Interferon Gamma Release Assays (IGRAs): Yesterday, Today and Tomorrow

Grand Rounds – April 14, 2010

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SAFER • HEALTHIER • PEOPLE
I have no conflicts of interests to disclose

Overview

• Development of interferon-gamma release assays (IGRAs)
• FDA-approved IGRAs
• Current recommended use: CDC guidelines
• Research questions
• Future possibilities
Development of IGRAs

In the beginning . . .
The Tuberculin Skin Test (TST)

- 0.1 ml of 5 TU PPD tuberculin injected intradermally
- Induration in millimeters read 48-72 hours after injection

And it was not (completely) good . . .
TST Limitations

- Technical problems in administration and reading
- >1 visit needed
- False-negative responses
  - anergy (compromised immunity)
  - TST reversion at old age
- Repeated TST boost the immune response
  - Need 2-step approach in serial testing
- False positives
  - Nontuberculous mycobacteria (NTM)
  - Bacille Calmette-Guerin vaccination (BCG)

Is there an alternative?
TST Versus In-vitro Assays


Underlying Principle of IGRAs

- Expose peripheral blood lymphocytes of person with suspected tuberculosis infection to antigens from *Mycobacterium tuberculosis*
- If person has been infected with *M. tuberculosis*, lymphocytes will respond by producing IFN-g
- Measure total IFN-g produced or number of cells that produce IFN-g
History of IGRAs

- Initial assay developed for testing cattle in 1990 (marketed as Bovigam)
  - Used *M. bovis* PPD as test “antigen”
- Modified for human use (later marketed as QuantiFERON-TB)
  - Proprietary *M. tuberculosis* PPD substituted for *M. bovis* PPD
- First human study published in 1997
- Large CDC trial published and FDA approval in 2001

Original QuantiFERON-TB (QFT) versus TST

<table>
<thead>
<tr>
<th>QFT</th>
<th>TST</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 patient visit</td>
<td>2 patient visits</td>
</tr>
<tr>
<td>Measurement of IFN-γ by machine (more objective)</td>
<td>Induration measured by human (more subjective)</td>
</tr>
<tr>
<td>Antigen: PPD</td>
<td>Antigen: PPD</td>
</tr>
</tbody>
</table>
What is PPD (Purified Protein Derivative)?

- Old tuberculin: a sterile solution of a concentrated filtrate of *M. tuberculosis* in culture
- PPD: purified protein fraction precipitated from old tuberculin
- PPD contains many antigens
  - Some are also found in BCG and NTM
- IGRA that uses PPD does not address issue of false-positive results related to BCG or NTM cross-reactions

Alternatives to PPD: Specific Mycobacterial Antigens

[Diagram showing Venn diagram with circles for *M. tuberculosis*, BCG, and NTM, highlighting the target area](image)
Antigens Specific to *M. tuberculosis*


Genetic Region of Difference 1 (RD-1)

- Not found in BCG or most NTM
  - NTM exceptions: *M. kansasii*, *M. szulcii*, *M. marinum*, and *M. riyadhense*
- Codes for 9 proteins
- Two found to produce strong immunologic responses in persons infected with *M. tuberculosis*
  - 10-kDa culture filtrate protein (CFP-10)
  - 6-kDa early-secreted target antigen (ESAT-6)
Antigens for Newer Generation IGRAs

- Negative control or nil (e.g., saline, heparin)
- Positive control or mitogen: non-specific immune response stimulator (e.g., phytohemagglutinin)
- *M. tuberculosis*-specific antigens
  - Unlike PPD used in TST, do not cross-react with BCG or NTM (some exceptions)
  - ESAT-6, CFP-10, TB 7.7 (actually simulated using overlapping peptides)

FDA-Approved IGRAs
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 (T-Spot)**
  - FDA approved July 2008

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**QuantiFERON®-TB Gold (QFT-G)**

Stage 1 Whole Blood Culture in Tissue Culture Plate

- Draw blood with heparin
- Make 1 ml aliquots & add antigen
- Incubate overnight. IFN-γ from sensitized lymphs

Stage 2: Measure [IFN-γ] & Interpret

- Harvest plasma from above settled cells
- Measure [IFN-g] in ‘Sandwich’ ELISA
- Computerized interpretation
QuantiFERON®-TB Gold In-Tube (QFT-GIT)

Stage 1: Whole Blood Culture in special blood collection tubes
- Collect 1mL of blood in 3 tubes
- Incubate at 37ºC for 16-24 hours
- Centrifuge 5 minutes to separate plasma above gel

Stage 2: Measure [IFN-γ] & Interpret
- Collect 50 µL of plasma for ELISA
- Measure [IFN-γ] in 'Sandwich' ELISA
- Software calculates results and prints report

*Mtb = ESAT-6 + CFP-10 + TB 7.7

T-Spot.TB (T-Spot)
- Collect blood in CPT tube
- Recover, wash, & count PBMCs
- Aliquot 250,000 PBMCs to 4 wells with anti-IFN-γ
- Add saline, PHA, ESAT-6 or CFP-10 & incubate
- Wash away cells
- Develop & count spots where cells produced IFN-γ

Saline  ESAT-6  CFP-10  PHA
### Differences in Currently Available IGRAs

<table>
<thead>
<tr>
<th></th>
<th>QFT-G</th>
<th>QFT-GIT</th>
<th>T-Spot</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Format</strong></td>
<td>Stimulate whole blood by 12 hrs</td>
<td>Stimulate whole blood, 37ºC incubation by 16 hrs</td>
<td>Stimulate PBMCs by 8 hrs</td>
</tr>
<tr>
<td><strong>TB Antigen</strong></td>
<td>ESAT-6 &amp; CFP-10 Separate wells</td>
<td>ESAT-6, CFP-10, &amp; TB 7.7 Incubation in single blood drawing tube</td>
<td>ESAT-6 &amp; CFP-10 Separate wells</td>
</tr>
<tr>
<td><strong>Measurement</strong></td>
<td>Total IFN-g</td>
<td>Total IFN-g</td>
<td>Number of IFN-g producing cells (spots)</td>
</tr>
<tr>
<td><strong>Possible results</strong></td>
<td>Positive, negative, indeterminate</td>
<td>Positive, negative, indeterminate</td>
<td>Positive, negative, indeterminate, borderline</td>
</tr>
</tbody>
</table>

### What Result is Considered Positive?

- Depends on the test
- Based on calculation of IFN-g response to TB antigens relative to IFN-g response to nil
- Unlike TST, not risk stratified (i.e., there are not multiple cutoffs for different risk groups)
- Still somewhat complicated
  - Mitigated by software that performs calculations
Indeterminate and Borderline Results

- Indeterminate
  - Negative control result is too high
  - High background production of IFN-g
  - Positive control result is too low
  - Immunocompromised patients may not respond to mitogen
- Borderline (T-Spot only)
  - Falls within borderline zone close to negative/positive cut point

Example: Interpretation Criteria for the QFT-GIT Test

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>TB Response</th>
<th>[Nin]</th>
<th>Mitogen Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>≥ 0.35 IU/ml and &gt; 25% of [Nin]</td>
<td>≤ 8.0</td>
<td>any</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt; 0.35 IU/ml or &lt; 25% of [Nin]</td>
<td>≤ 8.0</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>&lt; 0.35 IU/ml or &lt; 25% of [Nin]</td>
<td>≤ 8.0</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td></td>
<td>any</td>
<td>&gt; 8.0</td>
<td>any</td>
</tr>
</tbody>
</table>
CDC Guidelines

Previous U.S. Guidelines for FDA-Approved IGRAs

Vol. 52 / RR-2

Guidelines for Using the QuantiFERON®-TB Test for Diagnosing Latent Mycobacterium tuberculosis Infection

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Vol. 54 / RR-15

Guidelines for Using the QuantiFERON®-TB Gold Test for Detecting Mycobacterium tuberculosis Infection, United States

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Division of Tuberculosis Elimination, National Center for HIV, STD, and TB Prevention
Guidelines Development Process

- Focus on two tests approved since 2005 (QFT-GIT, T-Spot)
- Domestic and international expert consultants convened in August 2008
- Coordination with professional societies (ATS, IDSA, AAP) to harmonize guidance
- Drafts by CDC staff reviewed by peer experts and revised based on their feedback
- At MMWR Editorial Office awaiting publication

Data Reviewed

- Over 150 published articles
- Supplemented by unpublished data presented at August 2008 consultation
- Only published articles used and cited as evidence basis in guidelines
Summary of Data Review and Interpretation

Sensitivity

• No gold standard for latent TB infection
• TB disease used as a surrogate
  – Problematic
• Overall, tests are comparable
  – Trend for increased sensitivity with T-Spot, but limited head-to-head comparison
### Sensitivity Data

<table>
<thead>
<tr>
<th>Pooled data</th>
<th>TST</th>
<th>QFT-GIT</th>
<th>T-Spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>QFT-GIT</td>
<td>NA</td>
<td>82%</td>
<td>NA</td>
</tr>
<tr>
<td>T-Spot</td>
<td>NA</td>
<td>NA</td>
<td>93%</td>
</tr>
<tr>
<td>TST vs QFT-GIT</td>
<td>89%</td>
<td>83%</td>
<td>NA</td>
</tr>
<tr>
<td>TST vs T-Spot</td>
<td>91%</td>
<td>NA</td>
<td>91%</td>
</tr>
<tr>
<td>All 3</td>
<td>95%</td>
<td>84%</td>
<td>91%</td>
</tr>
</tbody>
</table>

### Specificity

- No gold standard
- Measured in persons with low or no identifiable risk for *M. tuberculosis* infection
- Variation in population from study to study
- Trend toward increased specificity with QFT-GIT, but head-to-head comparison data lacking
- Limited published specificity data on T.Spot in general
### Specificity Data

<table>
<thead>
<tr>
<th>Pooled data</th>
<th>TST</th>
<th>QFT-GIT</th>
<th>T-Spot</th>
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<tbody>
<tr>
<td>TST vs QFT-GIT</td>
<td>84%</td>
<td>99%</td>
<td>NA</td>
</tr>
<tr>
<td>TST vs T-Spot</td>
<td>86%</td>
<td>NA</td>
<td>88%</td>
</tr>
</tbody>
</table>

### Special Situations and Populations
Contact Investigations

- In contacts tested, exposure characteristics associated with increased risk of infection correlate better with IGRAs than TST
  - E.g., duration of exposure to infectious patient, infectiousness of patient
- Particularly true in BCG-vaccinated contacts

Children

- Limited data, especially children < 5 y.o.
- Some studies show increased percentage of indeterminate results
- Blood drawing more difficult in very young children
- More difficult to confirm diagnosis of TB disease in children
Prediction of Future Disease

- Two studies (Germany – close contacts; Austria – HIV-infected persons) suggest QFT-GiT is better than TST at predicting future TB disease
  - Very small number of TB cases (3-6 per study)
  - Confidence intervals overlap between TST and QFT-GiT
- One study from Netherlands found TST with 10 mm cutoff was better than QFT-GiT or T-Spot at predicting TB in immigrant contacts
  - Only 9 cases of TB
  - Not all differences statistically significant
- Study in Gambia showed no difference between Elispot assay (similar to T-Spot) and TST in predicting future TB

Immunocompromised Patients

- Do not appear to be significant differences in the number of positive QFT-GiT and TST results in HIV infected
- Nevertheless, discordance between QFT-GiT and TST is often high in immunocompromised
- Indeterminate results for QFT-GiT associated with low CD4 count
- Tend to be more positive test results with T-Spot across various immunocompromised populations
- More inconsistency in study results among immunocompromised populations
Periodic Screening (e.g., Healthcare Workers)

- Some studies have shown considerable variation in IFN-g response with serial testing over time
- Uncertainty about magnitude of change in result that is likely caused by new infection versus expected test variation
- Questionable significance of conversions and reversions when initial test result is near cut point
- Frequency of false-positive conversions may be higher with IGRAs because of less stringent criteria for conversion compared to TST

Cost

- U.S.-based cost effectiveness data are lacking
- Some non-U.S. studies have suggested limiting IGRAs to testing of persons who are TST + is most cost-effective strategy
  – Analysis highly dependent on prevalence of TB in population
- Cost of IGRA materials is greater than TST materials, but may be offset by labor costs and fewer positive test results with IGRAs
Provisional Recommendations

• TST or IGRAs should be used as aids in diagnosing infection with *M. tuberculosis*
  – Both the standard qualitative test interpretation and the quantitative assay measurements should be reported
Provisional Recommendations 2

• As with the TST, IGRAs generally should not be used for testing persons who have a low risk of infection and a low risk of disease due to *M. tuberculosis*

Provisional Recommendations 3

• Selection of the most suitable test or combination of tests for detection of *M. tuberculosis* infection should be based on the reasons and the context for testing, test availability, and overall cost effectiveness of testing
Provisional Recommendations 4

• IGRAs may be used in place of (and not in addition to) TST in all situations in which CDC recommends tuberculin skin testing as an aid in diagnosing *M. tuberculosis* infection, with preferences and special considerations as follow

• Despite the indication of a preference, use of the alternative test (IGRA or TST) is acceptable and still considered good medical and public health practice

Provisional Recommendations 5

• Populations/situations in which IGRAs are preferred
  – testing persons from groups that historically have poor rates of return for TST reading
  – testing persons who have received BCG (as a vaccine or for cancer therapy)
Provisional Recommendations 6

- Populations/situations in which TST is preferred
  - testing children younger than 5 years old

Provisional Recommendations 7

- Populations/situations in which there is no preference between IGRAs and TST
  - testing recent contacts of persons with infectious tuberculosis
  - periodic screening that addresses occupational exposure to TB (e.g., surveillance programs for healthcare workers)
Provisional Recommendations 8

• Routine testing with both TST and an IGRA is not recommended
• Results from both tests may be useful when the initial test is negative if increased sensitivity is desired (considered infected if either test is positive)
  – risk of infection, the risk of progression, and the risk of a poor outcome are increased
  – clinical suspicion of active tuberculosis and confirmation of *M. tuberculosis* infection is desired

Provisional Recommendations 9

• Results from both tests may be useful when the initial test is positive if increased specificity is desired (considered infected only if both tests are positive)
  – additional evidence of infection is required to encourage compliance (such as in foreign-born healthcare workers who believe their positive TST is due to BCG)
  – in healthy persons who have a low risk of both infection and progression
Provisional Recommendations 10

• 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

Provisional Recommendations 11

• Each institution and TB control program should evaluate the availability, overall cost effectiveness, and benefits of IGRAs in prioritizing IGRA use in their setting
Provisional Recommendations 12

• A diagnosis of *M. tuberculosis* infection, and the decisions about medical or public health management should include epidemiological, historical, and other clinical information when using IGRA or TST results
  – Decisions should not be based on IGRA or TST results alone

Need for Additional Research

• 16 research questions identified by CDC
• Examples
  – Are IGRAs better at predicting subsequent tuberculosis disease than TST?
  – What magnitude of change in IFN-g response indicates new infection?
  – After exposure, how long does it take for an IGRA to become positive?
More Examples of Research Questions

• Do IGRAs perform differently in children as compared to adults?
• Why do simultaneously performed TST, QFT-GIT, QFT-G, and T-Spot results differ?

Possible Future Directions
Alternatives to IFN-g?


Future Possibilities

• Tests employing cytokines other than IFN-g
  – Combinations of cytokines, i.e., cytokine profile (e.g., multiplex bead immunoassay)
• Tests employing *M. tuberculosis* antigens other than ESAT-6, CFP-10 and TB 7.7
• Automation
  – Current IGRAs require many manual steps
Multiplexed Bead-Based Immunoassays

Example: A = bead with antibodies to detect IFN-g, B = TNF-a, C = IL-12, etc

Illustration from Becton Dickinson

Acknowledgements

• TB Controllers in U.S.
• Professional Organizations (ATS, IDSA, AAP)
• CDC
  – Jerry Mazurek
  – Andy Vernon
  – Ken Castro
  – John Jereb
  – Stefan Goldberg
Expert Consultants (August 2008)

Col. Paul Barnicott  Masae Kawamura, MD  Rick O’Brien, MD
John Bernardo, MD  Lisa Keep, MD  Randall Reves, MD
Henry M. Blumberg, MD  Stephen Kralovic, MD MPH  Luca Richeldi, MD PhD
Helene Calvet, MD  Michael Leonard, MD  Neil Schluger, MD
Chuck Daley, MD  David Lewinsohn, PhD  John Segerson
Susan Dorman, MD  Debbie Lewinsohn, MD  Kim Smith, MD
Ed Graviss, PhD  Kathleen Moser, MD  Jeff Starke, MD
Tiffany Harris, PhD  Edward Nardell, MD  David Warshauer, PhD
Philip Hill, MD  Masa Narita, MD  Gail Woods, MD

Questions?