TB Updates
San Antonio, Texas
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Interferon Gamma Release Assays:
Understanding the Test
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March 22, 2013

David Griffith, MD has the following disclosures to make:

• No conflict of interests
• No relevant financial relationships with any commercial companies pertaining to this educational activity
Understanding Interferon-Gamma (IFN-\(\gamma\)) Release Assays (IGRAs) (A Moving Target)

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Understanding IGRAs (Good luck)

• Sensitivity and Specificity
• Value of IGRAs for predicting progression from LTBI to active TB
• Variable IGRA results with serial testing
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-\(\gamma\)

• Measure total IFN-\(\gamma\) produced or number of cells that produce IFN-\(\gamma\)

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)
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 37°C for 16-24 hours
- Centrifuge 5 minutes to separate plasma above gel

Stage 2: Measure [IFN-γ] & Interpret

- Collect 50μL of plasma for ELISA
- Measure [IFN-γ] in ‘Sandwich’ ELISA
- Software calculates results and prints report

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

Interpretation Criteria for the QFT-GIT Test

<table>
<thead>
<tr>
<th>Nil (IU/mL)</th>
<th>TB Antigen minus Nil (IU/mL)</th>
<th>QFT-GIT (IU/mL)</th>
<th>Mitogen</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 8.0</td>
<td>≤ 0.35 or &lt; 25% of Nil value</td>
<td>Negative</td>
<td>≥ 5.0</td>
<td>M. tuberculosis infection unlikely</td>
</tr>
<tr>
<td>≤ 8.0</td>
<td>≥ 0.35 and ≥ 25% of Nil value</td>
<td>Positive</td>
<td>ANY</td>
<td>M. tuberculosis infection likely</td>
</tr>
<tr>
<td>≥ 8.0</td>
<td>ANY</td>
<td>Indeterminate</td>
<td>ANY</td>
<td>Indeterminate</td>
</tr>
<tr>
<td>≤ 8.0</td>
<td>≤ 0.35 and or &lt; 25% of Nil value</td>
<td>Indeterminate</td>
<td>&lt; 5.0</td>
<td>Indeterminate</td>
</tr>
</tbody>
</table>
### QuantiFERON-TB Gold

**TABLE 2. TEST SENSITIVITY AND SPECIFICITY FOR CFP-10 AND ESAT-6 AT VARIOUS CUTOFFS IN WHOLE-BLOOD IFN-\(\gamma\) ASSAY**

<table>
<thead>
<tr>
<th>Cutoff, IFN-(\gamma) (IU/ml)</th>
<th>CFP-10 Specificity (%)</th>
<th>CFP-10 Sensitivity (%)</th>
<th>ESAT-6 Specificity (%)</th>
<th>ESAT-6 Sensitivity (%)</th>
<th>CFP-10 and/or ESAT-6 Specificity (%)</th>
<th>CFP-10 and/or ESAT-6 Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>92.5</td>
<td>81.4</td>
<td>94.8</td>
<td>94.9</td>
<td>89.4</td>
<td>97.5</td>
</tr>
<tr>
<td>0.10</td>
<td>94.4</td>
<td>77.1</td>
<td>96.2</td>
<td>90.7</td>
<td>92.0</td>
<td>95.8</td>
</tr>
<tr>
<td>0.15</td>
<td>95.8</td>
<td>72.9</td>
<td>97.6</td>
<td>88.1</td>
<td>93.9</td>
<td>93.2</td>
</tr>
<tr>
<td>0.20</td>
<td>96.7</td>
<td>71.2</td>
<td>99.1</td>
<td>86.4</td>
<td>96.2</td>
<td>91.5</td>
</tr>
<tr>
<td>0.25</td>
<td>97.2</td>
<td>67.8</td>
<td>99.1</td>
<td>84.7</td>
<td>96.7</td>
<td>91.5</td>
</tr>
<tr>
<td>0.30</td>
<td>97.7</td>
<td>66.8</td>
<td>99.1</td>
<td>83.1</td>
<td>97.2</td>
<td>89.8</td>
</tr>
<tr>
<td>0.35</td>
<td>98.6</td>
<td>65.3</td>
<td>99.5</td>
<td>81.4</td>
<td>98.1</td>
<td>89.0</td>
</tr>
<tr>
<td>0.40</td>
<td>98.6</td>
<td>61.9</td>
<td>99.5</td>
<td>79.7</td>
<td>98.1</td>
<td>88.1</td>
</tr>
<tr>
<td>0.45</td>
<td>98.6</td>
<td>60.2</td>
<td>100.0</td>
<td>78.8</td>
<td>98.6</td>
<td>86.4</td>
</tr>
<tr>
<td>0.50</td>
<td>99.1</td>
<td>60.2</td>
<td>100.0</td>
<td>75.4</td>
<td>99.1</td>
<td>83.9</td>
</tr>
</tbody>
</table>

Sensitivity was determined on the basis of data from 118 patients with culture-positive tuberculosis, and specificity was determined on the basis of data from 213 low-risk subjects. The chosen cutoff (0.35) is in boldface.

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### T-Spot.TB (T-Spot)

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

<table>
<thead>
<tr>
<th>Result</th>
<th>Nil*</th>
<th>TB Response ##</th>
<th>Mitogen++</th>
<th>Interpretation+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>≤ 10 spots</td>
<td>≥ 8 spots</td>
<td>Any</td>
<td><em>M. tuberculosis</em> infection likely</td>
</tr>
<tr>
<td>Borderline</td>
<td>≤ 10 spots</td>
<td>5, 6, or 7 spots</td>
<td>Any</td>
<td>Uncertain likelihood of <em>M. tuberculosis</em> infection</td>
</tr>
<tr>
<td>Negative</td>
<td>≤ 10 spots</td>
<td>≤ 4 spots</td>
<td></td>
<td><em>M. tuberculosis</em> infection unlikely</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>&gt; 10 ≤ 10</td>
<td>Any &lt; 5 spots</td>
<td>Any &lt; 20 spots</td>
<td>Uncertain likelihood of <em>M. tuberculosis</em> infection</td>
</tr>
</tbody>
</table>

### IFG-γ Release Assays in the Diagnosis of LTBI Among Immunocompromised Adults

Redelman-Sidi and Sepkowitz, AJRCCM 2012, Dec, e-pub

- No “gold-standard” for LTBI DX, therefore 1/4 approaches for evaluating IGRAs necessary:
  - Concurrent TST with comparison of results
  - Using clinical risk factors as a surrogate for LTBI
  - Longitudinal F/U after initial testing to document development of active TB
  - Performing IGRAs +/- TST in patients with active TB as a surrogate for LTBI
Sensitivity and Specificity of IGRAs for Diagnosing LTBI in Immune Competent Adults

- No gold standard for latent TB infection
- Overall, sensitivity of IGRAs and TST comparable
- Some lack of concordant results between QFT and TSpot
- In contacts tested, particularly BCG-vaccinated contacts, 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

IFG-γ Release Assays in the Diagnosis of LTBI Among Immunocompromised Adults
Redelman-Sidi and Sepkowitz, AJRCCM 2012, Dec, e-pub

- Diagnosis of LTBI in HIV-infected persons
- IGRAs have comparable sensitivity to TST
- TST and IGRAs appear less sensitive in HIV (+) patients than they are in HIV (-) persons with smaller difference with higher CD4+ counts
- T-Spot.TB may have an advantage over TST and QFT in patients with low CD4+ counts
- Either IGRA more specific than TST in BCG vaccinated populations (sensitivity!)
- TST and IGRAs are frequently discordant, and likely LTBI detected by one test and not the other
• Patients with inflammatory disorders who are candidates for treatment with TNF-α inhibitors
  – QFT, T-Spot.TB, TST comparably sensitive
  – IGRAs more specific than TST for BCG vaccinated
  – Sensitivity of QFT may be reduced in patients on immune suppressives with ↑ indeterminate results
  – T-Spot.TB appears less affected by immune suppression, consider for patients on steroids
  – For high risk patients simultaneous testing with TST and IGRA may be optimal with diagnosis of LTBI if either test positive

• Conclusions:
  – Better tests are needed to diagnose LTBI in immune compromised patients
  – Current data does not suggest that IGRAs have a clear across the board advantage of TBT in these patients
  – IGRAS have some advantages in specific groups
    • T-Spot. TB: HIV, steroid
    • QFT: better sensitivity in dialysis
  – Sensitivity vs Specificity
Predictive Value of Recent QFT Conversion for TB Disease in Adolescents
Machingaidze et al AJRCCM 2012, 186; 1051

- Comparison of 534 adolescents with IGRA conversion and 629 adolescents with (-) IGRA over 2 years for TB disease incidence
- TB incidence rate for QFT converters 1.46 cases/100 person-years vs 0.17 cases/100 person-years for QFT non-converters.
- Recent QFT conversion was indicative of an approximately 8-fold higher risk of progression to TB disease compared with non-converters within 2 years of conversion in a cohort of adolescents in a high-TB burden population.

Predictive Value of IFG-γ Release Assays and TST for Progression for LTBI to Disease State (a meta analysis)
Diel Chest 2012, 142; 63

- Appraised 23 studies evaluating the PPV and NPV from a test-determined LTBI state (IGRAs and TST) for progression to active TB for persons not receiving preventive therapy (F/U 24 mos, 20/28 studies)
- Pooled PPV for progression using IGRAs 2.7% vs TST 1.5% (p<0.0001)
- PPV for progression increased to IGRA 6.8% vs TST 2.4% with only high risk groups considered (p<0.0001)
- Pooled values for NPV for progression for both IGRAs 99.7% vs TST 99.4% (p<0.01)
Predictive Value of IFG-γ Release Assays and TST for Progression for LTBI to Disease State (a meta analysis)

Diel Chest 2012, 142; 63

• “Commercial IGRAs have a higher PPV and NPV for progression to active TB compared with those ow the TST, especially when performed in high-risk persons.”

Predictive value of IFG-γ release assays for incident active TB: a systematic review and meta-analysis

Rangaka et al, Lancet Infect Dis 2012, 12; 45

• 15 longitudinal studies of the predictive value for active TB of IGRAs (adults, children with or without HIV)
• > 26K participants, incidence of TB over a median F/U of 4 years was 4-48 cases/1000 person-years
• IGRA results “showed a moderate association” between (+) results and subsequent TB (IRR 2.1)
• IGRA (+) and TST (+) results similar for risk of TB in 5 studies (IGRA IRR 2.1; TST IRR [10mm] 1.6)
Predictive value of IFG-γ release assays for incident active TB: a systematic review and meta-analysis
Rangaka et al, Lancet Infect Dis 2012, 12; 45
• “However, the proportion of IGRA (+) individuals in 7/11 studies was generally lower that TST (+) individuals.
• “Neither IGRA nor the TST have a high accuracy for the prediction of active TB…”
• “Existing tests for LTBI should be chosen on the basis of relative specificity in different populations, logistics, cost, and patient’s preferences rather than on predictive ability alone”

Monthly follow-ups of Interferon-γ release assays among HCWs in contact with patients with TB
Park et al, Chest 2012 142; 1461
• 49 HCWs with TB contact: monthly QFT for 1 yr
• Inconsistent IGRA results (<0.35 to ≥0.70) found in 13 (27%) patients
• In 5 patients fluctuations in IFN-γ levels showed levels > 0.70 2 or more times
• Consistency NOT associated with profession, degree of TB exposure or N95 mask use
• ?Poor reproducibility of the assay, repeated infection and true reversion, periodic secretion of MTB antigens
Periodic Screening (e.g., Healthcare Workers)

- Several studies have shown considerable variation in IFN-γ 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 IGRA due to less stringent criteria for conversion compared to TST

Test variability of the QuantiFERON-TB Gold In-Tube assay in clinical practice
Metcalfe et al, AJRCCM 2013, 187; 206

- Multiple IGRA testing using leftover stimulated plasma (i.e., retesting of the same patient sample)
- 1086 test results obtained from 543 subjects
- Result of 2nd test discordant with that of the first in 8% of patients with valid results including 13 with an initial negative result and 15 with an initial positive result
- “Substantial variability” with the IGRA outside the normal expected range of test variability
The Elusive “Gold” Standard for Detecting MTB Infection
Mancuso et al AJRCCM 2013, 187; 122

• IGRAs are a complicated assay that require 126 measurements for one test
• The study by Metcalfe et al encompasses only the ELISA portion of the test, greater variability is likely if all portions of the test are repeated: different reagent lots, operators, instruments, or laboratories or with serial testing of patients at different time points.

Serial Testing with TB INF-γ Release Assays
Toward a Nuanced Understanding
M Pai 2012 Chest 142; 1367

• “When simplistic definitions are used for conversion, conversion rates that are incompatible with what is epidemiologically expected for a given setting will always result.”
• “When tests are repeated…on the same individuals, more complex…phenotypes are seen including stable and unstable (transient) conversions, persistently positive and negative results and other complex patterns that defy description”.
• “There are no longitudinal data on the prognosis of such phenotypes and it is unclear which subgroup should be targeted for preventive therapy.”
Serial Testing with TB INF-γ Release Assays
Toward a Nuanced Understanding
M Pai 2012 Chest 142; 1367

- “None of the current guidelines on IGRAs adequately addresses these challenges and provide specific guidance on how to handle conversion, reversions, and borderline results that fluctuate over time.”
- “It is particularly important to consider the clinical context: risk profile of the HCW; TST results…and history of contact in making decision about preventive therapy.”
- “Thus regardless of the test used for serial testing, we need to rethink the general strategy of annual testing of HCWs in efforts and resources might be better spent on controlling TB in high-burden countries…”

Guidelines for Preventing the Transmission of MTB in Health-Care Settings, 2005
MMWR, Dec 30, 2005; 54

- Medium risk setting: LTBI testing at baseline and at least every 12 mos
  - \( \geq 3 \) TB patients/yr: inpatient < 200 beds
  - \( \geq 6 \) TB patients/yr: inpatient \( \geq 200 \) beds
  - Outpatient/nontraditional: \( \geq 3 \) TB pats /yr
- Low risk setting: Baseline LTBI testing only
  - < 3 TB patients/yr: inpatient < 200 beds
  - < 6 TB patients/yr: inpatient \( \geq 200 \) beds
  - Outpatient/nontraditional: < 3 TB pats /yr
The Elusive “Gold” Standard for Detecting MTB Infection
Mancuso et al AJRCCM 2013, 187; 122

- Metcalfe et al call for a “borderline” zone to address IGRA uncertainty (0.35 ± 0.24 IU/ml)
- Would reduce the number of false-positive test interpretations among uninfected patients but would also increase the number of false negatives among infected patients.
- Use of a borderline zone would reduce the number of reversions and conversions but would increase the number of patients with uncertain results.

Movement Toward a Change in IGRA Criteria for ‘Positive’

The Elusive “Gold” Standard for Detecting MTB Infection
Mancuso et al AJRCCM 2013, 187; 122

• “In light of IGRA variability, testing of low-risk patients should be avoided, as with the TST. The problems associated with testing low-risk patients have not been solved by the introduction of IGRA and will not be solved by the use of a borderline zone or risk stratified IGRA interpretation. “

IFG-γ Release Assays vs TST for the Diagnosis of LTBI: An overview of the Evidence
Rajman et al Pulmonary Medicine 2013 e-pub

• “Because protection from IPT is well established only among TST + subjects, and because spontaneous reversion of positive IGRA is very common if the TST is negative, the authors recommend not treating TST-/IGRA+ individuals, unless they have very high risk for disease, as in HIV + individuals, and are clearly exposed to TB, in which case treatment for LTBI should be considered.”
Interferon-γ ELISPOT as a Biomarker of Treatment Efficacy in Latent TB Infection
(Adetifa et al AJRCCM 2013, 187; 439)

- Randomized, blinded, placebo-controlled trial of INH in 189 ELISPOT and Mantoux test positive participants (case contacts)
- Participants received a 6-month course of INH 900 mg BIW (96) or placebo (93), urine tested for INH metabolites to confirm adherence
- The proportion of ELISPOT-positive subjects reduced over time but did not differ by study arm

Interferon-γ ELISPOT as a Biomarker of Treatment Efficacy in Latent TB Infection
(Adetifa et al AJRCCM 2013, 187; 439)

- In contacts with LTBI, INH therapy plays no role in observed decreased in MTB antigen-specific T-cell responses over time.
- “Our data strongly suggest that claims of INH-induced reversion in LTBI from uncontrolled before and after studies are false: the reversion seen is most likely caused by natural decline of T-cell frequencies and not therapy.”
CDC Recommendations

• Routine testing with both TST and an IGRA is not recommended: interpreting discordant results between TST and IGRA is VERY DIFFICULT

• 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

CDC Recommendations

• 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
CDC Recommendations

- 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.
- How many times should you repeat the test for borderline results?
- Clinical judgement!! (10 mm TST)

 CDC Recommendations

- 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
- Particularly relevant for managing discordant test results (e.g., TST+/QFT-)
IGRA Summary

- Easier to perform than TST
- Programmatically cheaper than TST
- Sensitivity comparable to TST (T-Spot)
- Specificity better in BCG vaccinated populations (majority of US TB cases)
- Avoid using both tests EXCEPT with definite sensitivity or specificity questions
- Be aware of variability of serial test results, boosting after TST, and spontaneous reversion to negative

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IGRA Summary

- Short term value of IGRAs for predicting progression from LTBI to active TB may be better than TST, longterm predictive value uncertain
- Serial IGRA testing variability may not be a consequence of a treatment effect
- Overall, not worse than TST