New tests and biomarkers for latent tuberculosis infection

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Disclosure of conflicts

• No financial conflicts

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

• I co-chair the Stop TB Partnership’s New Diagnostics Working Group (NDWG)
For a century, we have defined LTBI using the 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
Models of the TB spectrum

Figure 2. A model for tuberculosis epidemiology, following the pathogenesis of tuberculosis. Figure reproduced with the permission of Urban & Vogel from [2].

Hans L. Rieder, 1999
Models of the TB spectrum

- M. tuberculosis
- Aerosol infection
- Environment-adapted gene program
- Host-adapted gene program
- Dormancy gene program
- DocR
- Dormancy program
- Resuscitation gene program
- Metabolically active gene program
- Extracellular replication (10^8 organisms)

- Granuloma
- Productive granuloma
- Latency
- Reactivation

- Innate control (Hypoxia)
- Efficient control
- Continuum or distinct stages?
  - Dormancy
  - Resuscitation
  - Reactivation
  - Extracellular growth

- Draining lymph node
- Ag-specific effector T cells
  - CD4, CD8
  - Th1, Th2
  - IL-12, IFN-γ, IL-4, IL-10

- Memory T cells
- CD45RO
- Central

- Regional control (?)
- Immunoregulation (?)

- Immune control suppressed (?)
- Caseous lesion (pO2)

- Regional immune control impaired (?)

- Treg CD4^+ CD25^+ Foxp3^+
- Th3, Th1
- Th2 ≠ Th1
- IL-4, IL-10, TGF-β, IL-12, IFN-γ

Kauffman S. TRENDS in Immunology Vol.26 No.12 December 2005
Poor understanding of the TB spectrum contributes to fuzzy terminology

- Latent infection
- Active infection
- Inactive infection
- Subclinical infection
- Acute infection
- Chronic infection
- Persistent infection
- Dormant infection
- Recent infection
- Remote infection
- Quiescent infection
- Incipient disease
What exactly does the TST detect?

LTBI: latent tuberculosis infection or lasting immune responses to *M. tuberculosis*? A TBNET consensus statement


ERJ 2009

“Latency, as assayed by the tuberculin skin test and IGRA, is a state of persistent mycobacteria-specific T-cell responses in the absence of clinical evidence for tuberculosis disease”

TST and IGRA measure “lasting TB immune responses” and not “latent TB infection”
TST can be positive in all states except the first

IGRAs are likely to be positive in all states as well? Or spontaneous convert/revert?
Advances in Latent TB diagnosis

- Improving the interpretation of TST
- Improving the TST reagent
- Replacing the TST with in-vitro assays (IGRAs)
TST requires a 3-dimensional interpretation

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, Canada; † Massachusetts General Hospital, Harvard University, Boston, Massachusetts, USA; ‡ Division of Infectious Disease and Microbiology, Sir Mortimer B. Davis Jewish General Hospital, McGill University, Montreal, † Department of Epidemiology and Biostatistics, McGill University, Montreal, Canada

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 Syphilis 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:
   10-14 mm

2. Age:
   0

3. Country of birth:
   - If Country of birth is the USA:
     - Alabama

4. BCG status:
   - Never vaccinated or unknown
   - Vaccinated age < 2 years
   - Vaccinated age >= 2 years

5. Contact with active TB:
   - None
   - Close Contact
   - Casual

6. Please select all the conditions that currently apply to the patient:
   - Diabetes Mellitus
   - Chronic renal failure hemodialysis

http://meakins.mcgill.ca/respepi/homeE.htm
When BCG is given after infancy or repeated many times, it can affect TST results

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

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.

Please select a Country from the drop down box, or use the map to select a country to view all available information concerning that country’s BCG policies and practices.
In India, BCG has limited effect on TST

<table>
<thead>
<tr>
<th>Country</th>
<th>India</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
<td>South Asia</td>
</tr>
<tr>
<td>More than 100 cases of TB per year</td>
<td>1</td>
</tr>
<tr>
<td>TB Prevalance per 100,000</td>
<td>299</td>
</tr>
<tr>
<td>Number of TB cases per year</td>
<td>3444585</td>
</tr>
<tr>
<td>Income group (World Bank)</td>
<td>Low income</td>
</tr>
<tr>
<td>Current BCG vaccination?</td>
<td>Yes</td>
</tr>
<tr>
<td>A, B, or C?</td>
<td>A</td>
</tr>
<tr>
<td>Which year was vaccination introduced?</td>
<td>1948</td>
</tr>
<tr>
<td>Year BCG stopped?</td>
<td>N/A</td>
</tr>
<tr>
<td>Timing of 1st BCG?</td>
<td>At birth</td>
</tr>
<tr>
<td>Multiple BCG?</td>
<td>No</td>
</tr>
<tr>
<td>Timing of BCG #2</td>
<td>N/A</td>
</tr>
<tr>
<td>Timing of BCG #3</td>
<td>N/A</td>
</tr>
<tr>
<td>Multiple BCG in the past?</td>
<td>No</td>
</tr>
<tr>
<td>Timing of old BCG #2</td>
<td>N/A</td>
</tr>
<tr>
<td>Timing of old BCG #3</td>
<td>N/A</td>
</tr>
<tr>
<td>Year booster BCG stopped</td>
<td>N/A</td>
</tr>
<tr>
<td>BCG Strain</td>
<td>BCGV1 Chennai strain, BCG laboratory Guindy, Chennai, India</td>
</tr>
<tr>
<td>Is TST done post BCG?</td>
<td>No</td>
</tr>
<tr>
<td>Year of BCG coverage estimate</td>
<td>2006</td>
</tr>
<tr>
<td>BCG coverage (%)</td>
<td>99</td>
</tr>
</tbody>
</table>
In Uganda, BCG has limited effect on TST
In Japan, BCG has a major effect on TST
In Ukraine, BCG has a major effect on TST
**ABSTRACT**

Highly specific IGRA may be used to assess vaccination status in countries with high rates of TB. The authors analyzed the prevalence of IGRA in countries with high rates of TB and found that many countries have not implemented effective IGRA policies. The study recommends that countries implement IGRA policies to improve the accuracy of vaccination status determination.

**RESULTS**

The study found that many countries have not implemented effective IGRA policies. The authors recommend that countries implement IGRA policies to improve the accuracy of vaccination status determination.

**TABLE 1: Countries that currently recommend multiple BCG doses for children.**

<table>
<thead>
<tr>
<th>Country</th>
<th>Current BCG Vaccination</th>
<th>Age of 1st BCG</th>
<th>Timing of 2nd BCG</th>
<th>Timing of 3rd BCG</th>
<th>Year BCG was approved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vietnam</td>
<td>No</td>
<td>6 months</td>
<td>N/A</td>
<td>N/A</td>
<td>1957</td>
</tr>
<tr>
<td>China</td>
<td>No</td>
<td>6 months</td>
<td>N/A</td>
<td>N/A</td>
<td>1957</td>
</tr>
<tr>
<td>Colombia</td>
<td>3 doses at birth</td>
<td>6 months</td>
<td>N/A</td>
<td>N/A</td>
<td>1957</td>
</tr>
<tr>
<td>Mexico</td>
<td>3 doses at birth</td>
<td>6 months</td>
<td>N/A</td>
<td>N/A</td>
<td>1957</td>
</tr>
</tbody>
</table>

**TABLE 2: Countries that currently recommend IGRA testing.**

<table>
<thead>
<tr>
<th>Country</th>
<th>IGRA Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>Yes</td>
</tr>
<tr>
<td>Canada</td>
<td>Yes</td>
</tr>
<tr>
<td>UK</td>
<td>Yes</td>
</tr>
<tr>
<td>Australia</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**KEY FINDINGS**

- Many countries continue to use BCG vaccination, even in childhood, despite the increasing use of IGRA for vaccination status determination.
- IGRA have been shown to have higher specificity and sensitivity in distinguishing BCG vaccinated from non-vaccinated individuals.
- IGRA are recommended by the World Health Organization for the assessment of vaccination status, especially in countries with high rates of TB.
- IGRA may be a more useful alternative to IGRA in countries that currently or recently use multiple BCG doses per population.
Advances in the development of antigens specific to *M. tuberculosis*

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.

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Safety of ESAT-6

Henrik Aggerbecka,*, Søren M. Madsenb
Improved rdESAT-6 skin test

Tuberculosis 2008

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

X. Wu, L. Zhang, J. Zhang, C. Zhang, L. Zhu and Y. Shi
Institute for Tuberculosis Research, the Second Affiliated Hospital of Chinese PLA General Hospital, Beijing, China

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–Guerin (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 (rESAT6), 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 1 μg of the purified MTB rESAT6 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 rESAT6 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 Dessel, Birgit Thierry-Carstensen, Pernille Nyholm Tingskov, Karin Welding, Peter Andersen
Improved skin test: Diaskintest®

Masterpharm, Russia
IGRAs

T-SPOT. TB® [Oxford Immunotec, UK]

QuantiFERON-TB Gold® In Tube [Cellestis Ltd, Australia]
Annals of Internal Medicine


Drik Hennessy, MD, MSc; Madhukar Pai, MD, PhD; and George Cornick, MD, DPhil

Background: Until recently, the tuberculin skin test was the only test for detecting latent tuberculosis (TB) infection, but 2 in vivo interferon-γ-release assays (IGRAs) are now commercially licensed.

Purpose: To estimate sensitivity, specificity, and reproducibility of IGRA (commercial or research versions of Quantiferon Gold or T-SPOT.TB) for diagnosing latent TB in healthy and immune-suppressed persons.

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

Study Selection: Studies evaluated IGRA using Mycobacterium tuberculosis-specific antigens (RD1 antigens) and overnight (16- to 24-h) incubation times. Reference standards had to be clearly defined without knowledge of test results.

Data Extraction and Quality Assessment: Specific criteria for quality assessment were developed for sensitivity, specificity, and reproducibility.

Data Synthesis: When newly diagnosed active TB was used as a surrogate for latent TB infection, sensitivity of all tests was suboptimal, although it was higher with T-SPOT. No test distinguishes active TB from latent TB. Sensitivity of the tuberculin skin test and IGRA was similar in persons who were categorized into clinical gradients of exposure. Pooled specificity was 97.7% (96% CI, 96% to 99%) and 92.9% (96% CI, 86% to 99%) for QFT and for T-SPOT, respectively. Both assays were more specific than the tuberculin skin test in 3 studies of immunocompromised patients. Discordant tuberculin skin test and IGRA reactions were frequent and largely unexplained, although some may be related to varied definitions of positive test results. Resolution of IGRA results from positive to negative was common in 2 studies in which it was assessed.

Limitations: Most studies used cross-sectional designs with the inherent limitations of no gold standard for latent TB infection, and most involved small samples with a widely varying likelihood of true-positive and false-positive test results. There is insufficient evidence on IGRA performance in children, immunocompromised persons, and the elderly.

Conclusions: New IGRA [ interferon-γ-release assays (IGRAs) are new tests for the diagnosis of latent tuberculosis (TB)].

Article reviewed: 2001;146:249-254

Annals of Internal Medicine

Review


Madhukar Pai, MD, PhD; Alice Zuerling, MSc; and Drik Hennessy, MD, MSc

Background: Interferon-γ-release assays (IGRAs) are alternatives to the tuberculin skin test (TST). A recent meta-analysis showed that IGRA have high specificity, even among populations that have received bacille Calmette-Guérin (BCG) vaccination. Sensitivity was substantially for IGRA and IGRA.

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

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 evaluated Quantiferon Gold,
Quantiferon Gold T-SPOT.TB (both from Cellestis, Victoria, Australia), and T-SPOT.TB (Oxford Immunotec, Oxford, United Kingdom) or its precommercial ELISPOT version, when data on the commercial version were lacking. For assessing sensitivity, the study sample had to have microbiologically confirmed active tuberculosis. For assessing specificity, the sample had to comprise healthy, low-risk individuals without known exposure to tuberculosis.

Data Extraction: One reviewer abstracted data on participant characteristics, test characteristics, and test performance from 36 studies; these data were double-checked by a second reviewer. The original investigations were contacted for additional information as needed.

Data Analysis: A fixed-effects meta-analysis with correction for overdispersion was done to pool data within specified subgroups. The pooled sensitivity was 78% (95% CI, 73% to 85%) for Quantiferon Gold, 74% (95% CI, 67% to 80%) for Quantiferon Gold T-SPOT.TB, Gold, and 92% (95% CI, 86% to 99%) for T-SPOT.TB.

Limitations: Most studies were small and had limitations, including no gold standard for diagnosing latent tuberculosis and variable TST methods and cutpoint values. Data on the specificity of the commercial T-SPOT.TB assay were limited.

Conclusion: The IGRA, especially Quantiferon Gold and Quantiferon Gold T-SPOT.TB, have excellent specificity that is unaffected by BCG vaccination. Tuberculin skin test specificity is high in non-BCG-vaccinated populations but low and variable in BCG-vaccinated populations. Sensitivity of IGRA and TST is not consistent across tests and populations, but T-SPOT.TB appears to be more sensitive than both Quantiferon tests and TST.

Quick 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 (>95%)
  - 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 (e.g. Japan)
- Sensitivity of IGRAs and TST is not consistent across tests and populations
  - QFT is as sensitive as TST (~80%)
  - 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
    - But this may partly be because of cut-offs used for T-SPOT vs QFT
- In low-incidence settings, IGRAs correlate well with markers of exposure
Variation in performance in high vs low endemic countries

T-cell interferon-γ release assays for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-burden settings

Keertan Dheda\textsuperscript{a,b,c}, Richard van Zyl Smit\textsuperscript{a}, Motasim Badri\textsuperscript{a} and Madhukar Pai\textsuperscript{d}

High incidence countries

<table>
<thead>
<tr>
<th>Study</th>
<th>Sensitivity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsiouris et al. 2008</td>
<td>0.65 (0.57–0.72)</td>
</tr>
<tr>
<td>Pai et al. 2007</td>
<td>0.73 (0.66–0.84)</td>
</tr>
<tr>
<td>Adetifa et al. 2007</td>
<td>0.64 (0.52–0.75)</td>
</tr>
<tr>
<td>Raby et al. 2008</td>
<td>0.74 (0.65–0.82)</td>
</tr>
</tbody>
</table>

Pooled sensitivity = 0.69 (0.64–0.73)
Chi-square = 3.94; df = 3 (P = 0.2683)
Inconsistency (I-square) = 23.8%

Low incidence countries

<table>
<thead>
<tr>
<th>Study</th>
<th>Sensitivity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominguez et al. 2008</td>
<td>0.79 (0.63–0.90)</td>
</tr>
<tr>
<td>Palazzo et al. 2008</td>
<td>0.82 (0.57–0.96)</td>
</tr>
<tr>
<td>Dstjan et al. 2007</td>
<td>0.93 (0.76–0.99)</td>
</tr>
<tr>
<td>Chee et al. 2008</td>
<td>0.83 (0.78–0.87)</td>
</tr>
<tr>
<td>Bertu et al. 2008</td>
<td>0.81 (0.68–0.91)</td>
</tr>
<tr>
<td>Harada et al. 2008</td>
<td>0.87 (0.70–0.93)</td>
</tr>
<tr>
<td>Ruhwald et al. 2008</td>
<td>0.81 (0.71–0.89)</td>
</tr>
</tbody>
</table>

Pooled sensitivity = 0.83 (0.80–0.86)
Chi-square = 4.37; df = 6 (P = 0.6270)
Inconsistency (I-square) = 0.0%
IGRAs in immunocompromised

• Immunocompromised groups are highly variable, and most studies are small:
  – All tests underperform in severely immunocompromised patients
    • Using both TST and IGRA might help increase sensitivity
  – Indeterminate IGRA results increase with level of immunosuppression (i.e. low CD4 counts)
  – T-SPOT.TB generally yields higher positivity rates than TST (this not always the case in QFT studies)
  – Very limited data on predictive value in immunocompromised
  – Utility as rule out test for active TB is not well established
What are the key unresolved issues?

• What is the predictive value of IGRAs for the development of active TB? Will this vary by high vs. low incidence setting?
• Will treatment of IGRA positive subjects reduce the future probability of active TB?
• What is the interpretation of IGRA conversions and reversions?
• Can IGRAs be used to rule out active TB diagnosis?
• What is the exact role of IGRAs in children and HIV+?
Serial IGRA testing shows highly dynamic patterns

These phenotypes are unlikely to have the same prognosis. Some are more likely to benefit from LTBI treatment than others.
Predictive value of IGRAs: promising but both tests may have only modest predictive abilities

Immune Responses to the Mycobacterium tuberculosis-Specific Antigen ESAT-6 Signal Subclinical Infection among Contacts of Tuberculosis Patients

T. Mark Doherty,1* Abebech Demissie,2 Joseph Oloba,3 Dowit Wolday,3 Sven Britton,4 Tewodros Egneke,2 Pernille Ravn,6 and Peter Andersen1
1Department of Tuberculosis Immunology, Statens Serum Institute, and Hvidovre Hospital, Copenhagen, Denmark; 2Armauer Hansen Research Institute; 3Black Lion Hospital; and 4Harrus Regional Hospital, Ministry of Health, Ethiopia; and Karolinska Institute, Stockholm, Sweden

High Incidence

Unpublished: S Africa, Senegal, Colombia

Low Incidence

Unpublished: Japan, Netherlands

Predictive Value of a Whole Blood IFN-γ Assay for the Development of Active Tuberculosis Disease after Recent Infection with Mycobacterium tuberculosis

Roland Dietl1, Robert Loddenkemper1, Karen Meywald-Walter1, Stefan Niemann1, and Albert Nienhaus1

1School of Public Health, University of Düsseldorf, Düsseldorf, Germany; 2German Central Committee against Tuberculosis, Lungenklinik Heeselfeld, HILUS, Klinikum Emil von Behring, Berlin, Germany; 3Public Health Department Hamburg-Mitte, Hamburg, Germany; 4National Reference Center for Mycobacteria, Research Center Borstel, Borstel, Germany; and 5Institute for Statutory Accident Insurance and Prevention in the Health and Welfare Services, Hamburg, Germany

Detection and Prediction of Active Tuberculosis Disease by a Whole-Blood Interferon-γ Release Assay in HIV-1–Infected Individuals

Maximilian C. Aichbaurg,1 Armin Rieger,1 Florian Breitenecper,1 Katharina Pfistershammer,1 Julia Tittos,1 Stefanie Eltz,1 Alexander C. Aichbaurg,7 Georg Stügl,1 Athanasios Makristathis,2 and Norbert Kohrgruber1,4

Incidence of Tuberculosis and the Predictive Value of ELISPOT and Mantoux Tests in Gambian Case Contacts


Bacterial Diseases Programme, Medical Research Council (MRC) Laboratories, Banjul, The Gambia

Annals of Internal Medicine

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ARTICLE

Prognostic Value of a T-Cell-Based, Interferon-γ Biomarker in Children with Tuberculosis Contact

Mustafa Bakir, MD, Kerry A. Millington, DPhil, Ahmet Soydas, MD, Jonathan J. Deeks, PhD, Serpel Efe, Yaseemin Aslan, SRN, Davinder P. Sohas, DPhil, and Aij Laivan, DMI
Performance and predictive value may differ between high vs low incidence settings

T-cell interferon-γ release assays for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-burden settings
Keertan Dheda*a,b,c, Richard van Zyl Smita, Motasim Badriα and Madhukar Paид

Purpose of review
The utility of T-cell interferon-γ (IFN-γ) responses to Mycobacterium tuberculosis specific antigens [interferon-γ release assays (IGRAs)] in high-burden settings remains unclear and there is growing evidence that IGRA performance varies across high tuberculosis (TB) burden vs. low TB burden settings. Here we review the evidence supporting the utility of IGRAs in specific subgroups and compare their performance in high-burden vs. low-burden settings.

Recent findings
Although the IGRA, compared with the tuberculin skin test (TST), has greater specificity in BCG-vaccinated individuals, treatment of latent tuberculosis infection is not a priority in high-burden setting. Nevertheless, in high-burden settings, the TST performs reasonably well and correlates as well, or better, with proxy measures of exposure.

Summary
IGRAs may still be useful in high-burden settings in specific subgroups at high risk of progression, including young children, HIV-infected individuals and healthcare workers, but this requires confirmation. Although the IGRAs cannot distinguish between latent and active TB, their utility as rule-out tests, when combined with smear microscopy or the TST, requires further study. Prospective studies are required in high-burden settings to confirm whether IFN-γ responses are predictive of high risk of progression to active TB, particularly in HIV-infected individuals.
Is gamma interferon adequate for protection/prediction?

Gamma interferon – key, but not sufficient for protection against TB?

*M. tuberculosis* is a classic intracellular pathogen – so macrophage activation by gamma interferon should be key to protection. But the picture may be more complicated, as Hazel Dockrell discusses.
Efforts to improve IGRAs

- Look for biosignatures based on multiple markers
- Inclusion of new antigens
- Measure additional cytokines/chemokines
- None are strong candidates at this time

Improved Diagnostic Evaluation of Suspected Tuberculosis

David D. Desouza, MD; Timothy S. C. Innes, MD; John A. Innes, MD; Jonathan J. Dekker, MD; Geoffrey Poxton, DPhil; Sarah Houghton, RGN; Huma Vakil, RGN; Kerry A. Millington, DPhil; Rukhannah Gunathilaka, MD; Valerie Cogot-Rouvi, PhD; and Ajit Labo, 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.

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

Interventions: Tuberculin skin testing, the enzyme-linked immunosorbent spot assay (ELISpot) incorporating early secretory antigens: target-6 and culture filtrate protein-10 (standard ELISpot), and ELISpot incorporating a novel antigen, rTB96 (ELISpot96) were performed during diagnostic assessment by independent persons who were blinded to results of the other test.

Results: Confirmed and highly probable tuberculosis was 88% (95% CI, 84% to 93%) with ELISpot96 and 85% (CI, 79% to 90%) with standard ELISpot. 79% CI, 72% to 85% with 15-mm threshold tuberculin skin testing, and 83% (CI, 77% to 89%) with stratified thresholds of 15 and 10 mm in vaccinated and unvaccinated patients, respectively. The ELISpot96 assay was more sensitive than tuberculin skin testing with 15-mm cutoff points (P < 0.001) but not with stratified cutoff points (P = 0.10). The ELISpot96 assay had a higher diagnostic sensitivity than standard ELISpot (P = 0.02). Combined sensitivity of ELISpot96 and tuberculin skin testing was 99% (CI, 95% to 100%), confirming a negative likelihood ratio of 0.02 (CI, 0 to 0.06) when both test results were negative.

Limitations: Local standards for tuberculin skin testing differed from those used internationally. The study sample included few immunosuppressed patients.

Conclusion: The ELISpot96 assay is more sensitive than standard

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

Jean-Michel Houngoubery, Hinda Schepers, Sammy Place, Apolin Drouant, Viviane Lecめる, Virginie Verschourew, Anne-Sophie Debé, T. Mark Doherty, Jean-Fred Van Varen, Camille Locht, and Françoise Mascart

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


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

Rabia Hussain, Najeebah Talat, Firdaus Shahid, and Ghaffar Dawood

IFN-g/IL-10 ratio
Humoral immune makers

- Antibody detection assays (serological tests) have largely failed
- They lack accuracy and discriminatory ability, especially in high burden settings
- Efforts are ongoing to improve them:
  - Seroprofiling the entire proteome
  - Antibody micro-arrays
  - Responses to a cocktail of antigens
  - More sensitive analytical methods
- No strong candidate yet
Asymmetric SROC
AUC = 0.8877
SE(AUC) = 0.0161
Q* = 0.9192
SE(Q*) = 0.0154

Figure 4. SROC Curve of Commercial Tests for the Diagnosis of Pulmonary TB

Figure 4. ROC curve of commercial rapid tests for the diagnosis of pulmonary tuberculosis (all patients, n=355)
Lack of discrimination in TB endemic settings

Figure 3. Dot-plot showing the optical density (OD) values obtained from 184 patients with active tuberculosis disease who resided in northern Tanzania (TZ-TB) and 32 healthy, bacille Calmette-Guérin–vaccinated, Danish resident volunteer donors with no known risk factors for tuberculosis (DK-BCG). The dotted line indicates the cutoff value, calculated as the mean OD + 3 SDs for the 32 healthy Danish resident volunteers.

Hoff et al. Clinical Infectious Diseases 2007; 45:575–82
Antigen/bacterial/DNA detection

- Antigen detection (e.g. LAM)
- DNA detection using NAATs, gene expression studies (transcriptomics)
- Detection of bacterial products or metabolites (proteomics, metabolomics, glycomics, and other ‘shotgun’ approaches)
  - Several exploratory initiatives are ongoing (e.g. GC6)

- Key issues:
  - Is the antigen/bacterial load in LTBI too low to be detected by these methods?
  - Will shotgun approaches identify the best biomarkers?
The search for biomarkers continues: lots of candidates have been identified, but…

Correlates of disease progression and prognosis during concurrent HIV/TB infection

Joel Fleury Djoba Sllawaya a, b, Morten Ruhwald c, Jesper Eugen-Olsen d, Gerhard Walzl e

Biomarkers for TB treatment response: Challenges and future strategies

Gerhard Walzl e a, Katharina Ronacher e, Joel Fleury Djoba Sllawaya b, Hazel M. Dockrell a

Biomarkers for Clinical and Incipient Tuberculosis: Performance in a TB-Endemic Country

Ajay Wanchal a, Yuxin Dong b, Sunil Sethi c, V. P. Myneedu d, Arthur Nadas e, Zhentong Liu a, John Belshe e, Suman Lal e
How close are we in differentiating infected from diseased in TB?

• Much work is being done, but no strong candidates yet
• Brute force, shotgun methods might provide some answers, but can also mislead
  – False positive results that are not replicated
  – Publication bias
  – Initially strong effects are often contradicted later
• There are lessons to be learnt from the genetic epi and genome-wide association studies!
Guidelines on IGRAs: A global survey

May 30 – June 1, 2009
Dubrovnik, Croatia
Results of the survey

• There are several countries that do not yet have an official guideline or statement on IGRAs; these include, for example:
  – China, India, Russia, Ukraine, Brazil, Belgium S Africa, Mexico, New Zealand, Finland, Ireland, Bulgaria, Croatia, Slovenia, Austria, Turkey, Viet Nam, Singapore, Portugal, Sweden, and Saudi Arabia.

• This does not mean IGRAs are not being used:
  – Some are using the tests (E.g. Singapore, Finland)
  – Some are developing guidelines (E.g. Finland, Saudi Arabia, Portugal)

• No high-burden, low-income country has published guidelines on IGRAs
  – But IGRAs are available in some high-burden countries (e.g. India, S Africa), and being used mostly in the private sector and in research settings
  – No NTP in a high burden, low-income country is using these assays
Results

• 17 countries that have at least one guidelines include:
  – USA, Canada, UK, Japan, France, Spain, Italy, Germany, Switzerland, Australia, Netherlands, Denmark, Czech Republic, Slovak Republic, Korea, Poland and Norway.

• Of the countries that have guidelines, 3 main approaches are discernable:
  – TST may be replaced by IGRA (i.e. only IGRA)
  – Either TST or IGRA may be used
  – Two-step approach of TST first, followed by IGRA

• Although the broad approach may fall into one of these, some guidelines recommend more than one approach, depending on the risk group tested
## Results*

<table>
<thead>
<tr>
<th>General testing approach</th>
<th>Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>TST may be replaced by IGRA (i.e. only IGRA is used)</td>
<td>Germany (anti-TNF-a), Swiss (anti-TNF-a), Poland (anti-TNF-a), Denmark (anti-TNF-a, BCG-vaccinated contacts/adults)</td>
</tr>
<tr>
<td>Either TST or IGRA may be used</td>
<td>USA (QFT preferred in BCG+), France, Australia (refugees), Japan (QFT preferred in all groups except in children &lt;5 y), Denmark (child contacts)</td>
</tr>
<tr>
<td>Two-step approach: TST first, followed by IGRA (either to improve specificity or sensitivity)</td>
<td>Canada, UK, Italy, Spain, Australia, Slovakia Germany (contacts), Swiss (contacts), Netherlands (contacts, immigrants), Norway, Korea (contacts)</td>
</tr>
</tbody>
</table>

* some guidelines recommend more than one approach, depending on the risk group tested (e.g. contacts, immunocompromised, children, etc)
North American Guidelines
CDC recommends that QFT-G may be used in all circumstances in which the TST is currently used, including contact investigations, evaluation of recent immigrants, and sequential-testing surveillance programs for infection control (e.g., those for health-care workers).
Given the high risk for progression to active disease in HIV-infected persons, any HIV-infected person with reactivity on any of the current LTBI diagnostic tests should be considered infected with *M. tuberculosis*.
At this time, neither an IGRA nor the TST can be considered a "gold standard" for diagnosis of LTBI. Current recommendations for use of IGRA in children are as follows:

- For immune-competent children 5 years of age and older, IGRA can be used in place of a TST to confirm cases of tuberculosis or cases of LTBI and likely will yield fewer false-positive test results.

- Children with a positive result from an IGRA should be considered infected with *M. tuberculosis* complex. A negative IGRA result cannot universally be interpreted as absence of infection.

- Because of their higher specificity and lack of cross-reaction with BCG, IGRA may be useful in children who have received BCG vaccine. IGRA may be useful to determine whether a BCG-immunized child with a reactive TST more likely has LTBI or has a false-positive TST reaction caused by the BCG.

- IGRA cannot be recommended routinely for use in children younger than 5 years of age or for immune-compromised children of any age because of a lack of published data about their utility with these groups.

- Indeterminate IGRA results do not exclude tuberculosis infection and should not be used to make clinical decisions.
To be released later this year

At the 2nd Global IGRA Symposium, it was announced that the new guideline will allow for the use of either TST or IGRA.

IGRA will be preferred over TST for BCG vaccinated

TST will be preferred over IGRA in young children <5 years of age
To be released

Will be broadly consistent with the new CDC 2009 recommendations

Will cover all TB diagnostics, not just LTBI
1. Not recommended for active TB in adults
2. Can be used as a supplementary aid in children with suspected TB
3. IGRAs may be used as a confirmatory test for a positive TST in contacts
4. IGRA may be performed in TST-positive, immunocompetent adults and children who are at relatively low risk of being infected and of progressing to active disease
5. In an immunocompromised person (adult or child), the TST should be the initial test; however, a clinician still concerned about the possibility of LTBI may perform an IGRA
6. Not recommended for serial testing of HCWs

Was updated again 2009, but very little was changed
Major global trends

- Two-step approach seems to be the most favored strategy for IGRA use
- Two-step approach is particularly favored in contacts, especially BCG-vaccinated contacts
- Trend towards using IGRAs alone prior to anti-TNF-a therapy
- Some guidelines are still cautious about IGRA use in young children
- Few guidelines recommend IGRAs for active TB, but some recommend as an adjunct, especially in kids
- Most guidelines do not mention use for serial testing of HCWs
Conclusions

• There is growing interest in the use of IGRAs, although most countries continue to recommend and use TST.
• More than 17 guidelines and statements have been published on IGRAs; many need to be updated.
  – considerable diversity in the approaches
• Guidelines are predominantly from high-income countries with established LTBI screening programs.
• Future guidelines and statements must aim to be explicitly evidence-based and be more transparent in disclosures
• Future guidelines will need to consider impact of IGRAs on patient outcomes and cost-effectiveness in various settings.
Evidence-Based Tuberculosis Diagnosis

A comprehensive resource for evidence syntheses, policies, guidelines and research agendas on TB diagnostics

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