartic

The following tool estimates the risk of active tuberculosis for an individual with a tuberculin skin test reaction of 10-mm, based on his/her clinical profile. It is intended for adults tested with standard tuberculin (5 TU PPDS, or 2 TU RT-23). Prevalence of tuberculosis infection is derived using the Stefano formula and incidence of smear positive TB in the country of origin (from WHO). The effects of NTM and BCG on TST positivity were compiled from a literature review as were the relative risks of various health conditions. For further information see references, or contact the authors.

Select:
1. TST reaction size:
2. Age: Age at immigration if applicable:
3. Country of birth:
4. BCG status:
5. Contact with active TB:
6. Please select all the conditions that currently apply to the patient:

http://meakins.mcgill.ca/respepi/homeE.htm
ESAT-6/CFP10 Skin Test Predicts Disease in *M. tuberculosis*-Infected Guinea Pigs

Karin Weldingh*, Peter Andersen
Department of Infectious Disease Immunology, Statens Serum Institut, Copenhagen, Denmark

Abstract

**Background:** Targeted preventive chemotherapy of individuals with progressive subclinical (incipient) disease before it becomes contagious would break the chain of tuberculosis transmission in high endemic regions. We have studied the ability of a skin test response to ESAT-6 and CFP10 (E6/C10) to predict later development of tuberculosis disease in the guinea pig model.

**Methods and Findings:** Guinea pigs, either vaccinated with BCG or unvaccinated, were infected with a low dose of *Mycobacterium tuberculosis* by the aerosol route and the development of delayed type hypersensitivity responses to E6/C10 and to purified protein derivative (PPD) were followed until the onset of clinical disease. We demonstrated a negative correlation between the size of the skin test response and the time to the onset of clinical disease; a large E6/C10 skin test response correlated to a shorter survival time post skin testing, while a small E6/C10 skin test reaction correlated with a longer survival time (r = -0.6 and P<0.0001). No correlation was found using PPD.

**Conclusions:** Our data suggest that it may be possible to develop a prognostic skin test based on E6/C10 that will allow the identification of individuals with incipient disease, who have the highest risk of developing active tuberculosis in the near future.

Safety of ESAT-6

Henrik Aggerbecka,*, Søren M. Madsenb

Tuberculosis (2006) 86, 363–373

http://intl.elsevierhealth.com/journals/tube
Improved rdESAT-6 skin test

Recombinant early secreted antigen target 6 protein as a skin test antigen for the specific detection of *Mycobacterium tuberculosis* infection

Summary

Although the delayed-type hypersensitivity skin test reaction to tuberculin purified protein derivative (PPD) is used worldwide for tuberculosis (TB) detection, it is incapable of distinguishing *Mycobacterium tuberculosis* (MTB) infection from bacille Calmette–Guérin (BCG) vaccination or infection with non-tuberculous Mycobacteria. As a result, there is an urgent need for a more specific diagnostic tool for TB. This study reports the skin reactions of guinea pigs and human volunteers to recombinant early secreted antigen target 6 (rdESAT6), a secretory protein found only in MTB, *M. bovis* and few other mycobacterial species. These volunteers had varying histories of BCG vaccination and exposure to MTB, allowing us to determine the specificity of their response to TB exposure. Our results show that 10 μg of the purified MTB rdESAT6 antigen elicited a positive skin response in both animals and humans exposed to MTB, as well as in animals exposed to *M. bovis* and *M. marinum*, all species of Mycobacteria that contain the gene for early secreted antigen target 6 (ESAT6). ESAT6 appears to be more specific to MTB infection than PPD, as demonstrated by the fact that we saw no skin responses in the BCG-vaccinated volunteers, nor in the guinea pigs sensitized with BCG vaccine, or with Mycobacteria that do not contain the gene encoding ESAT6. We believe that this is the first report of the use of a rdESAT6 protein in a skin test in human volunteers, and that these data support its use in the specific detection of MTB infection.

Double-blind randomized Phase I study comparing rdESAT-6 to tuberculin as skin test reagent in the diagnosis of tuberculosis infection

Sandra M. Arend, Willeke P.J. Franken, Henrik Aggerbeck, Corine Prins, Jaap T. van Dissel, Birgit Thierry-Carstensen, Pernille Nyholm Tingskov, Karin Weling, Peter Andersen.
Interferon-gamma release assays (IGRA)

T-SPOT.TB® [Oxford Immunotec, UK]

QuantiFERON-TB Gold® In Tube [Cellestis Ltd, Australia]
**Meta-analyses on IGRAs**

**Article**


Dobis Menares, MD, MSc; Madhukar Pai, MD, PhD; and George Comerford, MD, MPH

**Background:** Until recently, the tuberculin skin test was the only test for detecting latent tuberculosis (TB) infection, but it is non-specific. Interferon-gamma release assays (IGRAs) are now commercially licensed.

**Purpose:** To estimate sensitivity, specificity, and reproducibility of IGRAa commercial or research versions of Quantiferon Gold In-Tube (QFT-GIT) and Enzyme-linked Immunosorbent Assay (ELISPOT) for diagnosing latent TB infection in healthy and immunosuppressed persons.

**Data Sources:** The authors searched MEDLINE and reviewed citations of all original articles and reviews for studies published in English.

**Study Selection:** Studies that evaluated IGRAa using Mycobacterium tuberculosis-specific antigens in adults and overweight (≥10% or ≥64k) participants, or those with compromised immune status, were included.

**Conclusions:** Most studies used cross-sectional designs with the inherent limitations of no control group for latent TB infection, and most involved small samples with a wide range of specificity and false-positive test results. There is insufficient evidence on IGRAa performance in children, immunocompromised persons, and the elderly.

**Article**

**Systematic Review: T-Cell–Based Assays for the Diagnosis of Latent Tuberculosis Infection: An Update**

Madhukar Pai, MD, PhD; Alice Zwartling, MSc; and Dobis Menares, MD, MSc

**Background:** Interferon-gamma release assays (IGRAa) are alternatives to the tuberculin skin test (TST). A recent meta-analysis showed that IGRAa have high specificity, even among populations that have received BCG vaccination. Sensitivity was substantial for TST and IGRAa.

**Purpose:** To incorporate new evidence into an updated meta-analysis on the sensitivity and specificity of IGRAa.

**Data Sources:** PubMed was searched through 31 March 2008, and citations of all original articles, guidelines, and reviews for studies published in English were reviewed.

**Study Selection:** Studies that evaluated Quantiferon Gold, Quantiferon-TB Gold In-Tube (both from Cellestis, Victoria, Australia), and T-SPOT.TB (Oxford Immunotec, Oxford, United Kingdom) or its commercial version (T-SPOT version), were included. Studies that evaluated IGRAa in children or in populations with coinfection with HIV were excluded.

**Conclusions:** IGRAa are robust tests for diagnosing latent TB infection in adults, but performance in children, immuno-compromised persons, and the elderly is not well-studied.
Summary of Evidence

- TST specificity is high in BCG non-vaccinated; but low and variable in BCG vaccinated
- IGRAs (especially QFT) have very high specificity
  - IGRA specificity is higher than TST
  - IGRAs are not affected by BCG vaccination
    - Maybe very helpful in settings that give BCG after infancy or give multiple vaccinations
- Sensitivity of IGRAs and TST is not consistent across tests and populations
  - QFT is as sensitive as TST
  - QFT sensitivity is significantly higher in low incidence than high incidence countries
  - T-SPOT.TB appears to be more sensitive than both QuantiFERON tests and TST
    - Maybe helpful in evaluation of immunocompromised
- In low-incidence settings, IGRAs correlate well with markers of exposure
Summary of Evidence

- Diagnosis of active TB rests on microbiological detection of *M. tuberculosis*.
- Immune-based tests, such as IGRAs and TST, do not directly detect *M. tuberculosis*; they merely indicate a cellular immune response to recent or remote sensitization with *M. tuberculosis*.
- Because IGRAs cannot distinguish between LTBI and active TB, a positive IGRA result may not necessarily indicate active TB.
- Furthermore, a negative IGRA result would not conclusively rule out active disease in an individual suspected to have TB; this also applies to the TST.
Limitations of current evidence

- Almost all the available studies on IGRAs have limitations, namely lack of a gold standard for LTBI, cross-sectional design, use of sensitivity and specificity as surrogates for patient-important outcomes, and lack of adequate data on important outcomes such as accuracy of diagnostic algorithms (rather than single tests), incremental or added value of IGRAs, impact of IGRAs on clinical decision-making and therapeutic choices, and the prognostic ability of IGRAs.

- Thus, available evidence on IGRAs cannot be considered high quality, and further research is likely to have an important impact on current recommendations and guidelines.

- Ongoing studies should resolve these issues within the next few years and inform evidence-based guidelines on how to implement IGRAs in clinical practice.
Can IGRAs be improved?

- Inclusion of new antigens
- Measure additional cytokines/chemokines
- Include other biomarkers

Improved Diagnostic Evaluation of Suspected Tuberculosis

Davinder P.S. Desanp, DPhil; Timothy S.C. Hinks, MB; John A. Irwin, MD; Jonathan J. Dewks, PhD; Geoffrey Paxev, DPhil; Sarah Huckleberry, RGN; Himanshu Behari, RGN; Helen A. Millington, DPhil; Rubamator Gunathilas, MD; Valerie Cauty-Revel, PhD; and Ajit Labhani, MD

Background: The role of new T-cell-based blood tests for tuberculosis in the diagnosis of active tuberculosis is unclear.

Objective: To compare the performance of 2 interferon-γ assays and tuberculin skin testing in adults with suspected tuberculosis.

Design: Prospective study conducted in routine practice.

Setting: 2 urban hospitals in the United Kingdom.

Patients: 389 adults, predominantly of South Asian and Black ethnicity, with moderate to high clinical suspicion of active tuberculosis.

Intervention: Tuberculin skin testing, the enzyme-linked immunospot assay (ELSpot), and IFN-γ/IL-10 ratio.

Conclusion: The ELSpot assay was more sensitive than tuberculin skin testing with 15-mm cutoff points (P = 0.039) but not with stratified cutoff points (P = 0.10). The IFN-γ/IL-10 ratio had 4% higher diagnostic sensitivity than standard ELSpot (P = 0.02). Combined sensitivity of ELSpot and tuberculin skin testing was 99% (95% CI, 91% to 100%), confirming a negative likelihood ratio of 0.02 (95% CI, 0.00% to 0.00%) when both test results were negative.

Heparin-Binding-Hemagglutinin-Induced IFN-γ Release as a Diagnostic Tool for Latent Tuberculosis

Jean-Michel Houngbo, MD, Hinda Schepers, MD, Sammy Place, MD, Annie Bruneau, MD, Vincent Leclercq, MD, Virginie Verschueren, MD, Anne-Sophie Debije, MD, T. Mark Doherty, MD, Jean-Paul Van Vroozen, MD, Camille Lochu, MD, and Françoise Muscat, PhD

Accuracy of an immune diagnostic assay based on RD1 selected epitopes for active tuberculosis in a clinical setting: a pilot study

D. Gokkenz, MD, S. carnava, MD, D. Vinci, MD, C. Sultani, MD, E. Busi Rizzati, MD, F. Schillaci, MD, G. Ippoliti, MD, M. Amicosante, MD, and E. Girardi

Longitudinal Tracking of Cytokines after Acute Exposure to Tuberculosis: Association of Distinct Cytokine Patterns with Protection and Disease Development

Rabia Hussain, MD, Najeeha Talat, MD, Firdaus Shahid, MD, and Ghaffar Dawood, MD

IFN-γ/IL-10 ratio

Improving T-Cell Assays for the Diagnosis of Latent TB Infection: Potential of a Diagnostic Test Based on IP-10

Morten Ruhwald, MD, J. P. Petersen, MD, K. Kristian Kofod, MD, Hiroshi Nakaoka, MD, E. Eduardo Cuevas, MD, Lovett Lawson, MD, Stephen Bertil Squire, MD, J. Jesper Eugen-Olsen, MD, Pernille Ravn, MD
Immune-based biomarkers of latent TB

**M. tuberculosis**

- **Microbial factors**: Strain of *M. tuberculosis*, genotype, clade, virulence, etc.
- **Host factors**: Age, gender, nutrition, comorbid conditions, HIV, energy, genetic factors (ethnicity, polymorphisms, etc.), BCG status (BCG strain, timing, frequency of vaccination), environmental exposure (non-tuberculous mycobacteria, helminthic infections, etc.), immunosuppressive medications

**Human host**

- **Exposure factors**: TB exposure (nature, duration, frequency, intensity of contact), previous exposure
- **Disease factors**: Latent infection vs. active TB, previous disease, past latent or active TB treatment (type and duration), disease severity (cavities, smear-grade, dissemination, radiological extent), disease site (pulmonary vs. extra-pulmonary)

**Immune response to M. tuberculosis**

- **Innate immunity**
- **Adaptive immunity**

**Cellular immune response**: T-cell mediated immune response (measured using T-cell stimulation cytokine assays)

**Specimen**: Peripheral blood mononuclear cells (PBMC), whole-blood, others (e.g., broncho-alveolar lavage fluid)

**Types of cytokines**: IFN-γ, TNF-α, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18, etc.

**Measurement of cytokine response**: Skin test (e.g., Mantoux), ELISA, ELISPOT, Quantitative PCR, multiplex fluorescent bead assay, intracellular cytokine staining, etc.

**Incubation**: Short (e.g., 18 - 24 hours) to long incubation (e.g., 5 - 7 days)

**Antigens**: Non-specific (PPD M. tuberculosis, culture-filtrate proteins, BCG); Specific - RD1 (ESAT-6, CFP-10, Rv3873, etc.), non-RD1 (MPT64, MPB70, etc.); Antigen source: peptides vs. proteins, natural vs. recombinant, combinations of peptides, antigen contamination
The search for biomarkers continues…

Tuberculosis in Africa: Learning from Pathogenesis for Biomarker Identification

Stefan Kiehnert and Siegfrieda K. Pasiba
Max Planck Institute for Infection Biology, Department of Immunology, Charitéplatz 1, D-10117 Berlin, Germany
Correspondence: kiehnert@mpibi.mpg.de
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Stay tuned for more updates…

http://www.igrasymposium.com/
Disclosure of conflicts

- No financial conflicts
  - No stocks, no advisory boards, no speaker fees, no funds for research

- I consult for Foundation for Innovative New Diagnostics, a non-profit agency
  - FIND partners with several industries to develop new diagnostics for neglected diseases