Disclosures

• No relevant financial relationships
Learning Objectives

• Explain the use of TST and IGRA testing
• To learn the basis of tuberculin skin test (TST) and interferon-gamma release assays (IGRA) in LTBI
• To develop effective diagnostic strategies for LTBI in special settings
Case

• 24 yo US-born man referred for possible LTBI and h/o IBD
• Asymptomatic, no F/C/NSWL.
• PMHx:
  • Crohn’s disease x ~ 3 years with 2 flare-ups (diarrhea, bloody stools) on 6-MP and prednisone only for flare-ups
  • Primary sclerosing cholangitis (PSC) on liver biopsy
  • Prior h/o TST= 11 mm and (-)CXR: No LTBI treatment;
• Social Hx:
  • Denies prior TB exposures, traveled to Germany and finished college degree
  • More recent TST= 9 mm, but fluctuating LFTs due to PSC (Anticipated “problematic” LTBI treatment as per outside MD)
Case

**PMHx**
- Afebrile, no LAD, lungs CTA, otherwise unrevealing

**Labs:**
- Normal CBC
- MRI normal liver and bile ducts

**QFT: (11/17/10): (-) results**

**QFT (2/7/2012):**
- Nil = 0.02
- Ag-Nil = 0.01 (-) results
- Mitogen-Nil = 15.17

**TSPOT-TB:**
- Nil = 0; Panel A = 0; Panel B = 0;
- Pos control = >20: (-) results
TB Pathogenesis
What it does

Droplet nuclei containing tubercle bacilli are inhaled, enter the lungs, and travel to the small alveoli
• If the immune system CANNOT keep tubercle bacilli under control, bacilli begin to multiply rapidly and cause TB disease

• This process can occur in different places in the body
Natural History of TB Infection in Patients Without HIV

Exposure to TB

No infection (70-90%)

Infection (10-30%)

Latent TB (90%)

Never develop Active disease

Active TB (5-10%)

Treated

Die/Relapse/Recurr

Cured

Never develop Active disease
## Risk of Developing TB disease

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Risk of Developing TB</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB infection and no risk factors</td>
<td>About 10% over a lifetime</td>
<td>For people with TB infection, no risk factors, and no treatment, the risk is about 5% in the first 2 years after infection and about 10% over a lifetime.</td>
</tr>
<tr>
<td>TB infection and diabetes</td>
<td>About 30% over a lifetime</td>
<td>For people with TB infection and diabetes, and with no treatment, the risk is three times as high, or about 30% over a lifetime.</td>
</tr>
<tr>
<td>TB infection and HIV infection</td>
<td>About 7% to 10% PER YEAR</td>
<td>For people with TB infection and untreated HIV infection and with no LTBI treatment, the risk is about 7% to 10% PER YEAR, a very high risk over a lifetime.</td>
</tr>
</tbody>
</table>
Why Test for LTBI?

In 2014, a total of 9,412 new tuberculosis (TB) cases were reported in the US.

- Incidence: 3.0/100,000 persons
- Identifying and treatment of LTBI can prevent
  - Infected persons from developing TB disease
  - From infecting others
Who Should be Tested for LTBI?
Targeted Testing for LTBI: Why?

11 million LTBI cases—roughly 1 in 24 people—in the U.S.
• 550,000 - 1.1 million future TB cases

Unfocused population-based testing is not cost-effective or useful and leads to unnecessary treatment.

Targeted testing is used to focus program activities, provider practices, and financial resources on groups at the highest risk for LTBI

CDC Morbidity and Mortality Weekly Report (MMWR), March 20, 2015 / 64(10);265-269.
Patients at Increased Risk for Acquiring TB Infection

- Close contacts of persons known or suspected to have active tuberculosis
- Foreign-born persons from areas that have a high incidence of active tuberculosis
- Visitors of TB endemic countries, especially if visits are frequent or prolonged
- Residents and employees of congregate settings whose clients are at increased risk for active tuberculosis (e.g., correctional facilities, long-term care facilities, and homeless shelters)
- Health-care workers who serve patients who are at increased risk for active tuberculosis
- Populations defined locally as having an increased incidence of latent *M. tuberculosis* infection or active tuberculosis, possibly including medically underserved, low-income populations, or persons who abuse drugs or alcohol
- Infants, children, and adolescents exposed to adults who are at increased risk for latent *M. tuberculosis* infection or active tuberculosis

MMWR 2000;49(No. RR-6)
Patients at Increased Risk of Progression from LTBI to Active TB disease

<table>
<thead>
<tr>
<th>Persons at Increased Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Persons infected with HIV;</td>
</tr>
<tr>
<td>• Children younger than 5 years of age;</td>
</tr>
<tr>
<td>• Persons who were recently infected with <em>M. tuberculosis</em> (within the past 2 years);</td>
</tr>
<tr>
<td>• Persons with a history of untreated or inadequately treated TB disease, including persons with fibrotic changes on chest radiograph consistent with prior TB disease;</td>
</tr>
<tr>
<td>• Persons who are receiving immunosuppressive therapy such as tumor necrosis factor-alpha (TNF) antagonists, systemic corticosteroids equivalent to/greater than 15 mg of prednisone per day, or immunosuppressive drug therapy following organ transplantation;</td>
</tr>
<tr>
<td>• Persons with silicosis, diabetes mellitus, chronic renal failure, leukemia, or cancer of the head, neck, or lung;</td>
</tr>
<tr>
<td>• Persons who have had a gastrectomy or jejunoileal bypass;</td>
</tr>
<tr>
<td>• Persons who weigh less than 90% of their ideal body weight;</td>
</tr>
<tr>
<td>• Cigarette smokers and persons who abuse drugs and/or alcohol; and</td>
</tr>
<tr>
<td>• Populations defined locally as having an increased incidence of disease due to <em>M. tuberculosis</em>, including medically underserved, low-income populations.</td>
</tr>
</tbody>
</table>

CDC 2013: Core Curriculum on Tuberculosis
How Do We Test for LTBI?
Innate Immunity to *M. tuberculosis*

- Toll-like receptor
- Activate NK, T cells, macrophages
- NF-κB and cytokines
- Vit D Receptor
- Phagocytic killing of pathogens

Resp 2010.15:433
Latent TB Infection Diagnostics
Tuberculin Skin Test (TST)  
Interferon Gamma Release Assays (IGRAs)

# TST vs. IGRAs: Specificity

<table>
<thead>
<tr>
<th></th>
<th># Studies</th>
<th>Specificity (95% CI)</th>
<th>Chi-Square for heterogeneity (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TST</td>
<td>8</td>
<td>0.66 (0.46-0.86)</td>
<td>0.001</td>
</tr>
<tr>
<td>Not BCG vaccinated</td>
<td>3</td>
<td>0.98 (0.96-1.00)</td>
<td>NS</td>
</tr>
<tr>
<td>BCG Vaccinated</td>
<td>5</td>
<td>0.56 (0.34-0.78)</td>
<td>0.001</td>
</tr>
<tr>
<td>≥ 10 mm</td>
<td>6</td>
<td>0.58 (0.37-0.79)</td>
<td>0.001</td>
</tr>
<tr>
<td>≥ 15 mm</td>
<td>3</td>
<td>0.87 (0.70-1.00)</td>
<td>0.001</td>
</tr>
<tr>
<td>QuantiFERON-TB Gold</td>
<td>9</td>
<td>0.97 (0.95-0.99)</td>
<td>0.01</td>
</tr>
<tr>
<td>Not BCG vaccinated</td>
<td>2</td>
<td>1.0 (0.94-1.0)</td>
<td>NS</td>
</tr>
<tr>
<td>BCG Vaccinated</td>
<td>7</td>
<td>0.96 (0.94-0.99)</td>
<td>0.01</td>
</tr>
<tr>
<td>Elispot or T-SPOT.TB</td>
<td>4</td>
<td>0.92 (0.88-0.95)</td>
<td>0.01</td>
</tr>
</tbody>
</table>
## TST vs. IGRAs: Sensitivity

<table>
<thead>
<tr>
<th></th>
<th># Studies</th>
<th>Sensitivity (95% CI)</th>
<th>Chi-Square for heterogeneity (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TST</strong></td>
<td>14</td>
<td>0.71 (0.65-0.74)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Size of reaction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mm</td>
<td>9</td>
<td>0.74 (0.66-0.82)</td>
<td>0.001</td>
</tr>
<tr>
<td>10 mm</td>
<td>4</td>
<td>0.72 (0.50-0.95)</td>
<td>0.01</td>
</tr>
<tr>
<td>15 mm</td>
<td>1</td>
<td>0.40 (0.25-0.56)</td>
<td>-</td>
</tr>
<tr>
<td><strong>QuantiFERON-TB Gold</strong></td>
<td>13</td>
<td>0.76 (0.70-0.83)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Elispot or T-SPOT.TB</strong></td>
<td>12</td>
<td>0.88 (0.81-0.95)</td>
<td>0.001</td>
</tr>
</tbody>
</table>


Mayo Clinic Center for Tuberculosis
IGRAs Advantages over TST

- IGRAs are more specific than TST in BCG vaccinated patients
- IGRAs correlate well with TST to detect LTBI in contact investigations
- IGRAs do not require a second visit
- Mitogen-negative “indeterminate” results can detect anergic cases
- IGRAs do not trigger an amnesic response (i.e., boosting effect)
IGRA limitations

- IGRAs cannot differentiate LTBI vs. active TB
- Differences in blood T-cell cytokine and functional signatures between LTBI and active TB\(^1,2\)
- TST+/IGRA- results in LTBI are probably not always related to false + TST results
  - Discordant TST+/IGRA- cases can show T-cell activation to RD1-antigens using assays with longer stimulation times\(^3,4\)
  - Moderate concordance (\(\kappa = 0.69\)) between 2 IGRAs\(^5\)
- IGRA results variability over time\(^6\)
- Low positive predictive value for TB reactivation\(^7\)

(1) Berry MP, *Nature* 2010;
(2) Sutherland JS et al. *Eur J Immunol* 2009
(4) Butera O, at el. *BMC Infect Dis* 2009;
(6) van Zyl-Smith RN, *Am J Respir Crit Care Med* 2009
(7) Pai M et al. CMR 2014
IGRAs in Health Care Workers

• Prospective study in the US (N=2,418)
• Overall QFT and TSPOT:
  • Conversion:
    • QFT= 6.1%
    • TSPOT= 8.3%
    • TST= 0.9%
  • Reversions:
    • QFT= 76.4%
    • TSPOT= 77.1%
• With the use of a “borderline zone” (0.2-0.7):
  • Conversion rate: 2.3%
• Conversion rate with 2 subsequent (+) IGRAs:
  • QFT= 1.1%
  • TSPOT= 1.3%
• Unclear risk of TB by using this approach

IGRAs in People Living with HIV

• In active TB and HIV(+), in low-middle income countries
  • Sensitivity:
    • QFT= 61% (95%CI, 47 to 75%)
    • TSPOT= 72% (95%CI, 62 to 81%)
    • Neither IGRA was consistently more sensitive than TST
  • Indeterminate results
    • QFT= 8.2%
    • TSPOT= 5.9%
    • ↑ with CD4 < 200 (11.6 vs. 11.4%)
    • Lower with CD4 > 200 (3.1% vs. 7.9%)

• In suspected LTBI and HIV(+), IGRAs perform similarly to TST

• Both TST and IGRAs have suboptimal sensitivity, suggesting potential role for using both tests, especially in severely immunosuppressed individuals

IGRAs in Patients with Immune-Mediated Inflammatory Diseases

- Risk of TB in patients on immunosuppressive agents

- Systematic Review in LTBI
  - Small studies and high variability in immunosuppressive drugs
  - Neither IGRA was consistently more sensitive than TST
  - Risk factors for TB are predictive of IGRA(+) and TST(+) results
  - Immunosuppressive therapy significantly reduce QFT and TST positivity
    - OR for QFT = 0.37 (95%CI, 0.16 to 0.87)
    - OR for TST = 0.28 (95%CI, 0.10 to 0.80)
  - Indeterminate results are similar for QFT and TSPOT (~5%)
  - Limited prospective study data

- Both TST and IGRAs have suboptimal sensitivity in pts on immunosuppressive drugs, suggesting potential role for using both tests

Smith R et al. Curr Opin Rheumatol 2011
Shahidi N et al. Inflamm Bowel Dis 2012
Chang B et al. Clin Rheumatol 2011
IGRAs in non-HIV immunosuppression (Such as IBD)

Arias-Guillen M et al. Inflamm Bowel Dis 2014
US-CDC Guidelines

• High rate of false(+) IGRA if prevalence of TB infection <1%

• IGRA preferred, but TST is acceptable:
  • Low likelihood to return for TST reading (e.g. homeless, drug abusers)
  • Prior BCG vaccination (improve acceptance of LTBI Tx)

• TST preferred, but IGRA is acceptable:
  • Children < 5 yo

• Either TST or IGRA may be used without preference
  • Recent TB contacts with active case (Repeat testing 8-10 wks after end of exposure if negative test)
  • Periodic screening of HCW (?)

• Both TST and IGRA can be considered:
  • High risk of TB infection and progression, and risk of poor outcome
    • HIV (and severe immunosuppression)
    • Children < 5 yo
  • Investigation of active TB (?)
  • To enhance compliance to LTBI Tx (?)

Mazurek GH et al. MMWR 2010
**QuantiFERON-TB Plus**

- **New QFT test version**
  - 4\(^{th}\) tube (TB2) to detect detection of CD8 T-cells
  - TB7.7 antigen was removed from 3\(^{rd}\) tube (TB1)
  - Either or both TB1 and TB2 ≥ 0.35 IU/mL above nil

- **QFT-Plus vs. QFT-IT in close TB contacts with TST+ (N=119)**
  - High agreement ($\kappa = 0.80$), but QFT-Plus had stronger association with surrogate measures of TB exposure
  - Contact time >12 h/day: OR= 6.9 vs. 4.6
  - TB1-TB2 results >0.6 IU/mL: ↑ likelihood for contacts to be sleeping in the same room (OR= 4.3) and European origin (OR= 3.2) –recent exposure to TB?

- **QFT-Plus vs. QFT-IT in TB(N=162) vs. low-risk for TB (N=212)**
  - IFN-ϒ concentrations of QFT-Plus were lower in TB patients
  - Both QFT-Plus and QFT-IT had high diagnostic accuracy (AUC=0.99), but QFT-Plus had lower sensitivity (91.1%) with the 0.35 IU/mL cut-off. ↑ Sensitivity to 96.2% with a cut-off of 0.168

- **Characterization of CD4 & CD8 T-cell responses (27 TB vs 30 LTBI)**
  - TB1 mainly elicited CD4 T-cell responses
  - TB2 induced both CD4 and CD8 responses
  - TB2-specific CD8 responses more often seen in active TB vs. LTBI (44% vs. 20%)

Proposed Assessment with Combined IGRA and TST for suspected LTBI

Careful assessment of risk factors for TB infection and TB progression

- **TST+/IGRA+**
- **TST+/IGRA- (*)**
- **TST-/IGRA+ (*)**
- **TST-/IGRA-**

Repeat IGRA at 3-6 mo?

- **IGRA+**
- **IGRA-**

**LTBI**

**False+ TST due to BCG?**

**IGRA level? Possible LTBI**

**False+ IGRA? or transient infection?**

**No LTBI (**)**

(*) Either TST+ or IGRA+ can be a significant test in immunosuppression
(**) TST-/IGRA- does not rule out LTBI in immunosuppression

Adapted from Lalvani A, Pareek M Brit Med Bull 2010
Special Considerations: Boosting

• Some may have negative (waned) TST reaction when tested years after infection (e.g., older adults)

• Initial skin test may stimulate (boost) ability to react to PPD

• Subsequent positive boosted reaction may be misinterpreted as a new infection

• May still be considered for treatment if currently at high risk for TB disease
Special Considerations: Two-Step Testing

- Used for initial skin testing of adults to be retested periodically, to reduce likelihood that boosted reaction will be misinterpreted as recent infection
- If 1st test positive, consider infected; if negative, give 2nd test 1–3 weeks later
- If 2nd test positive, consider infected; if negative, consider uninfected
Diagnosis of Latent TB

Interpretation Criteria for T-Spot. TB Test (T-Spot)

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Nil*</th>
<th>TB Response†</th>
<th>Mitogen§ (Positive Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive†</td>
<td>≤10 spots</td>
<td>≥8 spots</td>
<td>Any number of spots</td>
</tr>
<tr>
<td>Borderline**</td>
<td>≤10 spots</td>
<td>5, 6, or 7 spots</td>
<td>Any number of spots</td>
</tr>
<tr>
<td>Negative††</td>
<td>≤10 spots</td>
<td>≤4 spots</td>
<td>≥20 spots</td>
</tr>
<tr>
<td>Indeterminate**</td>
<td>&gt;10 spots</td>
<td>Any</td>
<td>Any number of spots</td>
</tr>
<tr>
<td></td>
<td>≤10 spots</td>
<td>&lt;5 spots</td>
<td>&lt;20 spots</td>
</tr>
</tbody>
</table>

Interpretation Criteria for QuantiFERON-TB Gold in-Tube Test (QFT-GIT)

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Nil*</th>
<th>TB Response†</th>
<th>Mitogen Response§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive†</td>
<td>≤8.0</td>
<td>≥0.35 IU/ml and ≥25% of Nil</td>
<td>Any</td>
</tr>
<tr>
<td>Negative**</td>
<td>≤8.0</td>
<td>&lt;0.35 IU/ml or &lt;25% of Nil</td>
<td>≥0.5</td>
</tr>
<tr>
<td>Indeterminate††</td>
<td>≤8.0</td>
<td>&lt;0.35 IU/ml or &lt;25% of Nil</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td></td>
<td>&gt;8.0</td>
<td>Any</td>
<td>Any</td>
</tr>
</tbody>
</table>

* Nil: No reaction
† TB Response: Tuberculin reaction
§ Mitogen: Positive control

- HIV positive persons
- Recent contacts of persons with active tuberculosis
- Fibrotic changes on chest radiograph, consistent with tuberculosis
- Patients with organ transplants and other immunosuppressed patients
- Immigrants from high-prevalence areas
- Injection drug users
- Residents and employees* of high-risk congregate settings
- Personnel in mycobacteriology laboratories
- Persons with clinical conditions that place them at high risk
- Children: ≤4 years of age; all exposed to adults at high-risk
24 yo M (+)/(-)TST, (-)/(-)IGRAs and IBD

02/2012
Diagnosis?

(+)/(-) TST & (-)/(-) IGRA... LTBI??
Take Home Points

- Immunodiagnostics for LTBI remains challenging and areas of controversy exist.

- IGRAs are more specific than TST, but a TST+/IGRA- probably does not always represent a false (+)TST.

- Both TST and IGRAs cannot differentiate between active TB, subclinical TB, LTBI and host-cleared TB infections.

- Serial testing with IGRA is associated with a high rate of conversions and reversions.

- New diagnostics should not only accurately detect T-cell reactivity against MTB but also improve determination of LTBI with viable MTB at actual risk of TB reactivation.
Questions?