IGRAs for Detection of *M. tuberculosis* Infection

Focus on LTBI
Curry International Tuberculosis Center
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**Forecasting TB Disease with Tests for LTBI**

- Can a positive result indicate who will get TB?
- Can a negative result indicate who will not?

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**Overview**

- What makes a good test for diagnosing LTBI?
- Why not just settle for the tuberculin skin test?
- Introduction to Interferon Gamma Release Assays (IGRAs)
- Recommendations
Prevalence of Latent TB Infection in U.S., 1999–2000 (Based on NHANES with TST)

<table>
<thead>
<tr>
<th>Population</th>
<th>LTBI Prevalence</th>
<th>Population Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>4.2%</td>
<td>302 million</td>
</tr>
<tr>
<td>U.S.-born</td>
<td>1.8%</td>
<td>274 million</td>
</tr>
<tr>
<td>Foreign-born</td>
<td>18.7%</td>
<td>28 million</td>
</tr>
</tbody>
</table>


Strategy for Targeted Testing:
Focus #1, Prevalence of Infection

- Efficiency or productivity
- Positive predictive value
  - Prevalence rate of infection ("pretest probability")
  - Specificity of the test

Focus #1: Groups with Greater Prevalence of M. tuberculosis Infection

- Close contacts of persons with TB disease
- Persons from areas with high incidence of TB
- Persons who visit areas with a high prevalence of TB, especially if visits are frequent or prolonged
- Residents and employees of high-risk congregate settings
- Health care workers who serve high-risk clients
- Populations defined locally as having an increased incidence of infection or disease due to TB
- Infants, children, and adolescents exposed to adults in high-risk categories
Strategy for Targeted Testing: Focus #2, Prevention Value

• “You can prevent TB only in persons who would otherwise get sick with TB.”
  – Attack rate for TB disease after LTBI ("risk factors for progression")
• Positive predictive value of the test

Focus #2: Persons Who are Most Likely to Get Sick with TB after *M. tuberculosis* Infection

• Persons with HIV infection*
• Infants and children aged <5 years*
• Persons receiving immunosuppressive therapy*
• Persons recently infected with *M. tuberculosis* (within 2 yrs)
• Persons with history of untreated or inadequately treated TB
• Persons with silicosis, diabetes mellitus, chronic renal failure, leukemia, lymphoma, or cancer of the head, neck, or lung
• Persons with gastrectomy or jejunoileal bypass
• Persons who weigh less than 90% of ideal body weight
• Populations defined locally as having an increased incidence of infection or disease due to *M. tuberculosis*.

* Indicates groups at increased risk of a poor outcome such as meningitis, disseminated disease, or death due to *M. tuberculosis*

Diagnostic Tools for *M. tuberculosis* Infection

Tuberculin Skin Test (TST)  Interferon Gamma Release Assays (IGRAs)
Why Not Just Settle for the Tuberculin Skin Test?

- Specificity—poor to good
  - Mycobacteria besides *M. tuberculosis*
  - Bacillus Calmette-Guérin (BCG) vaccine
- Boosting
- Reliable tuberculin (PPD) antigen solution
- Technique—(skills)—poor to not-so-poor
  - Injection
  - Measurement and interpretation
- Two healthcare encounters for one result
- Morbid fear of retesting after a positive result

Purified Protein Derivative (PPD)

“Old tuberculin” was discovered by Robert Koch, 1890

Autoclaving in vitro grown *M. tuberculosis* at 100° C for two hours

PPD is a purified tuberculin

Mixture of denatured peptides/proteins

Chemical composition:
- 93% proteins
- 1% nucleic acid
- 6% carbohydrate

Antigens

- M. tuberculosis antigens shared with NTM, & BCG
- Antigens specific to M. tuberculosis, e.g., ESAT-6 & CFP-10

Ganguly et al, 2008: 88, 510-517

Interferon Gamma (IFN-γ) Release Assays

- Indirect tests for M. tuberculosis infection
- Do not differentiate latent infection from disease
- Two FDA-approved test kits in the market
  - “as an aid in the diagnosis of infection with Mycobacterium tuberculosis”

Two Basic Methods for IGRA

- Measure a difference, IFN-γ concentration
  - Measure IFN-γ concentration by ELISA
  - Whole blood stimulated with & without antigen
  - QuantiFERON-TB Gold
- Count a difference, # of cells releasing IFN-γ
  - Count cells by ELISpot
  - PBMCs* stimulated with & without antigens
  - T-SPOT.TB

*peripheral blood mononuclear cells
FDA Approved IGRAs

- QuantiFERON-TB (QFT)  
  - FDA approved Nov 2001 but no longer available
- QuantiFERON-TB Gold (QFT-G)  
  - FDA approved May 2005
- QuantiFERON-TB Gold In-Tube (QFT-GIT)  
  - FDA approved Oct 2007
- T-SPOT.TB  
  - FDA approved July 2008

Antigen Specificity by Species

<table>
<thead>
<tr>
<th></th>
<th>ESAT-6</th>
<th>CFP-10</th>
<th>ESAT-6</th>
<th>CFP-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. tuberculosis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. bovis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. africanum</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BCG substrain</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gothenburg</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moreau</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rice</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Tokyo</td>
<td>-</td>
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<tr>
<td>Danish</td>
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<tr>
<td>Glaxo</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Montreal</td>
<td>-</td>
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<tr>
<td>Pasteur</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. abcessus</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. avium</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>M. branderi</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>M. celatum</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>M. chelonae</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. fortuitum</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. gordonii</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. intracellular</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>M. kanssasi</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>M. malmoense</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>M. marinum</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>M. xenopi</td>
<td>-</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. scrofulaceum</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. szulgai</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

ESAT-6 and CFP-10 are specific antigens for M. tuberculosis. The table shows the antigen specificity by species, with + indicating a positive reaction and - indicating a negative reaction.


QuantiFERON®-TB Gold In-Tube (QFT-GIT)

Stage 1 Whole Blood Culture

1. Collect 1 mL of blood in 3 tubes
2. Incubate within 16 hr at 37°C for 16-24 hr
3. Centrifuge 5 minutes to separate plasma above gel

Stage 2: Measure [IFN-γ] & Interpret

1. Collect plasma for ELISA
2. Measure [IFN-γ] in 'sandwich' ELISA
3. Software calculates results and prints report
### T-SPOT.TB

- Collect blood, 2–8 mL
- Process within 8 hr
- Recover, wash, & count PBMCs
- Aliquot 250,000 PBMCs to four wells with anti-IFN-\(\gamma\)
- Add media alone, ESAT-6, CFP-10 or PHA, & incubate
- Wash away cells
- Develop & count spots where cells produced IFN-\(\gamma\)

#### T-SPOT.TB Interpretation

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>TB Response*</th>
<th>Nil</th>
<th>Mitogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>&gt; 8 spots</td>
<td>≤ 10 spots</td>
<td>any</td>
</tr>
<tr>
<td>Borderline</td>
<td>5, 6, or 7 spots</td>
<td>≤ 10 spots</td>
<td>any</td>
</tr>
<tr>
<td>Negative</td>
<td>≤ 4 spots</td>
<td>≤ 10 spots</td>
<td>≥ 20 spots</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>&lt; 5 spots</td>
<td>≤ 10 spots</td>
<td>&lt; 20 spots</td>
</tr>
<tr>
<td></td>
<td>any</td>
<td>&gt; 10 spots</td>
<td>any</td>
</tr>
</tbody>
</table>

*TB Response is the higher number of spots resulting from stimulation of PBMCs with two separate cocktails of peptides representing ESAT-6 or CFP-10, minus the number of spots resulting from incubation of PBMCs with saline (i.e., Nil).
**IGRA Test Interpretation**

- Based on IFN-γ response to TB antigens relative to nil value
- Depends on the product
- Unlike TST, not risk stratified (i.e., only one cutoff for different risk groups)
- Still somewhat complicated

**IGRA and TST**

- *in vitro*
- specific Mtb antigens
- no boosting
- 1 patient visit
- phlebotomy
- stimulate within hours
- results possible in 1 day
- complex laboratory test
- potential for database

- *in vivo*
- purified protein derivative
- boosting
- 2 patient visits
- intracutaneous injection
- injected = done
- results in 2–3 days
- point-of-care test
- data storage—varies
Characterization of IGRAs

- Problems:
  - No "gold standard" for *M. tuberculosis* infection
  - Inconsistent methods and test interpretation

- Sensitivity—Compare to culture-confirmed TB
  - Sensitivity = # positives / # infected people tested

- Specificity—In subjects at low risk for LTBI
  - Specificity = # negative / # uninfected people tested

- Agreement with TST
- Association with exposure
- Forecast subsequent TB disease

IGRA Sensitivity

- 80% in subjects with untreated TB disease
  - Similar for different IGRAs
  - Ranges in studies from 57 to 100%
  - Similar to TST sensitivity
- 95% if either +IGRA or +TST

IGRA Sensitivity for LTBI

- Extrapolated from results with TB disease
- Immune differences: LTBI* is not TB disease
- Conclusion: unable to accurately measure

*at most, 5%–10% with LTBI experience progression to TB
IGRA Specificity for LTBI

- 89%–99.6% in subjects at low risk for LTBI
- Similar to TST (15 mm cutoff)
- More specific than TST? (10 mm cutoff)
  - After BCG vaccination
  - With NTM disease

IGRA Discordance with TST

- Agreement varies from good to very poor
- Poor agreement may be what we wanted?
- Positive TST & negative IGRA discordance
  - BCG, NTM: suggests IGRA more specific?
  - TB Prevalence: suggests TST more sensitive?
- Negative TST & Positive IGRA discordance
  - Uncommon & more random
  - Suggestive of a testing error?

Association with Exposure

- IGRA results are associated with recent exposure more than TST results are
  - Fewer unexposed are IGRA+ than TST +
  - Similar number positive
Forecast of TB Disease

• Can a positive result indicate who gets TB?
• Can a negative result indicate who will not?

Would you ever not believe an IGRA result?

What could go wrong?

Preanalytical IGRA Variability

• Patient
  – Diurnal cycle
  – Illnesses
• Specimen collection and management
  – Label ID
  – Volume
  – Mixture and agitation
  – Shipment time and temperature
• Test kit
  – Freedom from contamination
  – Accuracy of all reagents
Analytical IGRA Variability

• Incubation
• Reagent preparation and management
• Process measurements (dozens)
• Output measurements

Available evidence
+ Expert opinion
= Guidelines

Recommendations (1)

• TST or IGRAs (QFT-GIT; T-SPOT) should be used as aids to diagnose infection with *M. tuberculosis*.
  – using FDA approved test formats, and in compliance with CCLIA standards.
  – reporting both the qualitative interpretation and quantitative measurements.
  – arranging for IGRA testing prior to blood collection.

• As with the TST, IGRAs should not be used for testing persons with low risk of infection and low risk of disease due to *M. tuberculosis* (with noted exception for those likely to be at increased risk in the future).
Recommendations (2)

• IGRAs may be used in place of (and generally not in addition to) TST in all situations in which CDC recommends tuberculin skin testing (with noted preferences and special considerations).
  – Despite the indication of a preference, use of the alternative test (FDA-approved IGRA or TST) is acceptable medical and public health practice.
  – Test selection should be based on the reasons for testing, test availability, and overall cost effectiveness of testing.

Recommendations (3)

• An IGRA is preferred for testing persons from groups that historically have poor rates of return for TST reading.
• An IGRA is preferred for testing persons who have received BCG (as a vaccine or for cancer therapy).
• TST is preferred for testing children younger than 5 years old.

Recommendations (4)

• IGRAs or TST (without preference) to test recent contacts of persons with infectious tuberculosis with special considerations for follow-up testing.
  – Negative results prior to 8 wks typically should be confirmed by repeating the test 8–10 weeks after the end of exposure
  – Repeating the same test minimizes misclassification attributable to test discordance
• IGRAs may be used in place of TST (without preference) for periodic screening that addresses occupational exposure (with special considerations regarding conversions and reversions because criteria for interpreting changes in IGRA results that identify new infection remain uncertain).
Recommendations (5)

• The use of both TST & IGRA may be useful if the initial test is negative and
  – the risk of infection, the risk of progression, and the risk of a poor outcome are high (such as when persons with HIV infection, or children < 5 years old are at increased risk for *M. tuberculosis* infection), or
  – active tuberculosis is suspected (such as in persons with symptoms, signs, or radiographic evidence suggestive of active tuberculosis) and confirmation of *M. tuberculosis* infection is desired.

Recommendations (6)

• The use of both TST & IGRA may be useful if the initial test is positive and
  – additional evidence of infection is required to encourage treatment (such as in foreign-born healthcare workers who believe their positive TST is due to BCG); or
  – in healthy persons who have a low risk of both infection and progression.

Recommendations (7)

• Repeating an IGRA or performing a TST may be useful when the initial IGRA result is indeterminate, borderline, or invalid, and a reason for testing persists.

• Each institution and TB control program should evaluate the availability, overall cost effectiveness, and benefits of IGRAs in prioritizing IGRA use in their own setting.
Recommendations (8)

• A diagnosis of *M. tuberculosis* infection, and decisions about medical or public health management should include epidemiological, historical, and other clinical information when using IGRA or TST results.

• Persons with a positive TST or IGRA result should be evaluated for
  – likelihood of *M. tuberculosis* infection
  – risks of progression to tuberculosis disease if infected
  – symptoms and signs of tuberculosis disease.
  • With these risks, symptoms, or signs, additional evaluation is indicated.

Recommendations (9)

• A diagnosis of LTBI requires that tuberculosis disease be excluded by medical evaluation.

• In persons with symptoms, signs, or radiographic evidence of TB disease, and in those at high risk of progression to TB disease if infected, a positive result with either an IGRA or TST may be taken as evidence of *M. tuberculosis* infection.
  – However, negative IGRA or TST results are not sufficient to exclude infection in these persons.

Recommendations (10)

• In healthy persons who have a low likelihood both of *M. tuberculosis* infection and of progression to TB disease if infected, a single positive IGRA or TST result should not be taken as reliable evidence of *M. tuberculosis* infection.
  – Reevaluate to confirm lack of risk and consider repeat testing on a case-by-case basis, or
  – Alternatively, assume, without additional testing, that the initial result is falsely positive.
Recommendations (11)

• In persons with discordant test results (one positive and the other negative) decisions about medical or public health management requires individualized judgment in assessing
  – the quality & magnitude of each result,
  – the probability of infection,
  – the risk of disease if infected, and
  – the risk of a poor outcome if disease occurs.

Conclusions

• TST and IGRAs are aids for diagnosing *M. tuberculosis* infection.
• IGRAs offer some useful improvements over tuberculin skin testing.
• Be a wise consumer—a diagnostic test gives a result but not “the” answer or “the” diagnosis. Know the purpose of testing and the use of the result. Understand the consequences of false-positive or false-negative results.